Advanced Biology through Inquiry

Teacher Guide

PASCO scientific®
10101 Foothills Blvd.
Roseville, CA 95747-7100
Toll Free 800-772-8700
916-786-3800
Fax 916-786-8905

Published by
PASCO scientific
10101 Foothills Blvd.
Roseville, CA 95747-7100
800-772-8700
916-786-3800
916-786-8905 (fax)
www.pasco.com

ISBN 978-1-937492-16-8
First Edition
First Printing
Printed in the United States of America
Part Number: PS-2852

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

** The IB Diploma Program is an official program of the International Baccalaureate Organization (IBO) which authorizes schools to offer it. The material available here has been developed independently of the IBO and is not endorsed by it.

CONTRIBUTORS

PASCO Development Team

- Freda Husic, *Director of Education Solutions*, *Program Manager*
- Cynthia Sargent, *Curriculum and Training Developer*, *Lead Author*
- Mike Blasberg, *Biology Product Manager, Author*
- Brennan Collins, *Graphics and Production*
- Fran Zakutansky, *retired teacher/PASCO Training Expert, Lab Testing and Review*

Contributing Authors

- Ryan Reardon, *AP Biology teacher, Shades Valley High School*
- Susan Park, *AP Biology teacher, Hotchkiss School*
- Marilyn Pendley, *Instructor, Caldwell Community College*
- Debbie Noyes, *AP Biology teacher, retired*
- Kelcey Burris, *AP Biology teacher, Union High School*

Editor

- Janet Miller, *Lead Editor*

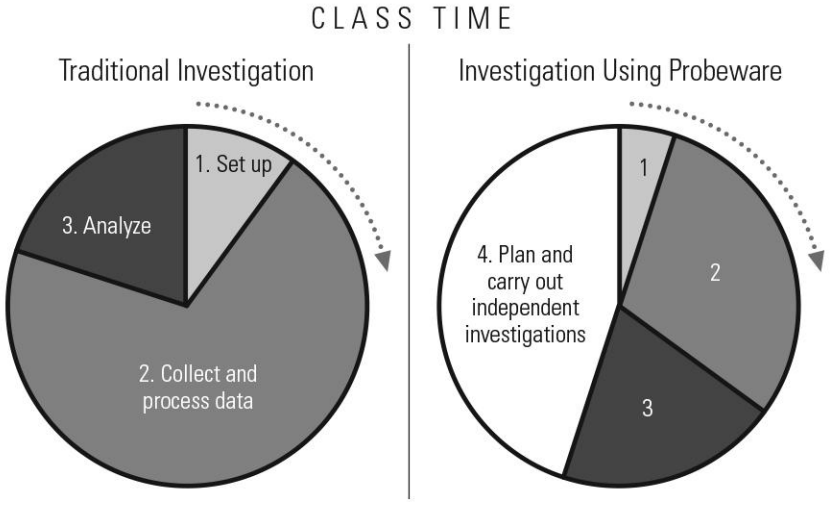
CONTENTS

INTRODUCTION

PASCO scientific's *Advanced Biology through Inquiry* investigations move students from the low-level task of memorization or confirmation of science facts to higher-level tasks of experiment design, data analysis, concept construction, and application. For science to be learned at a deep level, it is essential to combine the teaching of abstract science concepts with "real-world" science investigations. Hands-on technology-based laboratory experiences serve to bridge the gap between the theoretical and the concrete, driving students toward a greater understanding of natural phenomena. Students also gain important science process skills that include: developing and using models, planning and carrying out independent investigations, interpreting data, and using mathematics—including statistics.

The lab activities in this manual are designed so students complete brief guided-inquiry investigations that help them become familiar with the use of a particular sensor or technique and engage them in thinking about a core topic of biology. Following these Initial Investigations, students are able to move more easily into designing and conducting open-inquiry investigations related to the topic under study.

The use of electronic sensors (probeware) in investigations greatly reduces the class time required for set up and data collection, increases the accuracy of results, allows for richer analysis of data, and provides more time in the classroom for independent investigations.



Additionally, using electronic-sensor data collection, display and analysis devices allows students to:

- observe or reveal phenomena that cannot be observed, or is difficult to observe, through other methods
- perform investigations with reliable equipment that can be used repeatedly for years, reducing the need for consumable items
- focus not on the tedium of collecting data, but rather focus on the trends, patterns, and relationships which become immediately discernible when gathering real-time data
- easily apply basic statistics to data
- carry out multiple trials in a single class period
- practice using equipment and interpreting data measured by equipment that is similar to what they might use in their college courses and future careers

Teacher Resources and Student Handouts

All teacher and student materials are on the storage device accompanying the printed lab manual. Teacher resources are in pdf format, but the student handouts are in Microsoft® Word format, allowing you to customize the labs for your curriculum, students, and equipment.

Teacher Resources. Teacher resource files and printed labs contain all sections of the student handouts, such as the Initial Investigation, Data Analysis, and Synthesis Questions sections, as well as teacher-specific sections including time requirements, alignment to the AP®[1] Biology learning objectives and science practices, and teacher tips to help ensure success. Sample data is provided for the Initial Investigations and sample answers are provided for all questions. Most labs also contain sample data for suggested student-designed investigations.

Student Handouts. The handouts begin with brief background information and a driving question for the Initial Investigation. In many labs, the responsibility is placed on the student to design appropriate data tables for the investigation and determine appropriate analysis methods to support their claims regarding the results. Following the Initial Investigation, most labs prompt students to design and carry out an experiment of their own. Students analyze the data from their experiment and answer synthesis questions that require an application of their knowledge and the results of the investigation.

Scaffolding Inquiry Investigations

The investigations in this manual support a teacher's need to differentiate the level of scientific inquiry. *Guided inquiry* can involve activities in which students are provided a driving question and lab procedure, but the results of the procedure are not known ahead of time. That is, the lab is not a confirmation lab, but rather one that requires decision-making in analysis of data, problem-solving, and critical thinking.

Open inquiry is typically seen as an activity in which students make all decisions: define a driving question, design a procedure, determine a method for collecting data, make all data analysis decisions, process data to create graphs and figures to summarize results, and communicate the results to others in the science community. However, teachers should not look at inquiry in their classroom simply as "guided" or "open," but rather as a spectrum that ranges from less to more learner self-direction, and more to less direction from the teacher. (Appendix D of the College Board's lab manual, *AP Biology Investigative Labs: An Inquiry-Based Approach* provides an example of this spectrum.)

The lab activities developed by PASCO allow teachers to easily modify the level of inquiry appropriate for their students (student files are fully editable). Three examples of modifications follow:

- To increase the level of inquiry for Enzyme Activity, remove the lab procedure from the Initial Investigation. Provide students with the Background, Driving Question, Materials and Equipment, and Safety sections, and leave only a data table and analysis questions in the Initial Investigation section. Students are then tasked with developing their own procedure to address the driving question, data table, and analysis questions.
- To increase the level of inquiry for Diffusion, only provide students with the Design and Conduct an Experiment Worksheet for the lab. Introduce students to the topic of diffusion and the use of a pH sensor to measure diffusion by performing a demonstration similar to the Initial Investigation. Ask students to answer the analysis questions collaboratively for the demonstration in groups. After assessing students' readiness for open inquiry with the topic, provide them copies of the worksheet to design and carry out independent experiments.
- To increase the level of inquiry for Fermentation, inform students that they will use an ethanol sensor for their experiments. Provide them with the Experiment Design Plan to design their independent investigations and no other directions.

[1] AP is a trademark registered and/or owned by the College Board, which was not involved in the production of, and does not endorse, this product.

Lab Activity Components

This table identifies the sections in the teacher and student files.

TEACHER RESOURCES	STUDENT HANDOUT
Lab Overview	Background
Pacing and Length of the Lab	Driving Question
AP Connections	
Materials and Equipment	Materials and Equipment
Prerequisites	
Safety	Safety
Lab Preparation	
Teacher Tips	
Initial Investigation	Initial Investigation
Design and Conduct an Experiment[1, 2]	Design and Conduct an Experiment[1, 2]
Suggested Inquiry Questions	
Design and Conduct an Experiment: Data Analysis[2]	Design and Conduct an Experiment: Data Analysis[2]
Synthesis Questions	Synthesis Questions
Design and Conduct an Experiment Key	Design and Conduct an Experiment Worksheet
Sample Data	

[1]Some labs do not include a student-designed experiment.

[2]A few labs have a complex or lengthy Initial Investigation and these sections were reordered to account for this variation.

Overview of Lab Activity Components

LAB OVERVIEW OR BACKGROUND

The lab overview in the Teacher Resources provides a brief description of the lab and the topics addressed in the lab. For students, the lab activity begins with the Background, which provides a brief introduction to the topic and some prerequisite knowledge, and sets a purpose for the investigation.

DRIVING QUESTION(S)

This section, only in the Student Handouts, provides a specific, testable question that the Initial Investigation is designed to answer.

MATERIALS AND EQUIPMENT

This section lists all materials and equipment needed to carry out the activity procedure. If items in this list need to be created using additional materials, those are indicated as a footnote in the Teacher Resources version, and instructions for preparing them are in the Lab Preparation section. In the Teacher Resources, this section includes an additional table of items recommended for student-designed experiments.

SAFETY

This section lists the pertinent safety procedures for the lab.

INITIAL INVESTIGATION

The Initial Investigation is a guided investigation, as students are provided with the driving question, the basic procedural steps to complete the activity, and several embedded analysis questions. However, students are still tasked with higher-level thinking: deciding how to organize and present data in tables or graphs, deciding which analysis tools to use within the data collection software and whether to process the data further outside of the data collection system, and

answering analysis questions that require data interpretation and application of biological knowledge to explain data and support claims.

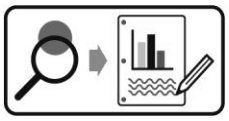
The Initial Investigation serves two main purposes: (1) to give students experience using a particular sensor or laboratory technique, and (2) to engage students in thinking deeply about a biological topic to help them construct meaningful questions they can pursue answers to through their student-designed experiments. Most of the Initial Investigations are brief, requiring a single lab period or less. As the school year progresses and students become more skilled in the science practices and more familiar with probeware, teachers may choose to reduce the amount of scaffolding in the Initial Investigation or remove this section entirely.

Embedded in this section of the Teacher Resources are sample data tables, graphs, computations and analysis, and answers to support teachers in their implementation of each lab activity with students.

DESIGN AND CONDUCT AN EXPERIMENT

This section directs students to plan and carry out an additional experiment. For teachers, the description of the experiment to be designed is more specific and includes suggested inquiry questions. Students are encouraged to come up with their own testable questions; however, teachers may choose to provide suggestions to students for labs performed early in the school year, or for labs in which additional equipment is limited.

This section contains the following instructions:



Design and carry out your experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan. Then complete the Data Analysis and Synthesis Questions.

A *Design and Conduct an Experiment Worksheet* is included in all labs that have a student-designed experiment option; it appears at the end of the Student Handout and provides questions to guide students in the planning of their experiment.

A more open-ended option is to provide students with the *Experiment Design Plan* handout. This page accomplishes the same goal as the worksheet, providing students some structure for planning an investigation, but it is not lab-specific. (The symbol shown above appears on both the worksheet and the Plan page to indicate their connection.) The Experiment Design Plan is included after this Introduction.

DESIGN AND CONDUCT AN EXPERIMENT: DATA ANALYSIS

Students are encouraged to analyze and present their data in various ways: perform calculations to average data from numerous trials, organize data tables to summarize results, and create graphs (either of the dependent variable as it appears on the sensor-measurement display or of processed data exported to Microsoft Excel® or other programs). For most labs the Data Analysis questions are open-ended, allowing students to determine how to evaluate the meaning and significance of their results. A few labs contain complex or lengthy Initial Investigations; these labs have Data Analysis sections devoted to the data of the Initial Investigation instead of a student-designed experiment.

NOTE: The College Board's AP® Biology Quantitative Skills Guide *is a good resource for supporting students' application of statistical methods to evaluate the significance of the results of their experiments.*

The Teacher Resources version of this section contains sample answers based on analysis of data collected for a sample inquiry that is described in detail in the Design and Conduct an Experiment Key. The data for the sample inquiry is presented as the first data set in the final section of Teacher Resources, entitled "Sample Data."

SYNTHESIS QUESTIONS

These questions require students to develop a deeper understanding of concepts and assesses whether students can transfer the knowledge learned in the lab to other situations. The questions are modeled after short-answer and free-response questions typical of advanced biology exams.

In addition to requiring students to synthesize information from different topics of biology, the questions often require students to use quantitative reasoning skills and apply mathematical or statistical knowledge. Most labs include data from a published scientific article, providing students an opportunity to analyze real and complex data for experiments difficult to carry out in the typical high school classroom.

DESIGN AND CONDUCT AN EXPERIMENT WORKSHEET OR KEY

The questions included on this Worksheet are designed to guide students in the development of a testable question and hypothesis, and in planning a controlled experiment to test their hypothesis. It offers a great opportunity for formative assessment before students embark on their independent investigations. When students are ready for a more open-ended option for planning their experiments, teachers should eliminate the Worksheet and provide students with copies of the Experiment Design Plan.

The Design and Conduct an Experiment Key provides a model of planning an investigation; it contains sample answers specific to one of the suggested inquiry questions listed in the Teacher Resources of a lab.

Additional sections in the Teacher Resources

PACING AND LENGTH OF THE LAB

This section provides teachers with estimated times that inform their lesson planning. For most labs, time estimates are provided for the Initial Investigation and for the time required for students to plan and carry out their own investigations.

AP CONNECTIONS

There are three components to the table provided in this section: Essential Knowledge, Science Practices, and Learning Objectives. The references in the table, such as "2.D.1" correspond to the *2012–2013 AP Biology Curriculum Framework* published by the College Board®[2].

The correlations table at the end of this introduction summarizes the connections between the Big Ideas of the curriculum framework and the labs included in this manual. The connections to the Big Ideas occur either directly in the data collection and analysis in the Initial Investigation or through the Synthesis Questions that require students to apply their understanding of concepts across domains.

PREREQUISITES

Students should have the prerequisite knowledge listed before performing the lab activity. These labs are *not* confirmation labs, so students can perform the lab without a large amount of prior knowledge in the topic. Through completion of the lab, they acquire content and deepen their understanding.

LAB PREPARATION

Directions for preparing solutions and other materials are provided in this section. Read through this section carefully in advance of the lab; some labs require materials to be prepared either the day before a lab or immediately before a lab. Most investigations make use of household items or materials that can be easily acquired or are common to a high school science stockroom. Some labs

[2] College Board®is a trademark registered and/or owned by the College Board, which was not involved in the production of, and does not endorse, this product.

do require materials that may need to be purchased by the school and the teacher should plan for this in advance.

TEACHER TIPS

This section provides a variety of information such as tips on the use of a particular sensor, or tips on a pedagogical approach for the activity. All tips help support the teacher in a successful implementation of the lab in the classroom.

SAMPLE DATA

The sample data is presented in tables or graphs that display expected patterns based on PASCO trials of sample inquiry questions. However, if students design experiments that address the same independent variable, their data may vary. Use these events for discussions of variation in scientific data as well as variation in approaches (experiment designs) taken to answer the same scientific question.

The Data Collection System

In this manual, "data collection system" refers to the system employed by students to record, visualize, and analyze sensor data during their experiments. The system consists of all components necessary to connect a sensor to a device containing the software that detects the sensor measurement and collects, records, and displays this data.

Some systems, such as the Xplorer GLX® or SPARK Science Learning System™, are stand-alone systems. These contain built-in software applications, and students simply attach a sensor and begin collecting data. Other systems use a computer or tablet with downloaded software applications. In these systems a USB or Bluetooth® interface is used to connect a sensor to the device. Software options for these include SPARKvue® 2 and PASCO Capstone™ software.

The activities are designed so that any PASCO data collection system can be used to carry out the procedures.

Getting Started with Your Data Collection System

To help you and your students become familiar with the many features of your data collection system, start with the tutorials and instructional videos available in the video library on PASCO's website (www.pasco.com). Each system's software also has a built-in help system.

There are free SPARKlab™ activities included in the SPARKvue software and performing one of these activities can be a good starting place for students to familiarize themselves with connecting a sensor, viewing data, saving their work, and other tasks related to probeware use.

PASCO scientific also has a terrific technical and teacher support team. They pride themselves on providing timely and comprehensive help to teachers and students using PASCO products.

Phone: 1-800-772-8700

Email: support@pasco.com

Web: www.pasco.com/support

Electronic Materials

A USB storage device was included with the purchase of this manual and is attached to its inside cover. Please view Overview.pdf for a list of the included resources.

In addition to the teacher and student versions of all lab activities, the storage device contains electronic files needed to perform the Mathematical Modeling of Evolution and BLAST into Bioinformatics activities.

International Baccalaureate Organization (IBO) Support

IBO Diploma Program

The International Baccalaureate Organization (IBO) uses a specific science curriculum model that includes both theory and practical investigative work. While this lab guide was not produced by the IBO and does not include references to the IB standards or internal assessment rubrics, the lab activities can be adapted easily to the IB classroom. For example, students can complete an Initial Investigation and the teacher grades their work using the "Data collection and processing" internal assessment rubric. The labs in this manual correlate closely to curriculum topics of the IB Biology higher level program: statistical analysis, cells, cell respiration and photosynthesis, genetics, human health and physiology, and others.

By the end of the IB Diploma Program students are expected to have completed a specified number of practical investigative hours and are assessed using the specified internal assessment criteria. Students should be able to design a lab based on an original idea, carry out the procedure, draw conclusions, and evaluate their results. These scientific processes require an understanding of laboratory techniques and equipment as well as a high level of thinking, skills that are developed and sharpened by completing the investigations in this manual.

AP Biology Correlations

The Big Ideas of the curriculum framework

Big Idea	Description
1	The process of evolution drives the diversity and unity of life.[3]
2	Biological systems utilize free energy and molecular building blocks to grow, to reproduce, and to maintain dynamic homeostasis.[3]
3	Living systems store, retrieve, transmit, and respond to information essential to life processes.[3]
4	Biological systems interact, and these systems and their interactions possess complex properties.[3]

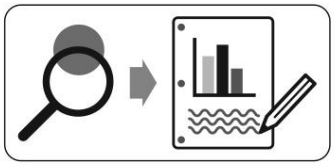
Connection between the Big Ideas and the activities in this manual

Activity	Lab Activity	Big Ideas			
		1	2	3	4
1	Enzyme Activity *Students use an oxygen gas sensor or pressure sensor to investigate the catalyzed decomposition of hydrogen peroxide by catalase.*	X	X		X
2	Diffusion *Students use a pH sensor to investigate the diffusion of hydrogen ions through a semipermeable membrane, comparing the rates of diffusion for two solutions that differ in their acidity.*		X		
3	Osmosis *Students use a colorimeter to determine which extracellular fluid is hypertonic to a model cell and which solution is hypotonic.*		X	X	
4	Plasmolysis *Students use a conductivity sensor to explain the results of different concentrations of salt water on plant tissue before they design an experiment to compare the water potential of different plant tissues.*		X		
5	Cell Size *Students use temperature probes to measure the effect of cell size on cell cooling rate using cubes of potato tissue.*	X	X		
6	Homeostasis *Students use multiple temperature probes simultaneously to investigate the body's ability to maintain homeostasis when subjected to a cold stimulus.*			X	X
7	Cellular Respiration *Students use a carbon dioxide gas sensor to investigate the rate of cellular respiration of germinating seeds.*	X	X		X
8	Fermentation *Students use an ethanol sensor to determine the ability of yeast to use different types of carbohydrates—sucrose and starch—for fermentation.*		X		X
9	Photosynthesis *Students use a carbon dioxide gas sensor to test the effect of the color of light on the rate of photosynthesis.*		X		X
10	Plant Pigments *Students analyze spinach pigments and chloroplasts using paper chromatography, a colorimeter, and a spectrometer to understand how plants capture light for photosynthesis.*		X		X

[3] *AP Biology Curriculum Framework: 2012–2013,* The College Board, 2011.iii.

Activity	Lab Activity	Big Ideas			
		1	2	3	4
11	Transpiration *Students use a low pressure sensor (barometer) and a weather sensor to investigate the rate of transpiration in plants under normal and humid conditions.*		X		X
12	Mitosis *After learning the technique for growing roots and preparing root tip squashes for microscope analysis, students observe the root tips for evidence of mitosis and statistically analyze the data.*			X	
13	Meiosis *Students use physical models of chromosomes to explore meiosis and genetic variation, and use cross over rates observed in Sordaria to calculate gene distance from the centromere.*			X	
14	Transformation *Students transform bacteria with a plasmid that contains an ampicillin resistance gene and a gfp gene that is regulated so only some transformed cells produce the green fluorescent protein.*			X	
15	Understanding Inherited Mitochondrial Disorders *Students use pedigree analysis and DNA analysis (electrophoresis) to confirm or refute the initial diagnosis of MELAS for two patients.*		X	X	X
16	Sickle Cell Gene Detection *Students use electrophoresis to analyze DNA samples from a child and the child's parents to determine if the child has inherited a mutation in the gene for hemoglobin B.*			X	X
17	Energy Dynamics *Students use EcoChamber containers and a carbon dioxide gas sensor to estimate energy flow and carbon cycling within a variety of detritus-based ecosystems.*		X		X
18	Artificial Selection *Students follow the growth and development of Wisconsin Fast Plants and determine if limiting cross-pollination to certain plants with a desired trait affects the frequency of that trait in the second generation.*	X			
19	BLAST Bioinformatics *Students analyze the DNA and protein sequences of beta globin of five mammalian species to determine their evolutionary relatedness.*	X			
20	Population Genetics *Students determine their phenotype for the PTC (phenylthiocarbamide) tasting trait and use class data to derive allele frequencies for a population.*	X			
21	Mathematical Modeling of Evolution *Students work with a mathematical model and computer simulation to explore how inheritance patterns and gene frequencies change in a population.*	X			
22	Animal Behavior *Students use a choice chamber to test the response of fruit flies to different stimuli and determine if there is a significant change in their behavior.*		X		X

Experiment Design Plan



Title:

Driving Question:

Hypothesis (If...then...):

Independent Variable:

Dependent Variable:

How will you manipulate the independent variable in each experimental group?

Experimental Group 1:

Experimental Group 2:

Experimental Group 3:

Experimental Group 4:

Control Group:

Controlled Variables
(quantify where possible):

Dependent Variable:

Data Collection Details:

Number of Trials (provide justification):

What data analysis will you perform
to evaluate your results and hypothesis?

Use the space below to create an outline of the experiment.

In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

MASTER MATERIALS AND EQUIPMENT LIST

Italicized entries indicate items not available from PASCO. The quantity indicated is per student or group. NOTE: The activities also require protective gear for each student (for example, safety goggles, gloves, apron, or lab coat).

Teachers can conduct some lab activities with sensors and probes other than those listed here. For assistance with substituting compatible sensors and probes for a lab activity, contact PASCO Teacher Support (800-772-8700 inside the United States or http://www.pasco.com/support).

Lab	Title	Materials and Equipment	Qty
1	ENZYME ACTIVITY	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO Oxygen Gas Sensor or PASCO Pressure Sensor	1
		Sampling bottle, 250-mL*	1
		Graduated cylinder, 25-mL	1
		Pipet, 1-mL	1
		Magnetic stirrer and stirring bar	1
		Base and support rod	1
		3-Finger clamp	1
		1.5% Hydrogen peroxide (H_2O_2)	20 mL
		Catalase suspension prepared from dried bakers' yeast	2 mL
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Hot plate or water bath	As needed
		Buffers, pH 3 to pH 10	As needed
		3.0% Hydrogen peroxide	As needed
		Catalase or peroxidase suspension from other sources (beef liver, turnips, rutabaga, other plants.)	As needed
		Ice	As needed

Lab	Title	Materials and Equipment	Qty
2	DIFFUSION	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO pH Sensor	1
		Graduated cylinder, 25-mL	1
		Beaker or cup, 250-mL–400-mL	1
		Dialysis tubing, 1 inch × 28-cm[1]	1
		Disposable pipet or 10-mL syringe	1
		Paper clip or binder clip	1
		Small cup to capture the 25 mL (or less) of fluid from the dialysis bag	1
		Apple cider vinegar	25 mL
		Pickle juice	25 mL
		Magnetic stir bar and plate (if available)	1
		Spring water (or distilled water)	200 mL
		Plastic wash bottle with distilled water	1
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		PASCO Conductivity Sensor	1
		Other commercially available solutions, such as: olive juice, jalepeño juice, beet juice	As needed
		Hot plates or warm water baths	As needed
		Other "extracellular" solutions to replace water	As needed
		Additional dialysis tubing , including tubing of a different diameter or with different pore sizes	As needed
		Ice	As needed
		Food coloring, different colors	As needed
3	OSMOSIS	**FOR EACH STUDENT STATION**	
		Data collection system	1
		PASCO Colorimeter	1
		Sensor extension cable*	1
		Cuvettes*	4
		Cups or beakers, 250-mL	2
		Small funnel	1
		Graduated cylinders, 25-mL	2
		Dialysis tubing, 12-cm piece	2
		Solution A (tap water)	100 mL
		Solution B (0.8 M sucrose)	20 mL
		Solution C (1.0 M sucrose)	100 mL
		Solution D (0.1 M sucrose)	20 mL
		Plastic pipets	2
		Small binder clips	2

Lab	Title	Materials and Equipment	Qty
4	PLASMOLYSIS	**FOR EACH STUDENT STATION**	
		Data collection system	1
		PASCO Conductivity Sensor	1
		Microscope, 400× magnification	1
		Microscope slides and cover slips (4)	4
		Plastic pipet or eye dropper	1
		Three NaCl salt solutions of unknown concentration	Several drops
		Red onion	Section
		Water	Several drops
		Paper towel	1
		TEACHER DEMONSTRATION	
		Electronic balance	1
		Celery stalks	2
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Sucrose solutions (1.0 M)	2 L
		Distilled water	600 mL
		Containers for preparing sucrose dilutions	As needed
		Electronic balance	1
		Small cups	As needed
		White potatoes	As needed
		Sweet potatoes or yams	As needed
		Celery, carrots, or other vegetables	As needed
		Apples or other fruits	As needed
5	CELL SIZE	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO Quad Temperature Sensor	1
		PASCO Fast-response temperature probes*	3
		Metric ruler	1
		Small knife or scalpel	1
		Cutting board or other appropriate surface	1
		Potato	1
		Plastic containers (for ice water), 24 oz or larger (approximately 700 mL)	1
		Water	~500 mL
		Toothpicks	2
		Permanent marker	1
		Tape	As needed
		Ice	~100 mL
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENT	
		Melon baller (to form spherical potato "cells")	1
		Shortening (or similar solid fat source)	As needed
		Cork borer (to form cylindrical potato "cells")	1
		Additional potatoes	As needed

Lab	Title	Materials and Equipment	Qty
6	HOMEOSTASIS	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO Quad Temperature Sensor	1
		PASCO Fast-response temperature probes *	2
		Large shallow bowl or pan[2] (for submerging a hand in ice water)	1
		Ice	As needed
		Water	As needed
		Adhesive bandages or medical tape for securing temperature probes to the skin	2 pieces
		Paper towel	As needed
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Non-latex disposable gloves	As needed
		PASCO physiology sensor(s) such as a hand-grip heart rate sensor, EKG sensor, spirometer sensor, and blood pressure sensor and cuff	As needed
7	CELLULAR RESPIRATION	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO Carbon Dioxide Gas Sensor	1
		Sensor extension cable*	1
		Sample bottle, 250 mL*	1
		Balance, readability: 0.01 g	1
		Paper towel	1
		Germinating pinto beans	20
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		PASCO Fast-response Temperature Sensor	1
		PASCO Oxygen Gas Sensor	1
		Solutions of different pH or salinity levels	As needed
		Additional germinating pinto beans	As needed
		Germinating and dormant seeds of other species, or small animals[3]	As needed

Lab	Title	Materials and Equipment	Qty
8	FERMENTATION	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO Ethanol Sensor	1
		Sampling bottle* or glass flask (125-mL or 250-mL)	2
		Graduated cylinders (2), 50-mL	1
		Plastic pipet	1
		Small beaker	1
		Magnetic stir plate and stir bar	1
		Rod stand and 3-finger clamp (optional)	1
		1% Ethanol (derived from anhydrous ethanol)	25 mL
		Yeast suspension, derived from active dry yeast	40–60 mL
		2% Sucrose solution	30 mL
		2% Starch solution	150 mL
		Iodine indicator (IKI)	5–10 drops
		Water from germinating seeds	5 mL
		2% Starch solution mixed with amylase (optional)	30 mL
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		PASCO Oxygen Gas Sensor	1
		PASCO pH Sensor	1
		PASCO EcoChamber™ container	1
		Additional yeast suspension	As needed
		Different types of yeast	As needed
		Additional energy sources: glucose, fructose, lactose, artificial sweeteners	As needed
9	PHOTOSYNTHESIS	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO Carbon Dioxide Gas Sensor	1
		Sensor extension cable*	1
		Sampling bottle, 250-mL*	1
		Box, foil, or cloth for shading the setup	As needed
		Light source	1
		Compact fluorescent light bulb, 60 W equivalent(or higher), red	1
		Compact fluorescent light bulb, 60 W equivalent (or higher) green	1
		Fresh spinach leaves[2]	As needed
		Forceps or pencil	1
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		PASCO Oxygen Gas Sensor	1
		PASCO EcoChamber container (to accommodate larger plants)	1
		Variety of leaf types, such as: non-green or less-green, and needles	As needed
		Variety of light sources, such as a grow light and other types of fluorescent bulbs	1

Lab	Title	Materials and Equipment	Qty
10	PLANT PIGMENTS	**FOR EACH STUDENT STATION – PART 1**	
		Data Collection System	1
		PASCO Colorimeter	1
		PASCO Wireless Spectrometer and spectrometry software	1
		Colorimeter cuvette	2
		Spectrometer cuvette (1-cm glass cuvette)	2
		Plastic pipets, 1-mL	3
		Capillary tube or eye dropper without a bulb	1
		Chromatography chamber with solvent[2,3]	1
		Chromatography paper	1 sheet
		Ethanol	30 mL
		Pigment extract:	10 mL
		Spinach leaves	3
		Ethanol	5–10 mL
		Beaker, small	1
		Mortar and pestle	1
		Cheesecloth or coffee filter paper	1
		Scissors	1
		Small stapler or paper clips	1
		Ruler	1
		Pencil	1
		Kimwipes®	As needed
		FOR EACH STUDENT STATION – PART 2	
		Data Collection System	1
		PASCO Colorimeter	1
		Colorimeter cuvettes	3
		Plastic pipets (4), 1-mL	4
		Chloroplast suspension:	9 drops
		Spinach leaves	Handful
		0.5 M Sucrose	100–200 mL
		Cheesecloth	1
		0.1 M Phosphate buffer (KH_2PO_4 and K_2HPO_4[3]	3 mL
		DPIP (2,6-dichlorophenolindophenol) solution,	2 mL
		Lamp with a compact fluorescent (CFL) light bulb	1
		Kimwipes	As needed
		Aluminum foil, to cover a cuvette	Small piece
		Distilled water	10 mL
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Different types of leaves	As needed
		Different light sources: different colored lightbulbs or lightbulbs of different wattage	As needed
		Additional chromatography paper and solvent	As needed
		Hot plate	1
		Frozen or canned spinach	As needed

Lab	Title	Materials and Equipment	Qty
11	TRANSPIRATION	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO Barometer/Low Pressure Sensor	1
		PASCO Weather Sensor	1
		Sensor extension cables	2
		*Quick-release connector**	1
		*Clear plastic tubing, 40–50 cm**	1
		One-hole rubber stopper that goes on tubing	1
		Large tub or bucket (for water)	1
		Paraffin film or petroleum jelly (if available)	As needed
		Plant sample containing numerous leaves, such as ornamental pear, oleander, hydrangea, and gardenia	1
		Base and support rod	1
		3-finger clamps	2
		Test tube clamp	1
		Clear plastic bag, 1 gallon	1
		Spray bottle with water	1
		Electronic balance, centigram	1
		Small syringe, 60-mL or larger, without needle	1
		Pipet	1
		Metric ruler	1
		Large scissors or small pruning shears	1
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		PASCO EcoChamber containers, including stoppers	At least 2
		PASCO Weather Sensor for each EcoChamber container	At least 2
		PASCO Carbon Dioxide Gas Sensor	1
		Small plants that fit in the EcoChamber container, such as pansy, marigold, and impatiens	As needed
		Clear plastic bags and twist-ties to cover the root ball (or quart or gallon zip-close bags)	As needed
		Additional plant samples (different species) that fit in the tubing of the potometer[2]	As needed
		Electronic balance, centigram	1
		Small fan	1
		Lamp with incandescent or UV bulb that provides heat	1
		Lamp with a CFL bulb that remains cool	1
		Lamp with a CFL bulb that remains cool	1

Lab	Title	Materials and Equipment	Qty
12	MITOSIS	**FOR EACH STUDENT STATION**	1
		Dissection scissors	1
		Forceps	1
		Razor blade or scalpel	1
		Glass test tube	1
		Glass microscope slides	3
		Cover slips	2
		Compound microscope with 400× magnification	1
		Disposable pipets 1-mL	2
		Plastic cup, 16-oz	1
		Spot plate	1
		Personal protective equipment: Disposable gloves and chemical apron	1
		Carbol fuchsin solution	1 mL
		1 M Warm hydrochloric acid (HCl), 1 mL	1 mL
		Onion bulb (green onion, small white onion, or garlic)	1
		Paper towel	As needed
		Large toothpicks	4
		Pencil with eraser	1
		Plastic wrap	As needed
		Disposable plastic gloves	1 pair
		Permanent marker	1
		Distilled water	As needed
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Data Collection System	1
		PASCO Conductivity Sensor	1
		PASCO pH Sensor	1
		Herbicide samples	As needed
		Additional onion bulbs, or other plant samples (such as garlic)	As needed
		Plant food samples: fertilizers or root growth stimulants	As needed
13	MEIOSIS	**FOR EACH STUDENT STATION**	
		Drosophila *Chromosome Sheet (included in the lab)*	1
		Karyotype of Offspring Fly Sheet (included in the lab)	1
		Scissors	1
		Tape	1
		Pop beads for chromosomes (4), 2 colors, 2 sizes, plus enough to make sister chromatids	150 (75 of each color)
		String, approximately 1 m and 0.5 m	2 pieces
		Cards with images or photographs of Sordaria *asci (Cards with images or photographs can be purchased from supply companies such as Flinn Scientific or Ward's Science.)*	As specified
		or	
		Sordaria *crossing over kit (Crossing-over kits can be purchased from many different science supply companies. Refer to the documentation included with the kit for additional preparation directions if students prepare their own slides to observe asci.)*	1

Lab	Title	Materials and Equipment	Qty
14	TRANSFORMATION	**FOR TEACHER PREPARATION**	
		Edvotek™ EDVO-Kit: 223/AP08	1
		Water bath, 60 °C	1
		Pipet pump and 10 mL glass pipet (optional)	1
		Sterile water	50 µL
		Micropipet with sterile tips (to aliquot plasmid)	1
		Microwave or hot plate and beaker with water	1
		Heat-resistant glove	1 pair
		Incubator	1
		Permanent marker, fine	1
		FOR EACH STUDENT STATION	
		LB (Luria Broth) Petri plate	1
		LB/Amp Petri plate (2)	2
		LB/Amp/IPTG Petri plate	1
		Inoculating loops (2), sterile	2
		Transfer pipets (4), 1-mL, sterile	4
		Micropipet with a sterile tip	1
		Microcentrifuge tubes (2)	2
		Small cup or beaker, 100-mL, for ice	1
		Tube with 0.5 M Calcium chloride ($CaCl_2$), 1 mL	1
		Tube with Recovery Broth, 1.5 mL	1
		Tube with pFluoroGreen™ (pGFP) plasmid, 12 µL	1
		Toothpick, sterile	1
		Ice	50 mL
		Permanent marker, fine	1
		Masking tape	As needed
		ONE PER CLASS	
		E. coli *host cells*	5 large Petri plates
		Warm water baths, 37 °C and 42 °C	2
		Incubator (37 °C)	1
		Long wave UV light source	1
		Disinfectant	As needed
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Additional E. coli *and other bacteria species*	As needed
		Additional Petri plates and LB agar	As needed
		Filter paper	As needed
		Hole punch	1
		Other antibiotics: kanamycin, penicillin, or others	As needed
		Other plasmids: pUC18, pBLU®, pKAN, or others	As needed
		Sterile forceps	As needed

Lab	Title	Materials and Equipment	Qty
15	UNDERSTANDING INHERITED MITOCHONDRIAL DISORDERS	**FOR TEACHER PREPARATION**	
		Mitochondrial Genetics Kit (BP-6946)	1
		Erlenmeyer flasks, 500-mL	2
		Large beaker or container, 3-L (to dilute buffer)	1
		Balance	1
		Microwave or hot plate	1
		Heat-resistant gloves	1 pair
		Gel casting trays	1 per group
		Scissors	1
		Plastic wrap or aluminum foil	1
		Distilled water, 3 L	3 liters
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		QuickStrip™ DNA samples	1
		InstaStain® Blue card	1
		Horizontal gel electrophoresis apparatus	1
		DC power supply	1
		Automatic micropipet, 5 to 50 µL, with tips	1
		Tray with 0.8% agarose gel	1
		Plastic tray for gel staining	1
		Plastic wrap	1 piece
		Graduated cylinder, 100-mL	1
		Waste receptacles (for used tips)	1
		Disposable gloves	1 pair
		Distilled water or buffer for staining	75–100 mL
		OPTIONAL	
		Camera (USB or other)	1
		Permanent marker	1
		Transparency film (for tracing the results)	1
		ONE PER CLASS	
		DNA visualization system (white light)	1
		Spatula	1

Lab	Title	Materials and Equipment	Qty
16	SICKLE CELL GENE DETECTION	**FOR TEACHER PREPARATION**	
		Genetically Inherited Disease Detection Kit (BP-6947)	1
		Balance	1
		Distilled water, 3 L	3 L
		Erlenmeyer flasks, 500-mL	2
		Gel casting trays	1 per group
		Heat-resistant gloves	1 pair
		Large beaker or container, 3-L (to dilute buffer)	1
		Microwave or hot plate	1
		Plastic wrap or aluminum foil	1
		Scissors	1
		FOR EACH STUDENT STATION	
		InstaStain® Blue card[1]	1
		QuickStrip™ DNA samples[1]	1
		Automatic micropipet, 5 to 50 µL, with tips	1
		DC power supply	1
		Disposable gloves	1 pair
		Distilled water or buffer for staining	75–100 mL
		Graduated cylinder, 100-mL	1
		Horizontal gel electrophoresis apparatus	1
		Plastic tray for gel staining	1
		Plastic wrap	Piece
		Tray with 0.8% agarose gel	1
		Waste receptacles (for used tips)	1
		OPTIONAL	
		Camera (USB or other)	1
		Permanent marker	1
		Transparency film (for tracing the results)	1
		ONE PER CLASS	
		DNA visualization system (white light)	1
		Spatula	1

Lab	Title	Materials and Equipment	Qty
17	ENERGY DYNAMICS	**FOR EACH STUDENT STATION**	
		Data Collection System	1
		PASCO Carbon Dioxide Gas Sensor	1
		PASCO Sensor extension cable*	1
		EcoChamber container, with lid and stoppers	1
		Electronic balance, centigram	At least 1 per class
		Weigh boat	1
		Plastic pipet, 1-mL	1
		Disposable gloves	1 pair
		Small knife (for cutting fruit)	1
		Filter paper or coffee filter (9 cm diameter)	1
		Yeast suspension or water (yeast is used in 2 of the 3 chamber configurations, water is used in the third)	5 mL
		Mealworms (used in 2 of the 3 chamber configurations)	20
		Detritus: organic material such as apples and banana peels	60 g
		Plastic wrap (ditritus of one of the two control chambers is wrapped in plastic wrap)	As needed
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Additional sensors such as a PASCO Oxygen Gas Sensor or PASCO Temperature Sensor	One or more
		Additional EcoChamber containers	One or more
		Different detritivores (earwigs, earthworms, crickets, ants, and similar organisms)	As needed
		Different sources of detritus (various fruit or vegetable scraps such as potato)	60 g per chamber

Lab	Title	Materials and Equipment	Qty
18	ARTIFICIAL SELECTION	**FOR EACH STUDENT STATION**	
		Wisconsin Fast Plants® seeds, standard	18
		Seed-starting soil or germinating mix (such as Jiffy Mix®) (This type of soil mix is not *potting soil)*	Enough to set up 3 growing systems twice
		Fertilizer, Osmocote™ pellets or a water-soluble fertilizer (use as directed)	24 pellets
		Wicking material, #18 nylon mason twine	3 pieces
		Recycled plastic bottles, 0.5 L to 1 L	3
		Soda bottle cap with hole (or aluminum foil with holes, held in place with a rubber band)	3
		Plant vermiculite	Enough to set up 3 growing systems twice
		Labeling tape and markers	1
		Black plastic to cover the water reservoir(optional)	3 pieces
		Water in a rinse bottle	As needed
		Lighting system with fluorescent lights	1 per class
		Bee sticks or cotton applicators (for pollination)	3
		Plastic plant labels (3)	3
		Scissors	1
		12-inch ruler	1
		Stakes and holders (wooden splints and plastic straws)	As needed
		Dechlorinated water or nutrient solution (for the reservoir), if water-soluble fertilizer is used.	As needed
		Hand-held plastic magnifier	1
		Petri dish lid	1
		Paper envelope, small	1
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Data Collection System	1
		PASCO pH Sensor	1
		Transfer pipets	As needed
		1 M nitric acid (HNO_3)	As needed
		1 M sulfuric acid (H_2SO_4)	As needed
19	BLAST BIOINFORMATICS	**FOR EACH STUDENT STATION**	
		Computer with Internet access	1
		DNA Sequences Worksheet	1
		ABI BLAST Sequences.docx	1
		Highlighter	1
		Scissors (optional)	1
		Ruler or large index cards	1
20	POPULATION GENETICS	**FOR EACH STUDENT**	
		PTC (phenylthiocarbamide) paper	1
		Control paper (optional)	1
		Calculator with square root function	1
		Allele cards from the gene pool	2
		Class data page	1 per class
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Beads, 2 or more contrasting colors	100 or as needed
		Large cups	2 or more

Lab	Title	Materials and Equipment	Qty
21	MATHEMATICAL MODELING OF EVOLUTION	**FOR EACH STUDENT STATION**	
		Computer	1
		Mathematical model spreadsheet file: ABI Mathematical Modeling Spreadsheet.xlsx	1
		Spreadsheet program (such as Microsoft Excel®, Numbers®[4], or Google Docs™[5])	1
22	ANIMAL BEHAVIOR	**FOR EACH STUDENT STATION**	
		Clear drinking straw	1
		Droppers	2
		Cotton swabs	10
		Timer	1
		Sheet of white paper	1
		Wingless fruit flies , or similar small organism	10
		Mashed ripe banana	10 mL
		Mashed unripe banana	10 mL
		Distilled water	10 mL
		ADDITIONAL EQUIPMENT FOR STUDENT-DESIGNED EXPERIMENTS	
		Cold and warm packs	1
		Aluminum foil	As needed
		Light source	As needed
		Condiments (such as ketchup and mustard)	As needed
		Solution with low pH (HCl)	As needed
		Solution with high pH (NaOH)	As needed
		Ammonia	As needed
		Soil or sand	As needed

* These items are included with the specific kit, apparatus, or sensor used in the experiment.

[4] Numbers is a trademark of Apple Inc., registered in the U.S. and other countries.

[5] © 2012 Google Inc. All rights reserved. Google Docs is a trademark of Google Inc.

ACTIVITY BY PASCO SENSORS

This table indicates which lab activity uses the sensors or special equipment listed.

Items Available from PASCO	Qty	Activity Where Used
PASCO EcoChamber Container	1	8, 9, 17
PASCO EcoChamber Container	2+	11
PASCO Barometer/Low Pressure Sensor	1	11
PASCO Carbon Dioxide Gas Sensor	1	7, 9, **11**, 17
PASCO Colorimeter	1	3, 10
PASCO Conductivity Sensor	1	**2**, 4, **12**,
PASCO Ethanol Sensor	1	8
PASCO Oxygen Gas Sensor	1	1, 7, 8, 9, **17**
PASCO pH Sensor	1	2, **8**, **12**, **18**
PASCO Physiology Sensors such as a hand-grip heart rate sensor, EKG sensor, spirometer sensor, and blood pressure sensor and cuff	1	**6**
PASCO Pressure Sensor	1	1
PASCO Quad Temperature Sensor	1	5, 6
PASCO Temperature Sensor	1	**17**[1]
PASCO Weather Sensor	1+	11
PASCO Fast-Response Temperature Sensor	1	**7**
Mitochondrial Genetics Kit (BP-6946)		15
Genetically Inherited Disease Detection Kit (BP-6947)		16

[1]In this activity either the fast-response temperature sensor or the stainless steel temperature sensor can be used. A stainless steel temperature sensor fits in the hole in the stoppers that are used with the EcoChamber container. The fast-response temperature probe could be used under the filter paper close to the contents.

NOTE: **Bolded** lab numbers indicate the sensor may be used for student-designed experiments.

.

1. ENZYME ACTIVITY

Lab Overview

Students investigate the catalyzed decomposition of hydrogen peroxide by catalase or peroxidase according to the equation:

$$\text{enzyme(l)} + 2H_2O_2(l) \xrightarrow[\text{enzyme complex}]{} 2H_2O(l) + O_2(g) + \text{enzyme(l)}$$

Students can use either the oxygen gas sensor or pressure sensor to measure the oxygen gas produced as the reaction proceeds. Students design an experiment to investigate questions they devise about factors that can affect catalyzed reaction rates. Investigations that manipulate pH, temperature, enzyme concentration, or substrate concentration work well.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	15 min
Initial Investigation	20 min

Student-Designed Experiment	
Experiment Design	20 min
Experiment	45 min
Data Analysis	20 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	1.B.1, 2.D.1, 4.A.1, 4.B.1
Science Practices	4.2, 5.1, 6.1, 6.4, 7.2
Learning Objectives	1.9, 1.16, 2.24, 4.3, 4.17

Materials and Equipment

For Each Student Station

- Data collection system
- Oxygen gas sensor or pressure sensor
- Sampling bottle, 250-mL
- Graduated cylinder, 25-mL
- Pipet, 1-mL
- Magnetic stirrer and stirring bar
- Base and support rod
- 3-Finger clamp
- 1.5% Hydrogen peroxide (H_2O_2), 20.0 mL[1]
- Catalase suspension, 2.0 mL[1]

[1]To formulate the catalase suspension using dried bakers' yeast, refer to the instructions in the Lab Preparation section

Additional equipment recommended for the student-designed experiment:

- Hot plate or water bath
- Buffers, pH 3 to pH 10
- 3.0% Hydrogen peroxide
- Catalase or peroxidase suspension from other sources (liver, plants, etc.)
- Ice

Prerequisites

Students should be familiar with the following concepts:

- Enzymes are proteins. Like any protein, an enzyme's function depends upon its specific shape, as determined by the tertiary level of protein structure.
- Enzymes are organic catalysts. A chemical reaction proceeds at a much faster rate when an enzyme is present.
- The *induced fit model* describes enzyme function. Formation of an enzyme–substrate complex is a critical aspect of lowering the activation energy of the reaction (and thereby increasing the rate of the reaction).

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- If using a hot plate, use caution to prevent burns.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Preparing the enzyme suspension

    a. Initial Investigation: Provide each student group with 2.0 mL of catalase for the Initial Investigation. All sample data was collected using catalase from yeast, but catalase from other organisms can work equally well.

    CATALASE FROM YEAST

    Mix 7 g (1 pkg) of dried bakers' yeast with 500 mL of distilled water. For best results, use warm water (36–40 °C) to activate the yeast.

    NOTE: Keep the enzyme suspension stirring continuously to ensure uniformity.

    b. Student-designed experiment: For the student-designed experiment, either catalase or peroxidase may be used. Concentrations of enzyme will vary from species to species or even within a species, depending on the environment. To ensure consistency in the enzyme concentration, prepare a large enough sample for students to run all anticipated experiments from a single batch. Conducting accurate comparisons of enzymes across species using the procedure provided is extremely difficult.

    CATALASE FROM BEEF LIVER OR BEEF BLOOD

    Homogenize approximately 1.0 g of beef liver or blood in 100 mL of cool distilled water. Frozen liver or blood usually has few or no active enzymes. Obtain the freshest materials possible. Alternatively, laboratory grade catalase can be purchased from a science supply company.

    PEROXIDASE FROM TURNIP OR RUTABAGA

    Using a vegetable peeler, remove the outer skin of the turnip or rutabaga and liquefy approximately 20 g in 500 mL of distilled water using a blender. To purify, filter the mixture through a coffee filter or coarse grade filter paper.

2. Preparing 1.5% hydrogen peroxide

   Three percent hydrogen peroxide is available from most pharmacies and supermarkets. To create a 1.5% solution for the initial investigation, mix the 3% H_2O_2 with an equal part of distilled water. For the Initial Investigation, prepare 20.0 mL per student group. For the student-designed experiment, the amount needed depends on each group's experiment.

3. pH Buffers

   You can make your own buffers but for simplicity, pHydrion™ buffers are recommended. Solutions of pH 3 through pH 10 should produce a sufficient range of results. Buffers more acidic or basic will quickly denature the enzyme. For the student-designed experiment, the amount needed depends on each group's experiment.

Teacher Tips

Tip 1 – Mix the enzyme suspension continuously

Yeast will settle to the bottom of the suspension, causing the concentration of enzyme to vary between samples. For best results, place the enzyme suspension on a stir plate to provide more consistent results. Instruct students to take their samples from the center of the suspension.

Tip 2 – Loosely plug the sample bottle with the oxygen sensor

The reaction will produce a substantial amount of oxygen gas, increasing the pressure within the sampling bottle. If the sensor is pressed firmly into the bottle, it may pop off unexpectedly. In addition to being a safety concern, if the sensor pops off during data collection, the procedure will need to be repeated to collect accurate data. Despite introducing an uncontrolled variable by opening the system, this setup will produce the most accurate results.

Tip 3 – Stir plates

The data collected during the reaction is most accurate if the solution is stirred or agitated at a constant rate. Because the bottle is top heavy with the oxygen sensor, supporting the bottle or sensors with the 3-finger clamp (as illustrated in the Initial Investigation) is recommended to keep it from tipping. If no stir plates are available, students can swirl the bottle by hand and still obtain reasonably good data.

NOTE: Make sure that the solution does not come into contact with the sensor.

Tip 4 – Understanding parts per million (ppm)

Many life and environmental science measurements express units in *parts per million,* a dimensionless measurement most easily understood as a fraction:

$$\frac{\text{parts}}{\text{million parts}}$$

This can be easily converted into a percentage, which many students prefer to use. For example, an oxygen concentration of 219,000 ppm is equivalent to 21.9% [(219,000/1,000,000) × 100]. With a little practice, students will be able to use *percent* and *parts per million* interchangeably.

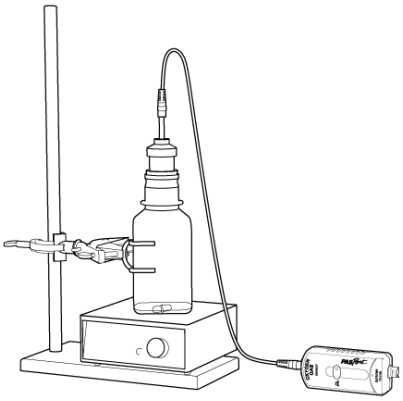
The data collection system can be set to measure in ppm or percent oxygen. Students may need to convert from one unit to the other to compare their data across groups.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

1. Put on your safety goggles.
2. Connect the oxygen gas sensor or a pressure sensor to your data collection system. Build a page to show a graph of the appropriate sensor measurement versus time in minutes.
3. Adjust the sample rate to 1 sample every 15 seconds. If possible, set an auto-stop condition for three minutes.
4. Set up the equipment as shown.
5. Calibrate the oxygen gas sensor.
6. Put the magnetic stirring bar in the sampling bottle.



Setup with O_2 gas sensor

7. Using a graduated cylinder, transfer 20.0 mL of 1.5% H_2O_2 into the clean 250-mL sample bottle. If the bottle is on a stir plate, set the stir speed to a medium setting.
8. Use a pipet to add 2.0 mL of catalase and quickly insert the sensor into the opening of the bottle. Begin data collection.

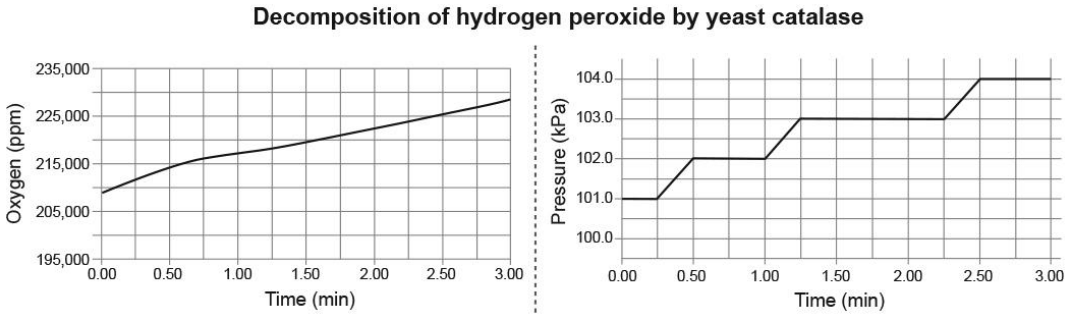
   NOTE: Loosely plug the sample bottle with the oxygen sensor. Keep the mixture stirring continuously on a medium setting or swirl the bottle gently by hand, making sure the solution does not come in contact with the sensor.

9. Why does the addition of the yeast suspension cause a change in the oxygen concentration inside the sampling bottle?

   Adding yeast to the sampling bottle increases the amount of oxygen gas because yeast contains the enzyme catalase. Catalase speeds up the breakdown of hydrogen peroxide; oxygen is a product of this reaction.

10. Copy Table 1 into your lab notebook to record the results for the sensor being used.

11. When data collection has stopped, calculate the rate of the reaction in (appropriate units)/min and record it in your copy of Table 1.



Data from an Initial Investigation using pressure and oxygen gas sensors.

The rate of reaction can be obtained from the slope of the line.

12. The spontaneous decomposition of hydrogen peroxide is very slow, less than 0.5%/day. When the decomposition was measured in a controlled experiment over several days with the pressure and oxygen sensors, the following data was obtained.

Table 1: Comparison of hydrogen peroxide decomposition rate with and without a catalyst

Sensor	Spontaneous Rate of Decomposition	Catalyzed Rate of Decomposition	Increase in the Catalyzed Rate
Pressure sensor	4.26×10^{-5} kPa/min	1.00 kPa/min	2.35×10^{4}
Oxygen sensor	0.33 ppm/min	6,524 ppm/min	1.98×10^{4}

NOTE: The sample data in the table came from yeast as the catalase source.

a. How much faster is the catalyzed reaction you observed compared to the spontaneous rate of decomposition?

Based on the sample data, the catalyzed reaction was between 17,000 and 24,000 times faster than the uncatalyzed reaction. Students' results may vary.

b. Explain why the reaction is so much faster when an enzyme is present.

The reaction is faster because the enzyme binds with the substrate, in this case hydrogen peroxide, and twists in such a way that the unstable bonds are broken. When the reaction is catalyzed by an enzyme, the activation energy of the reaction is much lower than that of the spontaneous reaction. That is, the energy needed to break the chemical bonds is lessened. With a lower activation energy, a greater number of hydrogen peroxide molecules decompose per second, resulting in a much faster reaction rate.

13. Is the rate of the reaction constant for the 180 seconds of data collection? Support your answer with evidence.

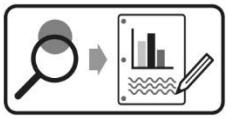
The rate is consistent for the duration of the data run. When a linear fit was performed on our data, the RMSE (0.0123) and R (0.994) values indicated a strong fit to the data.

14. If the reaction continued to run, do you predict the reaction rate to be constant? Explain your thinking.

As the reaction proceeds, the substrate concentration will decrease to the point where the rate of reaction will decrease.

Design and Conduct an Experiment

Based on the data, analysis, and discussion of the Initial Investigation, students should be able to identify factors that might change the enzyme-catalyzed rate of decomposition of hydrogen peroxide. Many factors that affect the structure and function of enzymes and the reaction rate of enzyme-catalyzed reactions can be easily manipulated in the lab.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- How does temperature affect enzyme activity?*
- How does pH affect enzyme activity*
- How does enzyme (or substrate) concentration affect the rate of reaction?*
- What is the optimal temperature or pH for the catalyzed reaction? How does this information correlate to the preferred environmental conditions of the species that you extracted enzyme from?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "How does temperature affect enzyme activity?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and data,

    a. Describe how the independent variable you manipulated affected the rate of decomposition of hydrogen peroxide. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

    In our test to determine the affect of temperature on enzyme activity (using 15 °C increments), we found that as the temperature of the yeast suspension increased, the reaction rate increased until the 45 °C trial group. In the next trial at 55 °C, the reaction rate was the lowest (0.1% O_2/min), suggesting that the enzyme could no longer catalyze the reaction. The data supports my hypothesis (see below) since the rate of oxygen generated by the decomposition of hydrogen peroxide increased from 0.3% to 1.6% O_2/min between 10 °C and 45 °C and indicates that the enzyme became denatured between 45 °C and 55 °C.

    (The hypothesis, from the Design and Conduct an Experiment Key: If temperature affects enzyme structure, then the rate of reaction will increase as the temperature increases until the enzyme is denatured.)

b. Based on the evidence you collected, explain why the results occurred.

The kinetic energy of the reaction increased with temperature, increasing the frequency of molecular collisions which increased the number of enzyme–substrate complexes formed, and consequently the reaction rate. However, between 45 °C and 55 °C, the enzyme structure was denatured as hydrogen bonds and R-group interactions changed and the shape of the protein was altered. Because of the hyper-specific nature of enzyme–substrate bonds, the catalase could no longer bind to the hydrogen peroxide and the reaction rate decreased.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

There is no evidence from the data that experimental error significantly impacted the results of this experiment.

3. Identify any new questions that have arisen as a result of your research.

Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. If you were to double the amount of catalase in the initial investigation, how would the reaction rate change? Explain your reasoning.

The reaction would proceed much faster at first but slow more quickly as substrate is consumed. In addition to substrate becoming less available as the reaction progresses, the increased concentration of products will reduce the chances of remaining substrate binding to the active site of the enzyme.

2. Many organisms, such as fungi, animals, and plants, have catalase.

a) What does this indicate about the enzyme?

Catalase must have been present in an early ancestor of living things and the gene that codes for catalase has been conserved through evolution. Hydrogen peroxide is a product of metabolism common to many organisms and catalase serves the vital function of rendering the toxic substance harmless.

b) Catalase is just one of thousands of different enzymes found in yeast cells and other organisms. Why do organisms need so many different types of enzymes?

Enzymes do not catalyze just any chemical reaction; each enzyme is specific to a particular reaction. For example, catalase only decomposes hydrogen peroxide; other enzymes are needed to break down other substances, such as the enzyme sucrase that breaks down sucrose. Additional enzymes are needed to build larger molecules from monomers, such as DNA polymerase that builds new nucleotide chains during DNA replication. Since thousands of different reactions occur within cells, organisms need thousands of different enzymes to help these reactions occur at the rates needed to sustain life.

3. The graphs below show the relative activity of α-amylase from two different species. Amylase is an enzyme that breaks down complex carbohydrates, like starch, into simple sugars that are used in cell respiration. Figure 1 shows data obtained using α-amylase samples from the bacterium *Bacillus subtilis*, found in the gut of termites across the southern United States.[6]

   Figure 2 shows data for an α-amylase sample taken from the copepod *Heliodiaptomus viduus*. This organism is found mainly in the Indian Ocean around hot vents. In each case, the enzyme was incubated at a given temperature and then tested for activity at regular intervals.[7]

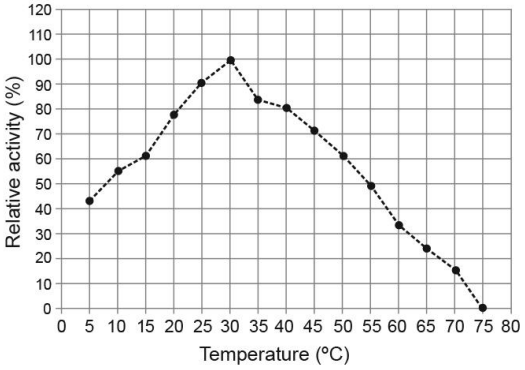


Figure 1. Amlyase activity in *Bacillus subtilis*

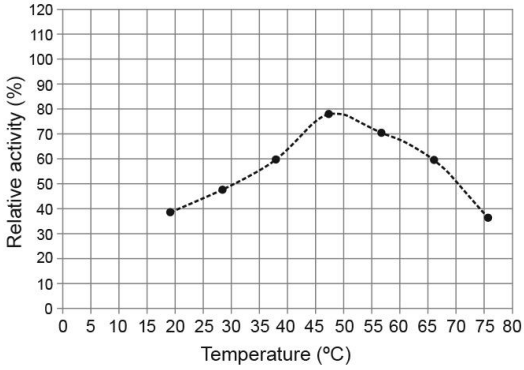


Figure 2. Amylase Activity in *Heliodiaptomus viduus*

   a. Discuss how and why temperature affects enzyme activity.

      Enzymes, like all proteins, have four levels of structure. The secondary and tertiary structure is the result of r-group interactions and hydrogen bonding. As kinetic energy increases with higher temperatures, these bonds can be disrupted, altering the hyper-specific shape of the enzyme and causing it to function poorly or not at all, at which point the enzyme is denatured.

   b. Explain why the optimal temperature for α-amylase is different for these species.

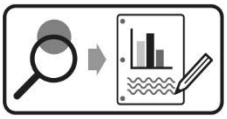
      The ability to break down starch at ambient environmental conditions presents a clear evolutionary advantage since it enables cellular respiration at higher rates in the existing environment. Because *H. viduss* lives near hot vents in the ocean, it has a form of amylase that does not denature until temperatures above 50 °C. If its amylase denatured at temperatures above 30 °C, like the amylase in *B. subtilis*, *H. viduus* would not be able to survive near hot vents.

[6] Femi-Ola, T. O.; Olowe, B. M. Characterization of Alpha Amylase from *Bacillus subtilis* BS5 Isolated from *Ameritermes evuncifer* Silvestri. *Research Journal of Microbiology* 6 (2011): 140–146.

[7] Dutta, T.K.; Jana, M; Pahari, P. R; Bhattacharya, T. The Effect of Temperature, PH, and Salt on Amylase in *Heliodiaptomus viduus* (Gurney) (Crustacea: Copepoda: Calanoida). *Turkish Journal of Zoology* 30 (2006): 187–195.

Design and Conduct an Experiment Key

Many factors that affect the structure and function of enzymes and the reaction rate of enzyme-catalyzed reactions can be easily manipulated in the lab. Identify one of these factors and design an experiment to determine how that factor affects the rate of an enzyme-catalyzes reaction.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of enzymes and reactions, what environmental factors (abiotic or biotic) could affect the rates of enzyme-catalyzed reactions?

   Protein structure and subsequently enzyme function are affected by changes in pH and temperature. Additionally, the reaction can be affected by the concentrations of the enzyme or substrate.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   How do changes in temperature affect the catalyzed decomposition of hydrogen peroxide?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   Catalase is a common enzyme in eukaryotic cells and functions at a range of temperatures. This experiment will examine how the enzyme function changes at different temperatures and determine if there is an optimal temperature.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable is the temperature of the solution. The temperature will be altered using a water bath to heat the yeast suspension to the desired temperature. The suspension will be added to room temperature hydrogen peroxide.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable is the rate of decomposition of hydrogen peroxide, which will be measured using the oxygen gas sensor. As shown in the Initial Investigation, the rate of decomposition of hydrogen peroxide is a function of enzyme activity, so measuring this rate while varying the temperature will provide the data to evaluate the hypothesis.

   The rate of decomposition of hydrogen peroxide at five different temperatures (at 15 °C increments) will be measured for two minutes and the rate at each temperature will be plotted as a point on a rate versus temperature graph. (The results of multiple trials at each temperature will be averaged.)

6. Write a testable hypothesis (If…then…).

   If temperature affects enzyme structure, then the rate of reaction will increase as the temperature increases until the enzyme is denatured.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   Ten milliliters of 1.5% hydrogen peroxide at the same temperature as the catalase will be used. The volume of yeast suspension will be 1 mL. The temperature will be manipulated by placing a test tube of yeast suspension with a temperature sensor into a water bath, heating it until it has reached the desired temperature. A test tube of hydrogen peroxide will be heated to the same temperature.

8. How many trials will be run for each experimental group? Justify your choice.

   Three trials will be run at each temperature. This will provide sufficient data for analysis and be achievable in the allotted time.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The reaction rate of the three trials at each temperature will be averaged, and the average reaction rate graphed versus temperature.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

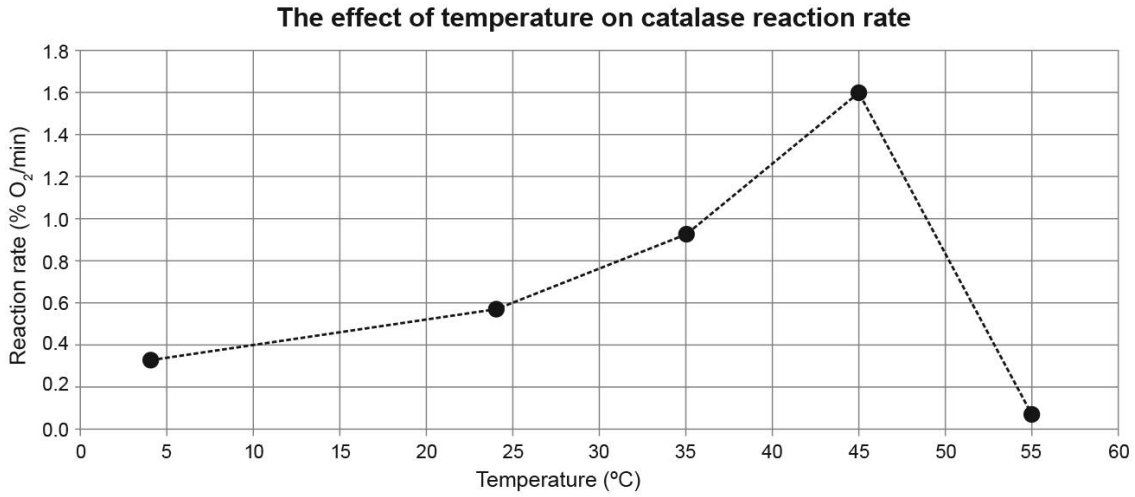
    The enzyme concentration can vary within a yeast suspension. To minimize this effect we will mix the yeast suspension constantly and collect data from multiple runs. The hydrogen peroxide can vary slightly with age, so we will conduct the entire experiment with the same batch and run multiple trials. Finally, the accuracy of the results depends on the sensor accuracy, which is +/-1%.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

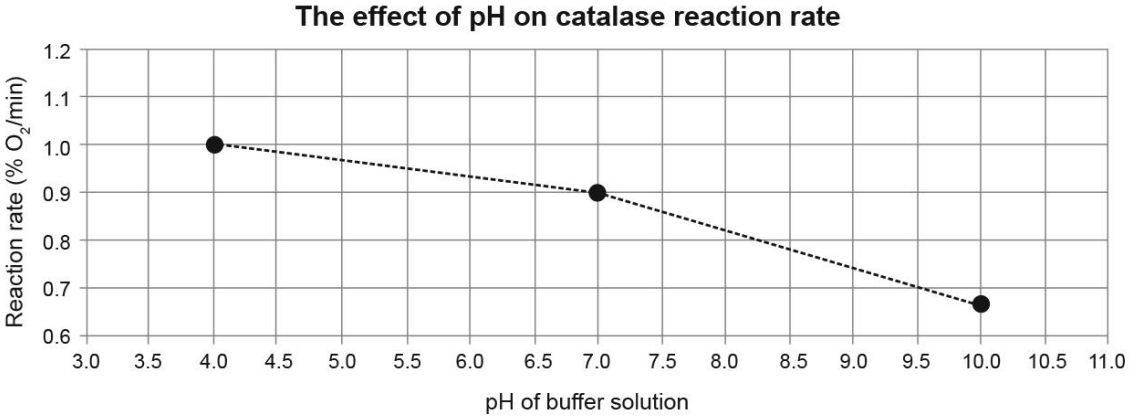
    1. Mix a batch of yeast suspension.
    2. Set up separate water baths at 4 °C, 22 °C, 35 °C, 45 °C and 55 °C. Monitor the water baths with temperature probes.
    3. Place 10 mL of yeast into a test tube and place the test tube in the water bath until it has reached the target temperature.
    4. Calibrate an oxygen gas sensor in an empty sample bottle and set up an experiment file to sample every 5 seconds for 2 minutes.
    5. Add 20 mL of 3% hydrogen peroxide to the 250-mL sample bottle.
    6. Pipet 3 mL of yeast suspension from the 25 degree test tube into the sample bottle, insert the sensor, and begin data collection. Make sure to swirl the sample bottle or use a magnetic stirrer.
    7. Rinse out the bottle. Repeat for each experimental group, with 3 trials at each temperature.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

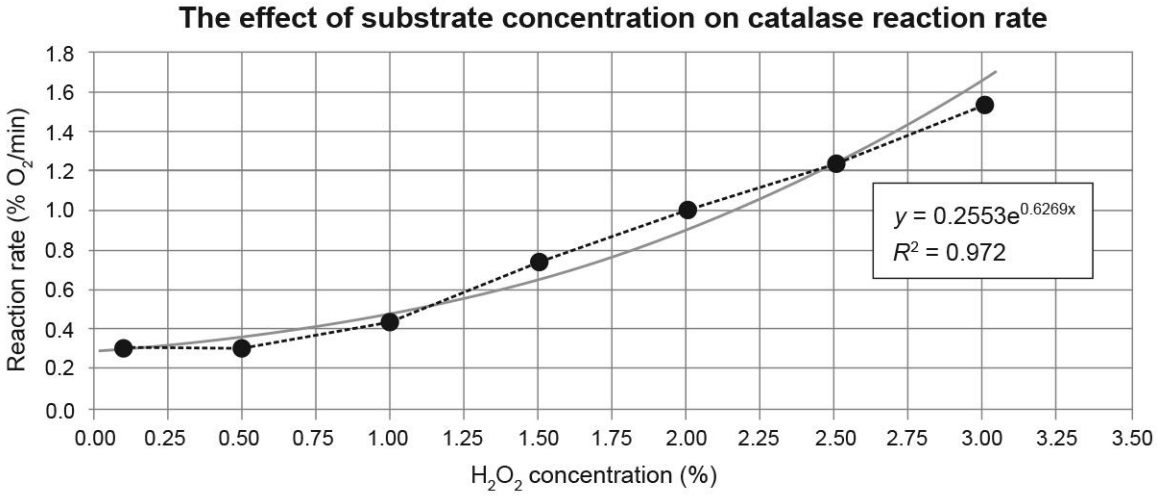


The above results are from an inquiry where the temperature was varied. For each trial, yeast and hydrogen peroxide were heated to a specific temperature: 4 °C, 22 °C, 35 °C, 45 °C, and 55 °C, before being added to the sample bottle. The 250-mL sample bottle contained 10.0 mL of 1.5% hydrogen peroxide and 1.0 mL of yeast catalase suspension.

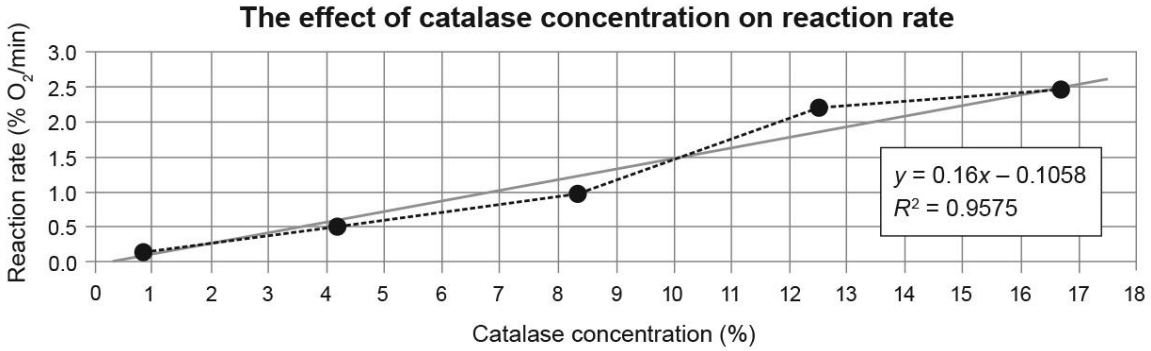


The above results are from an inquiry where the pH was varied. The hydrogen peroxide was diluted from 3% to 1.5% using buffers of differing pH: pH 4, pH 7, and pH 10. The final pH of the solution changed slightly when the 1.5% hydrogen peroxide and enzyme suspension were mixed. The 250-mL sample bottle contained 10.0 mL of 1.5% hydrogen peroxide and 1.0 mL of yeast catalase suspension.

NOTE: Students should use a pH probe to obtain the actual pH of their solution.



The above results are from an inquiry where the hydrogen peroxide concentration was varied. Each trial used a different concentration of hydrogen peroxide: 0.1%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%. The 250-mL sample bottle contained 10.0 mL of hydrogen peroxide and 1.0 mL of yeast catalase suspension.



The above results are from an inquiry where the yeast catalase concentration was varied. The 250-mL sample bottle contained 10.0 mL of 1.5% hydrogen peroxide and 2.0 mL of yeast catalase suspension with concentrations shown in the table below.

Catalase concentration preparation

Catalase (mL)	Water (mL)	1.5% H_2O_2 (mL)	*Total Volume (mL)*	Catalase Concentration (%)
0.1	1.9	10	12	0.83
0.5	1.5	10	12	4.17
1.0	1.0	10	12	8.33
1.5	0.5	10	12	12.50
2.0	0.0	10	12	16.67

2. DIFFUSION

Lab Overview

Students investigate the diffusion of ions through a semipermeable membrane using apple cider vinegar and a pH sensor. The vinegar is added to a dialysis bag to represent the intracellular solution of a model cell. Distilled water in a beaker represents extracellular fluid and students monitor the pH of the water over time to measure the rate of diffusion out of the model cell.

The color of the apple cider vinegar provides evidence of the semipermeable nature of the membrane; hydrogen ions easily diffuse through the membrane while pigments molecules do not. Student-designed experiments that test factors affecting the rate of diffusion can be accomplished with minimal materials and within the time frame of a single class period.

NOTE: Since hydrogen ions form bonds with water molecules, students are actually determining the rate of diffusion of hydronium ions: $H_3O^+(aq)$.

Diffusion within biological systems is affected by several factors. The most notable is the concentration gradient across a membrane. A large concentration gradient across the membrane speeds up diffusion. Other factors affecting diffusion rates within biological systems include temperature, distance between solute and membrane, surface area-to-volume ratios, and solute size. All these factors can be manipulated in student-centered investigations.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	30 min
Initial Investigation	30 min

Student-Designed Experiment	
Experiment Design	30 min
Experiment	30 min
Data Analysis	20 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.B.1, 2.B.2
Science Practices	1.4, 2.2, 3.1, 4.2, 4.3, 5.1, 6.1, 6.2
Learning Objectives	2.10, 2.11

Materials and Equipment

For Each Student Station

- Data collection system
- pH Sensor
- Graduated cylinder, 25-mL
- Beaker or cup, 250-mL–400-mL
- Dialysis tubing, 1 inch × 28-cm[1]
- Disposable pipet or 10-mL syringe
- Paper clip or binder clip
- Small cup to capture the 25 mL (or less) of fluid from the dialysis bag
- Apple cider vinegar, 25 mL[1]
- Pickle juice, 25 mL[1]
- Magnetic stir bar and plate (if available)
- Spring water (or distilled water), 200 mL
- Plastic wash bottle with distilled water

[1]Refer to the Lab Preparation section for information about obtaining or preparing these items.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Additional equipment recommended for the student-designed experiment:

- Conductivity sensor
- Other commercially available solutions, such as: olive juice, jalepeño juice, beet juice, as needed
- Hot plates or warm water baths
- Other "extracellular" solutions to replace water
- Additional dialysis tubing , including tubing of a different diameter or with different pore sizes
- Ice
- Food coloring, different colors

Prerequisites

Students should be familiar with the following concepts:

- Diffusion is the movement of substances from an area of higher concentration to an area of lower concentration and moves a system toward a state of equilibrium
- Biological membranes are comprised mainly of phospholipids and proteins. They are described as *semipermeable* in that they allow some substances to diffuse through them, while others are unable to diffuse through.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times
- Be sure to wear gloves or wash your hands after handling solutions. The solutions can irritate your skin and cause extreme eye irritation if you wipe your eyes with your hands after contact with them.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Dialysis tubing

   Cut enough dialysis tubing so that each group has two 28-cm lengths of tubing. Soak the sections of tubing in a large beaker with spring water or distilled water at least 20 minutes prior to students beginning the investigation.

2. pH sensors

   The pH sensors may need to be calibrated (refer to Teacher Tip 4).

3. Ensure that the pipets or syringes students will use to transfer solutions to the dialysis bag are rinsed and clean.

4. Apple cider vinegar and pickle juice

   Both of these solutions can be purchased from any grocery store. Remove the pickles from a large jar of pickles so students can easily acquire the pickle juice needed for the investigation.

Teacher Tips

Tip 1 – Have students rinse the outside of each dialysis bag prior to placing it in water

When students fill the dialysis bag with vinegar (or a similar solution), some of the liquid is likely to adhere to the outside surface of the bag. If the dialysis bag is not rinsed before placing it into the water, solution on the outside surface will diffuse into the water and produce inaccurate results.

Tip 2 – Close the dialysis bag securely at both ends

A leaky dialysis bag will produce inaccurate results. To avoid this problem do the following:

- Tie a knot in the bottom of the wet dialysis tubing prior to filling it. Alternatively, tie string or dental floss around the bottom of the tubing to ensure that the bag is securely closed.
- The top of the tubing can be twisted and closed with a paper clip or binder clip, and allowed to "flop over" the top of the beaker or cup. This method can make it easier to pour liquid out of the bag at the end of the investigation.

Tip 3 – Use a magnetic stir plate

Creating a situation where molecules and ions can be evenly dispersed within the beaker should lead to more consistent measurements during the experiment. Place a magnetic stir bar in the beaker of distilled water and set the stir plate to a medium speed. If a stir bar is used in the initial experiment, make sure a stir bar is used in student-designed experiments so students keep as many variables constant as possible.

Tip 4 – Calibrate the pH sensors

In this lab, the most important data is the change in pH and the rate of change of pH, as opposed to absolute pH values. However, if you find that a pH sensor measures significantly different values than expected, calibrate the sensor. For calibration instructions, refer to the sensor manual or visit the sensor webpage at www.pasco.com or the PASCO scientific channel on YouTube (www.youtube.com/user/pascoscientific) for a tutorial video.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

1. Put on your safety goggles.

2. Connect a pH sensor to the data collection system and build a page to display pH vs Time.

3. Pour 200 mL of water into a cup or beaker and set the beaker aside.

4. Add 25 mL of apple cider vinegar to a graduated cylinder. Rinse the tip of the pH sensor with water and place the sensor in the vinegar. Begin recording data to measure the pH of the vinegar. After 10–20 seconds, or when the pH stabilizes, end data collection and remove the sensor from the vinegar.

5. Obtain a piece of dialysis tubing that has soaked in water. Tie a tight knot in one end of the tubing to create a bag. Rub the other end of the tubing between your fingers to open the bag.

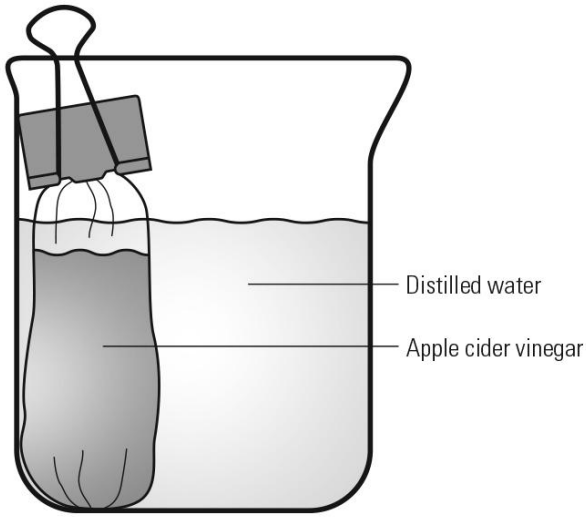
6. Use a clean pipet or syringe to add approximately 15–20 mL of vinegar from the graduated cylinder to the dialysis bag. Close the bag by tying a knot or by twisting the tubing and closing it with a binder clip. Rinse the outside of the bag with distilled water.

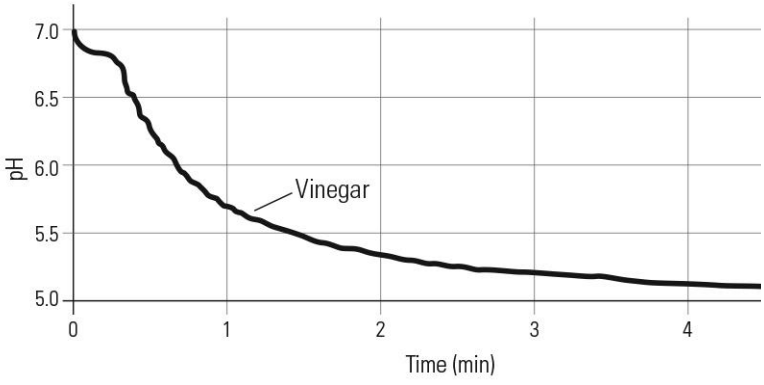
7. Rinse the pH sensor with water and place it into the beaker of distilled water. If a stir plate is available, add a magnetic stir bar to the beaker and set the stir plate to a medium spin speed.

   NOTE: If a stir plate is not used, gently swirl the beaker during data collection.

   NOTE: If using a cup instead of a beaker, be sure the pH sensor does not cause the cup to tip over. You may need to hold the sensor during data collection or use a base and support rod with a clamp to secure the sensor.

8. Start recording data and then slowly add the dialysis bag to the water. (If using a clip to close the bag, be sure the clipped end remains above the surface of the liquid when put into the water.) Continue recording data for 5 minutes or until the pH value stabilizes. Draw or print a record of the data.





9. At the end of the experiment, empty the contents of the dialysis tubing into a small cup and record the pH of the vinegar. Compare the pH after soaking the dialysis tubing in water to the initial pH of the vinegar. Explain the results.

   For sample data, the pH of the vinegar was 3.4 at the beginning of the experiment and 3.7 at the end of the experiment, indicating the vinegar solution became slightly less acidic. This is due to the movement of hydrogen ions out of the bag.

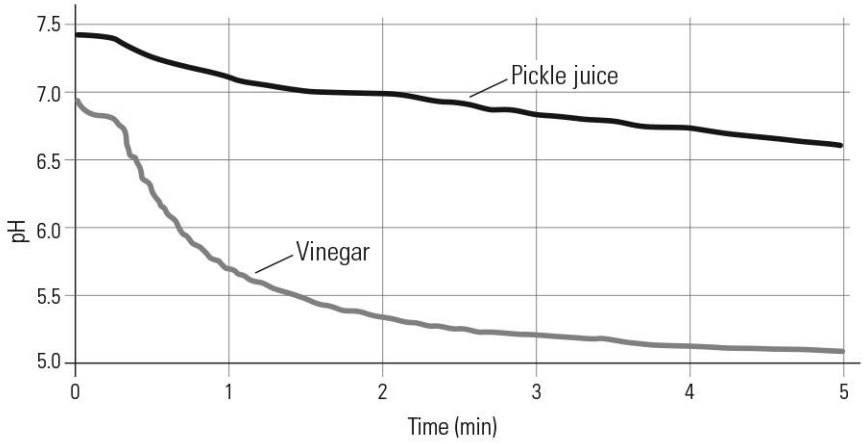
10. Is dialysis tubing a *semipermeable* membrane? Support your claim with evidence from the investigation.

    Dialysis tubing is semipermeable. Hydrogen ions diffused through the membrane, causing the pH of the water to decrease; however, the molecules responsible for the color of cider vinegar did not diffuse through the membrane. Those molecules remained in the bag and the water outside the bag remained uncolored.

11. If the experiment is repeated with 20 mL of pickle juice in a dialysis bag, how do you expect the results to compare to the first experiment with apple cider vinegar? Explain the basis for your prediction.

    Answers will vary. Students may know that pickle juice contains vinegar (or they may look at the ingredients label) and predict that the results will be similar to what occurred with apple cider vinegar. Advanced students may elect to test the pH of pickle juice and compare this value to the pH of the apple cider vinegar to make a prediction about the relative rate of diffusion.

12. Repeat the procedures, replacing apple cider vinegar with pickle juice in the dialysis bag. Draw or print a record of the data.



13. Explain any similarities or differences in the results for the two solutions. Consider general trends in the data as well as the relative rates of diffusion.

Both solutions cause the pH of the water to decrease. Apple cider vinegar and pickle juice are both acidic. When the bag is submerged, hydrogen ions diffuse from these solutions into the water. Pickle juice is less acidic than apple cider vinegar, so the rate of diffusion is slower. Both solutions are colored and in both cases the color remained in the bag.

Intracellular Solution	Initial pH of Intracellular Solution	Change in pH of Extracellular Environment after 60 seconds	Change in pH of Extracellular Environment after 5 minutes
Apple cider vinegar	3.40	–1.6	–1.9
Pickle juice	4.06	–0.3	–0.8

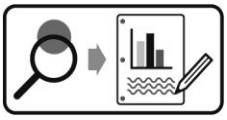
With the apple cider vinegar, the rate of diffusion was much greater during the first minute of data collection compared to the last minute of data collection. The rate of diffusion for the first minute of the experiment was –1.6 pH units/minute and the rate of change for the last minute was –0.1 pH units/minute. Because the hydrogen ion concentration was reaching equilibrium between the dialysis bag and the beaker, the rate slows over time.

At the beginning, the steep concentration gradient between the vinegar in the bag and water outside the bag causes the rate of diffusion to be much faster. With pickle juice, the rate of diffusion was almost constant over the course of data collection (the line is linear). The pickle juice is less acidic than the vinegar and the concentration gradient between the bag and water in the beaker was not as steep. There may be other factors, in addition to differences in acidity, which affect the rate of diffusion for pickle juice, such as salt content.

Design and Conduct an Experiment

The dialysis tubing fluid and the cup with distilled water were used to simulate intracellular and extracellular environments, respectively. The apple cider vinegar represents a solution containing some of the same materials as cytoplasm and interstitial fluid, such as hydrogen and sodium ions.

For this extension, students should identify components of this model system or environmental conditions that can be changed to test factors that affect diffusion.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If students are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- How does changing the concentration of a solution in the dialysis bag affect the rate of diffusion?*
- Does the temperature affect the rate of diffusion?*
- Do other commercial solutions (pickle juice, olive juice, coffee, or energy drinks) exhibit diffusion rates similar to vinegar?*
- How is the diffusion rate affected if a solution other than water is used as the extracellular fluid?*
- Do features of the dialysis tubing, such as diameter or pore size, affect diffusion rate?
- The food coloring of the cider vinegar did not move through the membrane. Will other food coloring particles move through?
- Does water move into the bag as ions move out? (In other words, is osmosis occurring simultaneously with diffusion?)*

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "How does changing the concentration of a solution in the bag affect the rate of diffusion?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected the rate of diffusion out of the dialysis bag and into the beaker. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

      The data suggest decreasing the vinegar concentration slows the rate of diffusion into the beaker, thus the hypothesis was supported. When the bag with full strength vinegar was placed into the beaker of distilled water, the pH of the solution in the beaker decreased from 5.6 to 4.6 within 60 seconds. When the bag with 1/4 strength vinegar was placed into the beaker of distilled water, the same change in pH in the beaker, from 5.6 to 4.6, took over twice as long: 146 seconds.

   b. Based on the evidence you collected, explain why the results occurred.

      The concentration gradient between the beaker of distilled water and the "full strength" vinegar was larger than the concentration gradient between the beaker of distilled water and the "1/4 strength" vinegar. Molecules (or in this case, hydrogen ions) diffuse faster across a membrane when the concentration gradient is steep.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   There is some evidence that one variable may have affected the results. Each time the dialysis bag was placed in the beaker of water, the pH measurement would spike slightly. Perhaps this was because the pH sensor was bumped or jostled. I still think the data is reliable, though, since the spike was seen repeatedly in trials with full strength, half-strength, and quarter-strength vinegar.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. The structure and properties of a biological membrane allows the membrane to carry out important functions for cells.

   a. What does it mean to say that the plasma membrane is semipermeable? Describe the structure of the plasma membrane and explain how it provides a selective barrier for cells.

      Semipermeable means that some molecules can diffuse across cell membranes while others cannot. The cell membrane is mainly a phospholipid bilayer—two layers of phospholipids with their "tails" facing inward and the "heads" facing the intracellular and extracellular environments. The hydrophobic nature of the phospholipids keeps many molecules from moving through the membrane. However, proteins embedded in the membrane provide hydrophilic channels for certain molecules to move through. Different cells can be permeable to different substances, depending on the types and amounts of transport proteins present in their membranes. In addition to proteins, other membrane components include cholesterol (animal cells), glycoproteins, and glycolipids.

b. Provide specific examples of molecules or other particles that enter or exit cells and for each example describe the mechanism of transportation.

Glucose: glucose molecules move through membranes by facilitated diffusion. Since glucose molecules are polar, they require a transport protein to facilitate their crossing of the membrane.

Oxygen: oxygen molecules move through the membrane by simple diffusion. Since they are small and nonpolar, they are able to move through the lipid bilayer.

Sodium ions: sodium ions can diffuse into cells through gated ion channels (a type of transport protein). They can also be pumped out of cells by the sodium–potassium pump. This mechanism requires ATP and is an example of active transport.

Water: water molecules move through transport proteins called aquaporins. The movement of water through the membrane is known as *osmosis*.

Neurotransmitters: these large molecules are released from cells through exocytosis, a form of transport that involves large molecules. The neurotransmitters are packaged into vesicles inside the cell and these vesicles fuse with the plasma membrane to release the molecules from the cell.

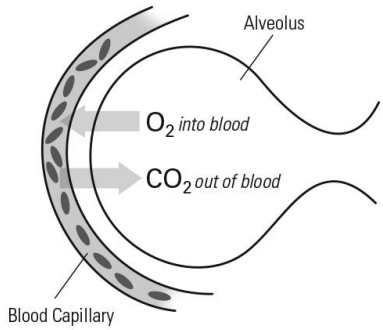
c. Eukaryotic cells have a number of membrane-bound organelles. Explain the function of these membranes within cells and describe the structures and functions of two organelles that consist of one or more membranes.

Membranes allow cells to compartmentalize intracellular activities, increasing the efficiency of these activities. Membranes also increase the surface area-to-volume ratio of organelles, which increases the organelle's ability to carry out necessary functions.

For example, placing the enzymes and other molecules needed to produce ATP through oxidative phosphorylation near one another within the membrane of a mitochondrion increases the rate at which ATP can be made. If the cell depended on random collisions between these enzymes and their substrates, the reactions would occur too slowly. In addition, the close proximity between molecules is required for electrons to move between electron carriers. The mitochondrial membrane is essential to aerobic respiration and its many folds (cristae) provide a huge SA:V ratio for respiration. Also, the double-membrane structure of the mitochondrion allows for a hydrogen ion gradient to build up; without that second membrane, hydrogen ions would diffuse to other areas of the cell, making respiration very inefficient.

Membranes can also sequester enzymes or other molecules that might cause harm if they were in the cytosol. Plant cells typically have large vacuoles: membrane-bound sacs that sequester wastes and other compounds. Animal cells have lysosomes that contain digestive (hydrolytic) enzymes and play a role in recycling a cell's organic materials.

2. There are many examples of diffusion in living things. One example is the gas exchange that occurs in the alveoli of the lungs.



a. Describe the concentration gradients that exist between the alveoli and the blood within capillaries surrounding the alveoli and explain how these gradients facilitate gas exchange. Use evidence to support your explanation.

Gasses, like all molecules, diffuse along their specific concentration gradients. The concentration of oxygen within the alveoli is four times higher than the oxygen concentration within the pulmonary capillaries—160 millimeters of mercury (mmHg) vs 40 mmHg. The concentration of carbon dioxide within pulmonary capillaries is slightly higher than the alveoli (45 mmHg vs 40 mmHg). These concentration gradients allow for the diffusion of oxygen from the lungs to the blood, and the diffusion of carbon dioxide out of the blood and into the lungs.

NOTE: It is difficult for students to measure the concentrations of oxygen and carbon dioxide in the blood. However, an activity using oxygen and carbon dioxide gas sensors can be quickly performed to determine differences in the concentrations of these gases between exhaled air from the lungs and atmospheric air. (See this activity demonstrated in the video "Carbon Dioxide and Oxygen Gas Sensors: Five Activity Ideas" on the PASCO scientific YouTube channel: https://www.youtube.com/watch?v=LBFKKQZo4kg)

b. Identify and describe two additional examples of diffusion in living things.

Similar to the diffusion that occurs between the alveoli and the blood, gas exchange occurs between the blood in the body's capillaries and the cells of the body's tissues. Cells continually use oxygen and produce carbon dioxide, and the blood is continually pumped through the lungs to replenish its oxygen content and eliminate carbon dioxide. This sets up a permanent concentration gradient, causing oxygen to move out of the blood into cells and carbon dioxide to move out of the cells and into the blood.

Plants acquire carbon dioxide for photosynthesis via diffusion. In the leaves of a plant are small openings, stomata, through which carbon dioxide molecules move. The concentration of CO_2 is higher in the air than inside the leaf, so the CO_2 moves into the leaf by diffusion.

Other examples of diffusion include: oxygen diffusing into the blood through the gills of a fish, glucose and other nutrients diffusing from the small intestine into the capillaries of the microvilli, and neurotransmitters diffusing across synapses.

3. Use the data presented in the table below to predict how altitude would affect a runner's ability to complete a five kilometer (5k) race in each of the given cities. Explain your predictions.

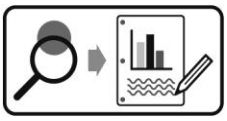
Table 1: Atmospheric oxygen concentration at various elevations

City	Elevation above Sea Level (feet)	Atmospheric O_2 Concentration (%)
Birmingham, AL	600	21
Boulder, CO	5,430	17.8
Nederland, CO	8,230	15.9
Breckenridge, CO	9,300	15.1

The runner would have the least difficulty completing the 5k race in Birmingham, AL, and the most difficulty completing the race in Breckenridge, CO. Aerobic exercise (like running) requires oxygen for working muscles. When less oxygen is available in the atmosphere, less oxygen is available to diffuse into the blood, and eventually less oxygen is available to the active tissues (in this case, skeletal muscle). The runner's heart rate and respiratory rate would likely increase in an attempt to increase oxygen levels in the blood, but eventually the runner's muscles would probably shift to anaerobic respiration. This would produce lactic acid, causing the runner to fatigue quickly.

Design and Conduct an Experiment Key

The dialysis tubing and the cup with distilled water were used to simulate intracellular and extracellular environments. The apple cider vinegar represents a solution containing some of the same materials as cytoplasm and interstitial fluid, such as hydrogen and sodium ions. How can you change a component of this model system, or change the environmental conditions, to test factors that affect diffusion?



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of membranes and diffusion, what factors could affect the rate of diffusion?

   Factors include: Concentration gradient, molecular weight of the solute, temperature, properties of the membrane, and movement of other molecules according to their gradient.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   How does changing the concentration of the solution inside a dialysis bag affect the rate of diffusion?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   Concentration gradients across membranes are essential to the function of biological systems. These systems include mitochondria, neurons, kidneys, lungs in animals, and bulk transport within plants.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable is the concentration of apple cider vinegar in the dialysis bag. The vinegar in the store-bought bottle will be considered “100%” and the vinegar will be diluted to 50% and 25% for comparison.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable will be the pH of the water in the beaker. The pH will be monitored for at least 5 minutes but the rate of diffusion will be determined from the change in pH during the first 120 seconds of the experiment.

6. Write a testable hypothesis (If…then…).

   If decreasing the concentration of the solution inside a dialysis bag decreases the rate of diffusion of substances out of the bag, then the pH will change more rapidly when undiluted vinegar is used, compared to 50% or 25% vinegar.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   The following will be held constant in all trials: 20 mL of vinegar in the bag, 100 mL of water in the beaker, water and vinegar are at room temperature, stirring with the stir bar at medium speed in the beaker, monitoring data for five minutes, using the same source and length of dialysis tubing.

8. How many trials will be run for each experimental group? Justify your choice.

   I plan to run three trials for each vinegar concentration. This allows me to see if results are repeatable and will provide data for calculating an average rate of diffusion.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The overall trend of the change in pH will be observed for each concentration during a 5-minute soak in water. The rate, though, will be determined from the first 120 seconds, since the rate tends to slow after 2 minutes. The three trials for each concentration will be averaged and the averages will be compared to determine if concentration affected diffusion rate.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    Vinegar might drip on to the outside of the dialysis bag, and rinsing the bag might not remove all of the vinegar.

    It may be difficult to add vinegar to the bag without spilling any and the volumes may not be exactly the same in all trials.

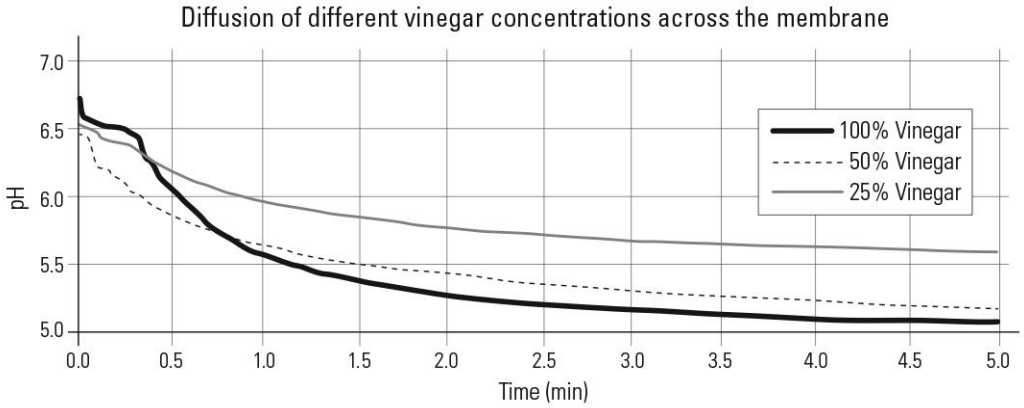
    The proximity of the pH sensor to the dialysis bag may vary slightly from trial to trial.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

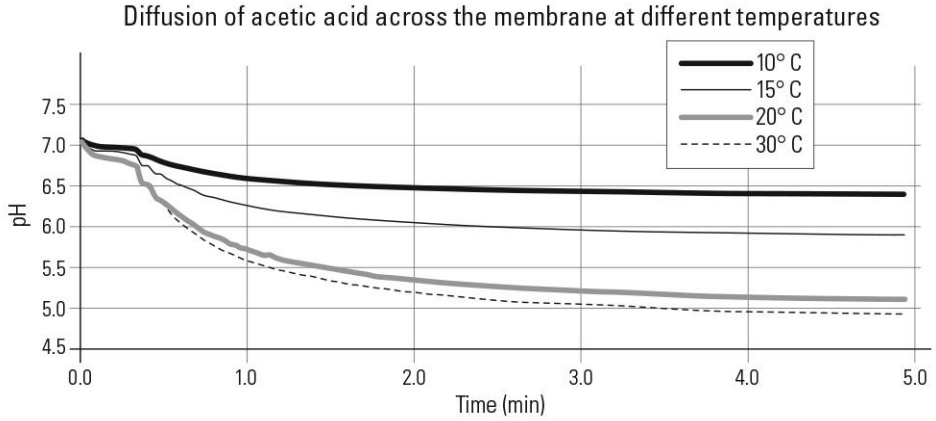
    1. Pour 200 mL of water into a cup or beaker.
    2. Add 20 mL of vinegar from a graduated cylinder to the dialysis bag. Close the bag and rinse the outside of the bag with distilled water.
    3. Rinse the pH sensor with water and place it into the beaker or cup.
    4. Start recording data and then slowly add the dialysis bag to the water. Continue recording data for five minutes or until the pH value stabilizes.
    5. Following the end of each trial, rinse the beaker and add 200 mL of fresh water, and repeat the experiment so there are 3 trials using the 100% concentration of vinegar.
    6. Carry out 3 trials for 50% vinegar (10 mL vinegar + 10 mL water) and 3 trials for 25% vinegar (5 mL vinegar + 15 mL water).

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

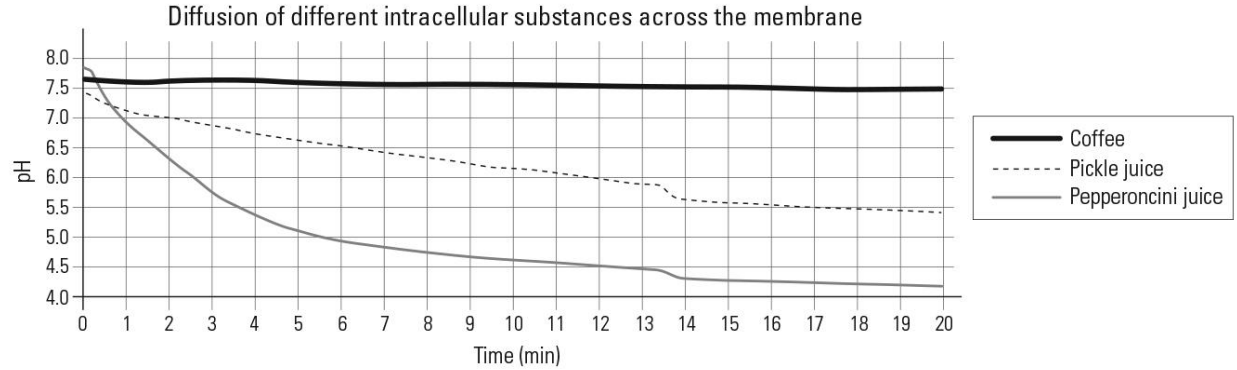
Sample Data



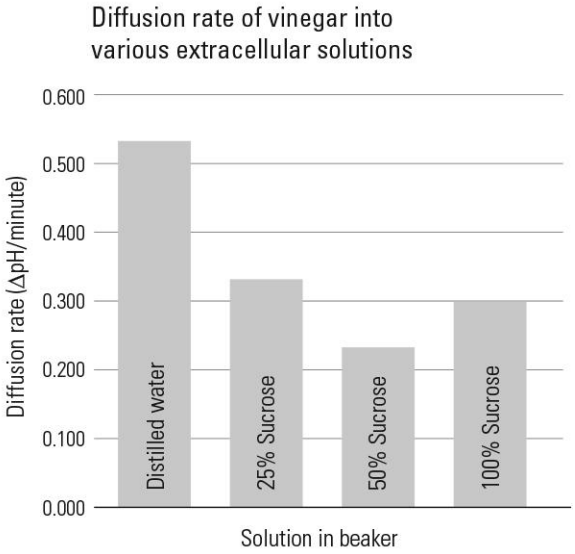
The results above are from an inquiry comparing the rate of diffusion through dialysis tubing using different vinegar concentrations. The temperature was maintained between 24–26 °C throughout the duration of the experiment. Distilled water was used for the extracellular fluid.



The results above are from an inquiry comparing diffusion from a dialysis bag containing a 100% vinegar solution into an extracellular environment maintained at different temperatures using a water bath. The temperature was monitored throughout the five minutes and adjusted as necessary.



The results shown above are from an inquiry measuring the diffusion of different substances through the model cell. The temperature was maintained between 24–26 °C throughout the duration of the experiment and there was no stirring of the water (extracellular fluid).



The results above are from an inquiry investigating the diffusion rate using different concentrations of sucrose solution as the extracellular fluid. Dialysis bags containing 100% vinegar were placed in beakers with the different sucrose solutions and the pH was recorded every second for three minutes. A linear fit was applied to the first 60 seconds of pH data to determine the rate of diffusion.

Determining if osmosis as well as diffusion occurs between pickle juice and water

Condition	Parameters Measured in the Extracellular Environment		Parameters Measured for the Solution in the Dialysis Bag	
	pH	Conductivity (µS/cm)	Mass of Bag and Fluid (g)	Absorbance of Blue Light (%)
Initial	6.6	12	22.1	1.019
Final	4.1	478	22.9	0.846

The results above are for an inquiry testing changes in both the intracellular fluid (pickle juice) and extracellular fluid (distilled water) to determine if osmosis also occurs during the diffusion experiment. Trials were run at room temperature.

3. OSMOSIS

Lab Overview

Students build models that help them understand how blood osmolarity and kidney function maintain homeostasis. Students determine which extracellular fluid is hypertonic to a model cell and which solution is hypotonic. The changes in a colored solution in the model cell (dialysis tubing) provide evidence to support a prediction about the net movement of water into or out of the cell: water moving into or out of the model cell will cause a change in the amount of light transmitted through the colored solution. Students use a colorimeter to detect this change.

Pacing and Length of the Lab

Investigation	
Teacher Preparation Time	15 min
Investigation	45 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.B.1, 2.B.2, 2.C.2, 2.D.3, 3.E.2
Science Practices	1.4, 2.2, 4.3, 5.1, 5.3, 6.1–6.2, 6.4, 7.2
Learning Objectives	2.9, 2.12, 2.16, 2.18, 3.44, 3.49

Materials and Equipment

For Each Student Station

- Data collection system
- Colorimeter
- Sensor extension cable[1]
- Cuvettes[1] (4)
- Cups or beakers (2), 250-mL
- Small funnel
- Graduated cylinders (2), 25-mL
- Dialysis tubing (2), 12-cm piece[2]
- Solution A[3], 100 mL
- Solution B[3], 20 mL
- Solution C[3], 100 mL
- Solution D[3], 20 mL
- Plastic pipets (2)
- Small binder clips (2)

[1]Included with the colorimeter.

[2]To prepare the dialysis tubing for student use, refer to the Lab Preparation section.

[3]To formulate Solutions A–D using blue food coloring, sucrose, and water, refer to the Lab Preparation section.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- The terms isotonic, hypotonic, and hypertonic describe the relative amount of solutes in a solution that surrounds cells.
- Osmosis is the net movement of water through a semipermeable membrane from a less concentrated solution (with a greater concentration of water) to a more concentrated solution (where there is a lower concentration of water).
- The amount of light transmitted through a colored solution depends upon the concentration of the solution.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Handle cuvettes carefully and alert your teacher if any break.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Prepare the dialysis tubing:

   Cut 2 pieces of dialysis tubing for each group. The length should be approximately 12 cm. Soak the cut sections of tubing in a beaker with water for at least 15 minutes.

2. Prepare solutions A–D:

   a. Solution A is tap water: Add 1 L of tap water to a beaker or flask labeled “A”.

   b. Solution B is 0.8 M sucrose: Add 68.4 g of sucrose to a 500-mL beaker. Fill the beaker to 250 mL with water and stir until the sucrose is dissolved. Add 3 drops of blue food coloring to the solution and mix. Label the beaker “B”.

   c. Solution C is 1 M sucrose: Prepare 1 L of sucrose solution, using 342 g of sucrose. Stir to dissolve all of the sucrose and label the container “C”.

   d. Solution D is 0.1 M sucrose: Add 8.6 g of sucrose to a 500-mL beaker and fill the beaker with water to the 250-mL mark. Stir until the sucrose is dissolved and then add 3 drops of blue food coloring and mix. Label the container “D”.

Teacher Tips

Tip 1 – Understanding the colorimeter

The colorimeter has four choices for color of light: red, green, blue, and orange. Each of these colors has a unique wavelength. There are two possible measurements: transmittance and absorbance. This lab makes use of the green transmittance measurement. Green light is absorbed by blue dye particles in the solution. Depending on the number of drops of food coloring added, 5–20% of green light will be transmitted through the solution. If the blue color is darker, the transmittance is less. If the blue color is lighter, the transmittance is greater.

Tip 2 – Transmittance of light through the blue-colored solutions

Blue food coloring particles are larger than the pore size of standard dialysis tubing. The particles will not move through the membrane. The movement of water into or out of the colored solution will change the amount of light transmitted through the solution. Best results are obtained if the solution is not too dark to start with. To check this, add approximately 5 mL of Solution B to a clean cuvette and place it into the colorimeter. Look at live data on the home screen to determine the transmittance of green light. Ideally, the transmittance will be between 10–20%. If it is less than 5%, the solution is too dark. Dilute the solution with additional 0.8 M sucrose solution. Check the initial transmittance of Solution D using this same method, but dilute it with additional 0.1 M sucrose solution if necessary.

Investigation

This investigation is designed to help students build mental models based on experimental evidence and does not include a student-designed experiment. Labs that address the related concepts of diffusion and plasmolysis do include student-designed experiments.

From the student handout:

1. Put on your safety goggles.

2. Prepare the 2 beakers of "extracellular" fluid: Add 100 mL of Solution A to a 250-mL beaker or cup. Add 100 mL of Solution C to a different beaker or cup.

3. Connect the colorimeter to the data collection device using the sensor extension cable. Monitor live data without recording.

4. Calibrate the colorimeter.

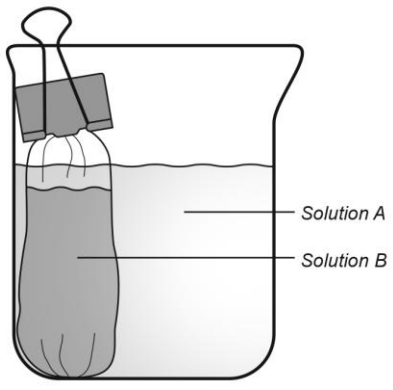
5. Obtain two graduated cylinders and pour 20 mL of Solution B into one and 20 mL of Solution D into the other.

6. Use a plastic pipet to fill a clean, dry cuvette with approximately 5 mL of Solution B. Place the cuvette into the colorimeter and record the transmittance of green light. Remove the cuvette.
   For the sample data, the initial transmittance of green light through Solution B is 7.1%.

7. Use a different pipet to fill a clean, dry cuvette with Solution D. Measure and record the transmittance of green light through the solution.
   For the sample data, the initial transmittance of green light through Solution D is 19.5%.

8. Obtain a piece of dialysis tubing that has been soaked in water. Rub the tubing between your fingers to open it. Twist the tubing at one end and tie a tight knot in it. Place a funnel in the opening at the opposite end. Pour 15 mL of Solution B into the tubing. Twist the tubing at the top and use a binder clip to keep it closed.

9. Place the tubing with Solution B into the beaker with Solution A. The tubing should be mostly submerged but it should rest upright, with the binder clip remaining above the surface of the solution.



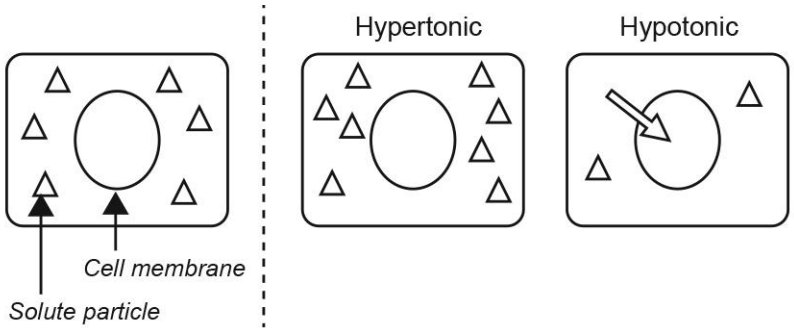
Dialysis bag, submerged in beaker

10. Prepare another dialysis tubing bag using 15 mL of Solution D. Place this bag into the beaker with Solution C. Label the cups or beakers, or place them on a labeled paper towel, to keep track of which solutions are present in each arrangement.

11. Let the dialysis bags remain in the beakers, undisturbed, for 30 minutes. While you wait, answer the questions that follow.

12. The diagram below represents a cell surrounded by extracellular fluid that is isotonic to the cell. Based on this model, draw solute particles in the other two diagrams to represent fluids that are hypertonic and hypotonic to the cell.

    NOTE: Draw the diagrams in your lab notebook.



13. a. In which situation will there be a net movement of water into the cell?

    In the hypotonic situation there will be a net movement of water into the cell.

    b. Draw an arrow on the diagram to indicate this.

    See the diagram.

    c. Imagine that the cell contains a colored solution. If osmosis causes water to move into the cell, what should happen to the transmittance of light through that solution? Explain your reasoning.

    If water moves into a cell, the colored solution will be diluted and become lighter in color. This would cause an increase in light transmittance through the solution.

14. For the remaining diagram, explain the net movement of water expected and describe the expected change in transmittance.

    In the hypertonic situation, water is expected to move out of the cell since the hypertonic solution has a lower concentration of water than the cell. Water leaving the cell would cause the colored solution to become darker and there would be a decrease in transmittance.

15. After 30 minutes, remove the binder clip from the dialysis bag soaking in Solution A. Untwist and open the dialysis bag so you can pipet solution out of the bag.

16. Use a pipet to fill a dry, clean cuvette with approximately 5 mL of Solution B from the bag. Place the cuvette into the colorimeter and record the transmittance of green light through the solution. Compare this to the initial transmittance for Solution B and calculate the change.

    For the sample data, the final transmittance is 8.4%. There was an increase of 1.3% (8.4% – 7.1%).

17. Open the dialysis bag in Solution C and fill a cuvette with a sample of Solution D from the bag. Determine the transmittance of green light for Solution D and compare this with the initial transmittance. Calculate the change.

    For the sample data, the final transmittance is 15.1%. There was a decrease of 4.4% (15.1% – 19.5%).

18. Create a data table to organize the measurements of transmittance of green light. Include a column in your table for percent change in transmittance. For Solutions B and D, record transmittance data and calculate the percent change.

$$\text{Percent change} = \frac{(\text{Final value} - \text{Initial value})}{\text{Initial value}} \times 100$$

Transmittance of green light through a blue-colored solution

Solution in the Dialysis Bag	Initial Transmittance (%)	Final Transmittance (%)	Percent Change in Transmittance
Solution B	7.1	8.4	18%
Solution D	19.5	15.1	–22.6%

Data Analysis

1. a. Which solution experienced a decrease in transmittance?

   Solution D experienced a decrease in transmittance.

   b. If transmittance decreased, did the solution become lighter in color or darker in color?

   It may not be noticeable to the naked eye, but the solution became darker in color, limiting the ability of light to pass through it.

   c. In this situation, did water move into the model cell or out of the model cell?

   The transmittance decreased due to water leaving the model cell and moving into the surrounding solution.

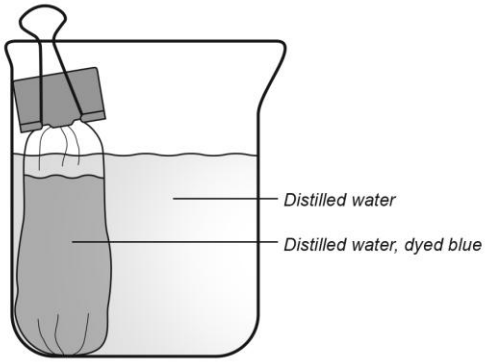
   d. Was the extracellular fluid in this situation initially hypertonic, hypotonic, or isotonic to the model cell? Explain your choice.

   In this situation, the extracellular fluid was hypertonic to the model cell. A hypertonic solution, due to a high concentration of solutes, has a relatively low concentration of water. In this situation, osmosis causes water to move out of a cell, which is what occurred to the model cell containing Solution D.

2. Solution A is tap water and Solution B is a 0.8 M sucrose solution. Based on this information, explain the change in transmittance for Solution B.

   Solution A is hypotonic to Solution B (which was inside the model cell). The extracellular fluid is low in solutes and has a high concentration of water. In this situation, water moves into a cell. Water from Solution A moved into the dialysis tubing and diluted Solution B. This caused the blue color to become lighter so more green light could be transmitted through the solution.

3. A student carries out this experiment and adds the following to the setup: a dialysis bag containing distilled water submerged in a beaker with distilled water. Explain the purpose of this dialysis bag and the value of the results, if any.



Dialysis bag, submerged in beaker

Table 1: Transmittance of light through a blue-colored solution

Condition	Transmittance (%) and % Change
Initial	15.4
Final	15.5
Percent change	0.6%

This setup acts as a control group. The water in the beaker is isotonic to the water in the bag, so this arrangement shows whether the blue dye particles added for coloring affect water movement into or out of the bag. There was a very small change in transmittance (0.6%), showing that the larger changes that occurred in the other bags were significant.

Synthesis Questions

Consider a hot day in which you have to run a mile in P.E. class. As you run, your body sweats to help maintain proper body temperature. Sweating leads to minor dehydration due to water lost from the sweat droplets that evaporate off the skin.

In this investigation, you monitored the water content of a model cell by detecting changes in transmittance of light through a colored solution. In the body, the water content of plasma (the liquid portion of blood) is monitored by specialized cells called *osmoreceptors*. These osmoreceptors are located in the hypothalamus.

1. When dehydration occurs, what change in the blood is able to be detected by osmoreceptors?

   Osmoreceptors detect changes in osmolarity. If dehydration occurs they detect an increase in the relative concentration of solutes (particularly sodium ions) in the plasma as a result of water loss or absorption.

2. The osmoreceptors help regulate the inhibition or stimulation of anti-diuretic hormone (ADH). In the case of dehydration, the hypothalamus signals the pituitary gland to release ADH.

   a. What effect does releasing ADH have on the kidneys?

      ADH causes the kidneys to increase reabsorption of water. The kidneys produce more concentrated urine in order to conserve water and counteract dehydration.

   b. Specifically, the target cells that ADH binds to add aquaporins to their cell membranes in response to the hormone. How does this explain the change that occurs in the kidneys to counter dehydration?

      Aquaporins are integral proteins that act as pores, allowing water molecules to move through the cell membrane. Adding aquaporins to the membrane increases the permeability of the membrane and water can move out of the tubule containing urine. The water is reabsorbed into the bloodstream, helping the body conserve water, and decreasing the volume of urine produced.

   c. ADH is a short peptide hormone that triggers a cAMP signal pathway in target cells. Why wouldn't ADH move directly into cells?

      Cell membranes are not permeable to peptide hormones, only to lipid-soluble hormones like steroids. Peptide hormones must bind to a receptor on the surface of target cells to initiate a cell signaling pathway.

3. Describe the negative feedback system that prevents overcorrection of dehydration.

   Due to water loss, ADH is released in response to increased osmolarity of blood plasma. As a result of the ADH and the kidneys' response, reabsorption of water is increased, decreasing osmolarity. When the blood flows through the vessels of the hypothalamus, osmoreceptors detect that the osmolarity has returned to normal levels. The hypothalamus then inhibits the pituitary gland from releasing ADH. This reduction in ADH causes target cells to decrease the number of aquaporins in their membranes and water reabsorption is lessened.

4. Caffeine is a diuretic. Explain the effect caffeine has on urine production and explain why excess caffeine consumption leads to dehydration.

   Caffeine interferes with ADH function and causes a large volume of urine to be produced. ADH would normally act to counter dehydration by increasing water reabsorption into the blood. However, with high levels of caffeine, too much water is excreted into the urine, which leads to dehydration.

4. PLASMOLYSIS

Lab Overview

Students examine red onion tissue, looking for evidence of plasmolysis in the cells. Based upon their observations of plant tissue in three solutions with different (unknown) concentrations of salt, students determine which of the solutions has the highest salt concentration. They test their conclusions with a conductivity meter. Students then design an experiment that applies their understanding of water potential and the equation for water potential to compare that property in different plant tissues.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	15 min
Initial Investigation	35 min

Student-Designed Experiment	
Experiment Design	20 min
Experiment (over 2 days)	30 min
Data Analysis	30 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.B
Science Practices	2.1, 2.2, 5.1–5.3
Learning Objectives	2.11, 2.12

Materials and Equipment

For Each Student Station

- Data collection system
- Conductivity sensor
- Microscope, 400× magnification
- Microscope slides and cover slips (4)
- Plastic pipet or eye dropper
- Electronic balance[3]
- Three salt solutions of unknown concentration, several drops[1]
- Red onion section[2]
- Water, several drops
- Paper towel
- Celery stalks[3]

[1]To formulate the salt solutions using sodium chloride and water, refer to the Lab Preparation section.

[2]Alternatively, *Elodea* leaves can be used as the plant tissue observed.

[3]These items are needed for a teacher demonstration. Refer to the Lab Preparation section.

Additional equipment recommended for the student-designed experiment:

- 1.0 M Sucrose ($C_{12}H_{22}O_{11}$), 2 L
- Distilled water, 600 mL
- Containers for preparing sucrose dilutions
- Electronic balance
- Small cups
- White potatoes
- Sweet potatoes or yams
- Celery, carrots, or other vegetables
- Apples or other fruits
- Cork borer

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- Plants cells have a cell wall as well as a cell membrane. If the volume of the cytosol decreases, the cell membrane will pull away from the cell wall; this is *plasmolysis*.
- The concentration of solutes in a solution influences the movement of water into or out of a cell. During osmosis, water moves down its concentration gradient, from a region of greater water potential to a region of lesser water potential.
- Solutions can be described as hypertonic, hypotonic, or isotonic relative to the cytosol of a cell; these terms describe the relative amount of solutes in the extracellular fluid.
- Salts contain charged particles—ions—that cause a solution to conduct electricity.
- Water potential is affected by solute concentration and pressure. The equation used to calculate water potential is

  $\Psi = \Psi_s + \Psi_p$

  where

  Ψ is the water potential

  Ψ_p is the pressure potential

  Ψ_s is the solute potential

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Use caution when cutting onion or vegetable samples with a knife.
- Take care not to break microscope slides. Tell your teacher if there is broken glass.
- Do not eat or drink any laboratory materials.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Red onion

   Purchase one or more red onions from the grocery store. Slice the onion into sections, one section for each student group.

2. "Unknown" salt solutions

   a. Prepare a 7% salt solution by dissolving 7 g of NaCl in 93 mL of water. Label this solution "A".

   b. Prepare a 10% salt solution by dissolving 10 g of sodium chloride (NaCl) in 90 mL of water. Label this solution "B".

   c. Prepare a 3% salt solution by dissolving 3 g of NaCl in 97 mL of water. Label this "C".

   d. Place a pipet in each container.

3. The day before the investigation, set up two containers with a celery stalk in each. This is needed for the teacher demonstration.

   a. Measure and record the initial mass of each celery stalk.

   b. Put one celery stalk in a beaker or cup containing 10% salt water. Put the other celery stalk in a beaker or cup containing distilled water.

   c. Leave the celery stalks in the liquids overnight. On the following day, obtain the mass of each stalk and share the results with your students.

4. For the student-designed experiments, prepare a large volume of 1.0 M sucrose solution.

   Dissolve 684.6 g of sucrose in 2000 mL of distilled water.

Teacher Tips

Tip 1 – Preparing the wet mounts

- Most students will have looked at onion cells under the microscope and prepared wet mounts before. Remind students that the tissue sample needs to be very thin (transparent). They should take their samples from the outer red-colored tissue of the onion so that the cytoplasm can be readily seen.
- To be sure students keep track of which slide goes with each solution, they can label a paper towel "A", "B", and "C", and place each prepared slide next to the corresponding letter.

Tip 2 – Observing plasmolysis

The concentrations of the unknown solutions do not need to be exactly the concentrations specified above. **Prior to students performing the investigation**, make wet mounts with each solution and observe the cells under the microscope. It is ideal if one solution (Solution "B") shows significant plasmolysis, while another (Solution "A") shows partial plasmolysis. The third can show little or no plasmolysis. If there is not enough difference in results, adjust the concentrations of the unknowns accordingly.

Tip 3 – Microscope use

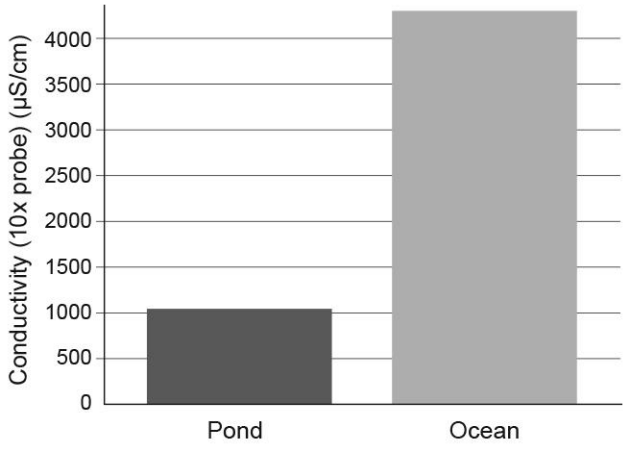
- If this is the first time in the current school year that students will use the microscope, review microscope skills with them. These skills include adjusting the light level and fine focus while on low or high power.
- Some digital microscopes, such as the ken-a-vision® Digital Monocular Comprehensive Microscope 2 (SE-7246), are compatible with SPARKvue software running on a computer or can be connected to the SPARK Science Learning System. Onion tissue samples can be viewed on the computer screen and images can be captured and saved. Students can also use measurement and annotation tools that can enhance this investigation. The use of a digital microscope to preserve images expedites data collection and preserves a record of the samples that can be analyzed later.

Initial Investigation

The Initial Investigation is designed to familiarize students with the water potential equation and the concept of plasmolysis. If students have a strong understanding of these concepts, this section may be removed. The student-designed experiment is guided-inquiry: students are asked to find the water potential of two or more plant tissues rather than devise a question completely on their own.

From the student handout:

1. Refer to the bar graph to answer the following questions.



   a. Why is the conductivity of the two water samples different?

      Ocean water has a higher conductivity due to the presence of a relatively high amount of salt. A fresh water sample, such as pond water, has very little salt dissolved in it.

   b. Which water sample is most likely to cause plasmolysis in a plant cell? Explain the basis of your answer.

      Ocean water is more likely to cause plasmolysis than pond water. Ocean water has a conductivity of over 4000 µS/cm while the conductivity of pond water is only slightly more than 1000 µS/cm. The greater conductivity of ocean water is due to ocean water having more salt dissolved in it, which means that the water potential of salt water is low. The ocean water, being hypertonic to a plant cell, would cause water to leave plant cells by osmosis, causing plasmolysis. Pond water, which has fewer solutes, is closer to being isotonic to plant cells.

2. Prepare a wet mount of a very thin layer of red onion tissue. Use water as the liquid for this wet mount.

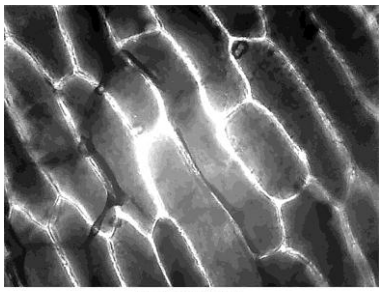
3. Prepare three additional microscope slides with onion tissue.

   a. For each of these slides, use one of the three "unknown" solutions (A, B, or C) as the liquid for the wet mount. Rinse the pipet or eye dropper with water after preparing each slide.

   b. Label the slides or place the slides on a labeled paper towel to keep track of the solution used for each wet mount.

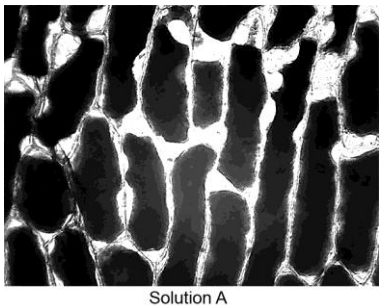
4. View the onion–water wet mount under the microscope. Select an area of the onion tissue that is thin and provides a good view of individual cells. Draw and record detailed observations; if possible, take a photograph of the cells.

The cells are square or rectangular in shape with red-colored cytoplasm filling the volume of the cell. The cell wall and cell membrane cannot be distinguished from one another.



Distilled water

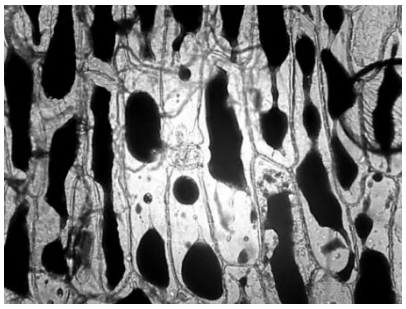
5. View the wet mount prepared with Unknown A. Draw 1–3 cells and record differences you notice between this slide and the original one.



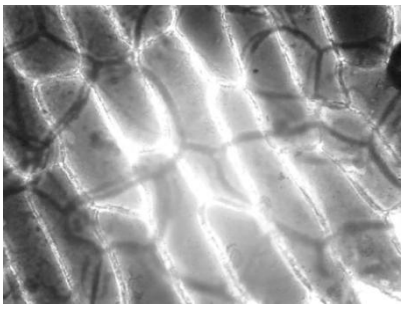
Solution A

The cytoplasm has decreased in volume. The red area appears more darkly colored. The cell membrane has moved inward, away from the cell wall.

6. Observe the Unknown B and Unknown C slides. Compare and contrast the cells of these samples with previous samples and record detailed observations. Draw or photograph the cells.



The Unknown B slide shows most or all cells have plasmolyzed. There is a large amount of clear area within the cells, indicating that the cytosol has decreased in volume and the cell membrane is no longer near the cell wall.



The Unknown C slide looks very similar to the onion–water wet mount. The cytosol fills the cell and it is difficult to distinguish between the cell membrane and cell wall.

? 7. The unknown solutions are all solutions of sodium chloride (NaCl). Based on your observations of the cells, which "unknown" solution has the highest salt concentration? Which has the lowest salt concentration? Explain the reasoning for your choices.

Unknown B has the highest salt concentration while Unknown C has the lowest. Solution B showed the most plasmolysis; these cells lost the greatest amount of water to the environment. The environment must have been hypertonic, with a lot of solutes. Unknown C did not appear to cause much plasmolysis, if any, so it must be lowest in salt concentration. (The cells didn't look much different from the cells surrounded by water.)

8. Connect a conductivity sensor to your data collection system. Set up the system to view live data, or begin an experiment if you plan to save or print the data. Press the green button next to the "ocean wave" on the front of the sensor.

9. Use the conductivity sensor to test the conductivity of each "unknown" solution. Record the results of the conductivity test.

Conductivity results

Solution	Conductivity (µS/cm)
Unknown A	90,000
Unknown B	97,611
Unknown C	31,650

10. Do the results confirm your earlier ranking of the salt concentration in the solutions? Explain your answer.

Solution B was predicted to have the highest salt concentration. This is confirmed by the high conductivity of the solution. Solution C has the lowest conductivity and this is consistent with it having the lowest salt concentration.

Use the information below about water potential to answer the questions that follow.

The equation used to calculate water potential is

$\Psi = \Psi_s + \Psi_p$

A solution in an open container has a pressure potential Ψ_p of 0 bars, so in many situations this component of the equation can be left out, so that $\Psi = \Psi_s$.

The solute potential Ψ_s is equal to $-iCRT$ where

i is the ionization constant

C is the molar concentration

R is the pressure constant [0.0831 liter bar/(mole K)]

T is the temperature in K (273 + °C of the solution)

For distilled water, $\Psi_s = 0$ bars since there are no solutes.

11. Unknown A is a 0.12 M salt (NaCl) solution. Calculate the water potential Ψ of this solution at 21 °C. (In an open container, $\Psi_p = 0$.)

$\Psi = \Psi_s + \Psi_p$

$\Psi = [-(2)(0.12\ M)(0.0831\ \text{liter bar/(mol K)})(294\ K)] + 0$

$\Psi = -5.9$ bars

12. Refer to the water potential you calculated for Unknown A to answer the following.

a. Is the water potential of a red onion cell greater than or less than the value you calculated for Unknown A? How do you know?

Unknown A caused onion cells to plasmolyze. Since water moved out of the cells, the cells must have had a greater water potential compared to Unknown A, which surrounded the cells, so the water potential of red onion cells is greater than –5.8 bars.

b. How does the water potential of Unknown C compare to that of Unknown A? Support your claim with evidence and clear reasoning.

Unknown C did not cause cells to lose much water, if any. It had the lowest conductivity value. This means that Unknown C would have a solute potential close to zero and would have a greater water potential than Unknown A. For example, if the concentration of NaCl in Unknown C is only 0.01 M, its water potential is –0.49 bars, which is greater than Unknown A's water potential, which is –5.8 bars.

13. Adding distilled water to plasmolyzed cells can return the cells to a turgid state. (Test this for yourself if you have time!) Why don't plant cells burst when placed in a hypotonic environment, such as distilled water?

In a hypotonic environment, there is a net movement of water into the plant cells. As the volume of the cell increases, pressure results from the cell membrane pushing against the cell wall. The cell wall is rigid and the pressure the wall exerts back on the membrane limits the movement of water into the cell. This pressure raises the water potential of the cell and eventually equilibrium is reached.

14. Yesterday your teacher placed celery stalks into distilled water and salt water. Record the initial and final mass of each celery stalk. Record observations of the stalks, comparing them to one another and to standard celery stalks you find in a grocery store. Use your understanding of osmosis and water potential to explain what occurred in each stalk overnight.

Celery results

Solution	Initial Mass (g)	Final Mass (g)
Distilled water	37.95	40.86
Salt water	34.41	29.96

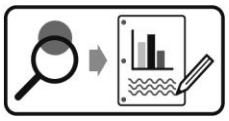
The stalk in distilled water gained mass and snaps and breaks when bent (it is crisp). The cells in the stalk gained mass due to water moving into the cells. (Cells have a lower water potential than distilled water.) The cells became turgid, due to pressure from the cell membrane pushing against the cell wall; the pressure increases as the cytosol volume increases due to osmosis.

The stalk in salt water lost mass. Salt water has a low water potential, causing water to move out of the cells. The plasmolysis of the cells caused the celery stalk to lose turgor pressure, so the stalk is flexible and does not snap when bent.

Design and Conduct an Experiment

A number of methods have been developed to experimentally determine the water potential of plant tissues. One method, the gravimetric technique, involves measuring the change in mass that occurs in tissue samples exposed to solutions of known concentration. The Potato Core Lab commonly done in biology courses is an example of an application of this technique.

For this extension, your students should choose the tissues to test and design their own procedures for their experiments. Depending on their experience, you may need to suggest analysis techniques to students, such as calculating the percent change in mass for the samples.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Which has a greater water potential, white potato or sweet potato?*
- How does the water potential of celery compare to the water potential of carrots (or other vegetables)?*
- Is the water potential of a fruit similar to that of a vegetable?*
- Is the water potential measurement affected by the solute used? For example, will sucrose and salt water provide similar results for the water potential of a potato?*

Design and Conduct an Experiment: Data Analysis

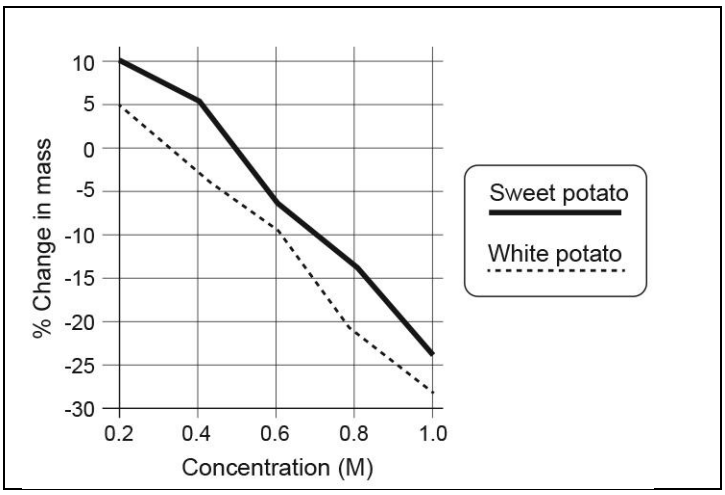
The answers in this section are sample answers that apply to the suggested inquiry question: "Which has a greater water potential, white potato or sweet potato?" Results of this experiment are shown in the Sample Data section.

1. From your observations and your data:

    a. Explain the effect of the different sucrose solution concentrations on plant tissue samples.

    Some samples gained mass due to water moving into the cells from a hypotonic solution—a solution with no solutes or a low concentration of solutes. Some samples lost mass due to the solutions that were hypertonic to the cells, causing water to move out of the cells. The hypertonic solutions had a relatively high amount of solute, so water moved from the cells to the region with lower water potential.

b. Determine the sucrose molarity at which each plant tissue sample would experience no change in mass (that is, there would be no net movement of water occurring.)



Students should create a graph of percent change in mass versus solute concentration. The *x*-axis value at which *y* = 0 is the concentration that causes no change in mass for the sample.

White potato: A sucrose solution of 0.32 M would cause no change in mass of a potato core.

Sweet potato: A sucrose solution of 0.49 M would be expected to cause no change in mass of a sweet potato core.

c. Calculate the water potential for each plant tissue sample tested.

Based on the sample data, the water potential for white potato is:

$\Psi = \Psi_s + \Psi_p = [-(1)\ (0.32\ M)(0.0831\ liter\ bar/(mole\ K))(294\ K)] + 0 = -7.8\ bars$

Based on the sample data, the water potential of the sweet potato is –12.0 bars.

NOTE: At equilibrium, the pressure potential is assumed to be zero, since the solutions are in an open container and there is no net movement of water into or out of the cells.

2. Do the results of the experiment support or reject your hypothesis? Support your claim with evidence.

Answers will vary based on student's predictions and their choice of samples to compare. Student hypotheses are expected to be unique and their answer should be based on their evidence, not on any scientific principle. For the sample inquiry described in the Design and Conduct an Experiment Key, the hypothesis is supported. Sweet potato cells didn't plasmolyze until the sucrose concentration reached 0.6 M, meaning that a solution with a relatively high amount of solute is isotonic to these cells. Therefore, sweet potato cells have a lower water potential than white potato cells, which plasmolyzed at a sucrose concentration of 0.4 M.

3. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

Answers will vary. If students carry out multiple trials for each experimental group of the procedure, their data should be consistent in trials that were replicated. If there is a high amount of variance in data, experimental error or other variables may be the cause. For example, in the PASCO trial using white potato, the first trial indicated that *y* = 0 at 0.33 M but in trials 2 and 3, *y* = 0 at 0.41 M and 0.42 M, respectively, showing natural variation in potato core water potential.

The data would be more reliable if all three trials showed similar concentrations at which there would be no change in mass. The data is sufficiently reliable for a comparison of white potato to sweet potato, since the white potato in all three trials had a higher water potential than the three trials with sweet potato.

Another indication of the reliability of data is the trend of the line for percent change in mass. The decrease in mass should be linear (the decrease in mass may level off, however, between 0.8 M and 1.0 M). If the data points do not indicate a linear relationship, error likely occurred during data collection.

4. Identify any new questions that have arisen as a result of your research.

Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. Salt is used as a preservative to prolong the shelf life of foods without refrigeration. Describe how a salty environment would affect the cells of organisms, such as bacteria, that typically cause food spoilage.

   Salt water causes water to leave cells due to its very low water potential. A high-salt environment would cause cells such as bacteria to die, due to shrinkage and dehydration.

2. Osmosis plays a role in regulating the opening and closing of stomata in leaves. Stomata are small openings through which a plant obtains the carbon dioxide molecules needed for photosynthesis. Guard cells are cells that are adjacent to stomata and change in shape to cause stomata to be open or closed. If guard cells swell due to osmosis, stomata open. It has been discovered that a high concentration of potassium ions (K^+) is needed for this to happen. Explain why an increase in K^+ concentration in guard cells causes the cells to swell.

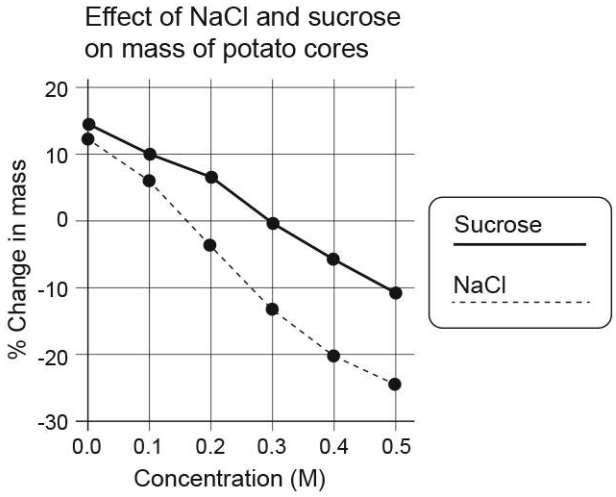
   Increasing the concentration of potassium ions in guard cells causes the water potential of that cell's cytosol to decrease (it becomes more negative due to the presence of more solute particles). This causes water to move from the surrounding solution into the guard cells, causing them to swell.

3. Use the concept of "free energy" to explain why water molecules tend to move from an area of higher water potential to an area of lower water potential during osmosis.

   An area of higher water potential has a greater number of unbound water particles. The particles have the ability to move freely. A solution with a low water potential has many solutes, which dissolve when water particles are attracted to and surround the solute particles.

   Because many water molecules become bound to solute particles in hydration shells, fewer water molecules have the ability to move freely. A solution with a large number of water molecules moving randomly has more free energy than a solution with bound water molecules, so the spontaneous direction of movement is from the area of higher water potential to an area of lower water potential.

4. Potato cores were placed in solutions with varying concentrations of sodium chloride (NaCl) and sucrose ($C_{12}H_{22}O_{11}$) for 24 hours. The percent change in mass of the cores in each solution was calculated and plotted on a graph.[8] Consider the equation for water potential and the properties of sodium chloride and sucrose as solutes, and explain the different results for the cores in the two solutions.



The sodium chloride solutions have a lower water potential than the sucrose solutions, even when comparing two solutions of the same molarity, such as 0.3 M. The water potential is equal to the solute potential ($\Psi_p = 0$ because the containers are open).

$$\Psi = \Psi_s = -iCRT$$

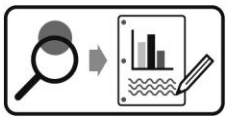
For sucrose, the ionization constant *i* is 1, since sucrose does not dissociate into ions when it dissolves. Sodium chloride, however, dissociates into Na^+ and Cl^-, so there are more particles present in a 0.3 M sodium chloride solution compared to a 0.3 M sucrose solution. The ionization constant for sodium chloride is 2, so the water potential will be 2 times lower for a sodium chloride solution than for a sucrose solution at the same concentration.

The cores immersed in sodium chloride solutions showed plasmolysis (loss of mass) at a lower concentration than did the cores in the sucrose solutions. The sodium chloride concentrations of 0.2 M to 0.5 M caused a loss of mass (going below 0% change in mass). The cores immersed in sucrose solutions didn't experience a loss of mass until the concentration reached 0.3 M, and even then it was a very slight decrease in mass. The sodium chloride solutions have lower (more negative) water potentials, which correspond to a greater water loss (plasmolysis) of potato cells in the salt solutions than in the sugar solutions.

[8] Kosinski, R.J.; Osmosis and the Water Potential of Potato Tissue. Clemson University. Last update: October 2013. Retrieved February 6, 2014 from http://biology.clemson.edu/bpc/bp/lab/110/osmosis.htm.

Design and Conduct an Experiment Key

A number of methods have been developed to experimentally determine the water potential of plant tissues. One method, the gravimetric technique, involves measuring the change in mass that occurs in tissue samples exposed to solutions of known concentration.



Develop and conduct your experiment using the following guide.

1. Consider the possible sources of plant tissue for this lab. List examples of tissues you might use.

   Examples include potatoes, sweet potatoes, celery, carrots, apples, cucumber, and zucchini.

2. Your teacher will provide you with a large volume of a 1.0 M sucrose solution. What range of diluted solutions do you think would be appropriate to use to determine the water potential of the plant samples?

   At least four concentrations should be used: 1.0 M, 0.75 M, 0.50 M, 0.25 M, for example. Better still would be a greater number of concentrations in that range: 0.2 M, 0.4 M, etc. Students should recognize that the water potential of a cell should be much less than a 1.0 M solution, and therefore should include very dilute solutions. Distilled water should be used as a control.

3. Write a driving question: choose two or more plant tissues, or choose other solutions in addition to sucrose, and develop a testable question for your experiment.

   Which has a greater water potential, white potato or sweet potato?

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable is the plant tissue. Different sources of tissue will be obtained and their water potential compared. A cork borer will be used to cut cylinders from two types of potato: white potato and sweet potato.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable is the change in core mass. The initial mass will be measured before putting the cores into solutions and the final mass will be measured after the cores have been in the solutions for 24 hours. Three cores will be placed in each solution and the initial and final masses will be the total mass of the three cores. The percent change in mass will be calculated from the change in mass.

6. Write a testable hypothesis (If…then…).

   If a sweet potato has a lower water potential than a white potato, then the sweet potato cores will be in equilibrium with a solution of greater molarity (more solutes) than a solution isotonic to the white potato.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   Temperature: all trials will be carried out at room temperature.

   Solution concentrations: the dilutions will be prepared first and the cores from different types of potatoes will be placed in cups using the same series of dilutions (0.2 M, 0.4 M, etc.)

   Number and size of cores: three cores of each type of tissue will be placed in each cup. The same cork borer will be used to make uniform-sized pieces. Pieces will be trimmed to the same length. The potatoes will be peeled first so there is no skin on the surface of the cores.

   Mass measurement: the initial mass and final mass will be the collective mass of all 3 cores placed into a cup. The same balance will be used for each mass measurement.

8. How many trials will be run for each experimental group? Justify your choice.

   Two trials for each experimental group (sweet potato and white potato) will be run. Two cups will be set up in an identical manner for each solution concentration and tissue type. Sometimes a cup is knocked over and solution spills. If this happens, a "backup" is in place. Also, there will be data that can be averaged.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The percent change in mass in the cores will be calculated: ($\%\ \text{Change} = \frac{|\text{Final Mass} - \text{Initial Mass}|}{\text{Initial Mass}} \times 100$). The average of the two trials of each tissue type and solution concentration will be calculated. For example, the percent change in mass of 3 white potato cores in 0.2 M sucrose solution will be averaged with the percent change in mass for the duplicate setup. The average percent change in mass versus sucrose molarity for each tissue type will be graphed. From the graph, the concentration *C* at which no change in mass occurs can be extrapolated. This value is needed to find the water potential of the plant samples, using the equation: $\Psi = \Psi_s = -iCRT$

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    There may be unknown variation in the water potential of white potatoes relative to each other, or variation in the water potential of sweet potatoes. Old potatoes may be "dehydrated." The age of potatoes bought from a store cannot be controlled.

    The initial and final masses of the cores may be affected by the handling of the cores before or after they are in the solution. The cores should be blotted dry so the mass is indicative of only the tissue and not any liquid adhering to the surface. However, leaving the cores on paper towel for a few minutes may draw water out of the core.

    Dilutions of the 1.0 M sucrose solution may not be prepared properly due to inexperience with this technique.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

    Prepare solutions of 0.2 M, 0.4 M, 0.6 M, and 0.8 M concentrations from the 1.0 M stock solution. Make 300 mL of each solution.

    0.2 M = 60 mL stock + 240 mL water

    0.4 M = 120 mL stock + 180 mL water

    0.6 M = 180 mL stock + 120 mL water

    0.8 M = 240 mL stock + 60 mL water

    Obtain 24 small plastic cups. Label four cups for each of the following: "distilled water", "1.0 M", "0.8 M", "0.6 M", "0.4 M", "0.2 M". Pour 70 mL of each solution into the corresponding cups.

    Peel a white potato and a sweet potato. Use a cork borer to cut three cores out of each potato. The cores should not have any skin on their surface. Place the cores next to each other and trim them to the same length.

    On an electronic balance, obtain the total mass of the 3 white potato cores and the total mass of the 3 sweet potato cores. Record the initial masses.

    Place the 3 white potato cores into one of the cups with distilled water and the 3 sweet potato cores into the other distilled water cup.

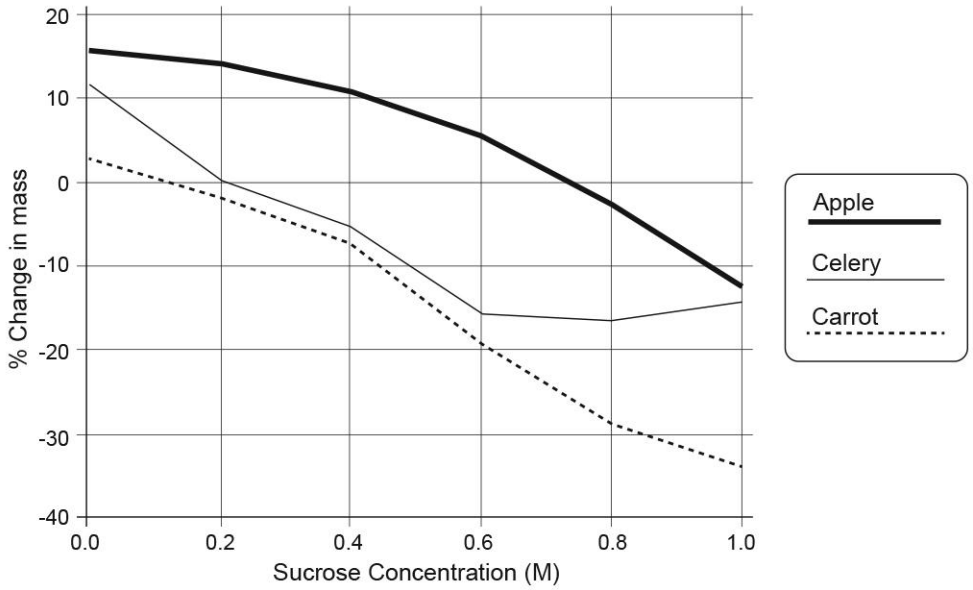
    Repeat the procedure until there are 12 cups with white potato (0.0 M–1.0 M solutions, in duplicate) and 12 cups with sweet potato cores in the same dilution series (in duplicate).

    Cover the cups with plastic wrap and leave them on a counter overnight.

    On Day 2, remove 3 cores from one of the distilled water cups. Gently and quickly blot the cores to remove excess liquid. Place the 3 cores together on the balance and record the collective mass as the "final mass" of the cores. Repeat the mass measurement procedure for all cups.

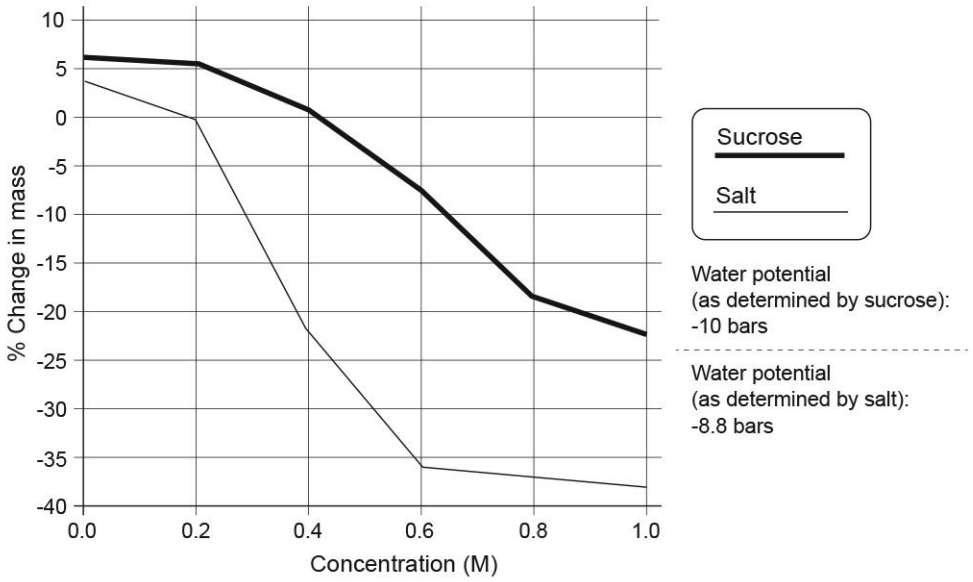
12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data



The results shown above are from an inquiry comparing the water potential of a fruit to that of two different vegetables. Slices of apple, celery, and carrot were immersed in sucrose solutions at room temperature for 24 hours.

The above graph shows the water potential of a variety of fruits and vegetables, determined by finding the concentration at which no change in mass occurred in sucrose solutions and then calculating Ψ at that concentration.



The results shown above are for an inquiry that used two types of solutes (ionic and covalent compounds) to determine the water potential of white potato. The mass of each potato core was obtained before and after the 24-hour immersion.

5. CELL SIZE

Lab Overview

Students model the effect of cell size on cell cooling rate using cubes of potato tissue. Potato cubes of different sizes are placed in ice water and multiple temperature probes are used to simultaneously measure the interior temperature of each cube and the temperature of the ice bath. Students determine if and how the difference in cooling rates relates to the difference in surface-area-to-volume ratios (SA:V) of the cubes. Students then consider variables other than size that might affect the SA:V ratio and design an experiment that tests the effect of that variable on the cooling rate of a cell, using potato tissue to model the cell.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	15 min
Initial Investigation	30 min

Student-Designed Experiment	
Experiment Design	20 min
Experiment	45 min
Data Analysis	20 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	1.B.1, 2.A.3, 2.C.2, 2.D.1
Science Practices	2.1, 2.2., 3.1, 3.3, 4.1–4.4, 5.1–5.3
Learning Objectives	2.6, 2.7, 2.9, 4.14

Materials and Equipment

For Each Student Station

- Data collection system
- PASCO Quad Temperature Sensor
- Fast-response temperature probes (3)[1]
- Metric ruler
- Small knife or scalpel
- Cutting board or other appropriate surface
- Potato
- Plastic containers (for ice water), 24 oz or larger (approximately 700 mL)
- Water, about 500 mL
- Toothpicks (2)
- Permanent marker
- Tape
- Ice, about 100 mL

[1]Included with the PASCO Quad Temperature Sensor.

Additional equipment recommended for the student-designed experiment:

- Melon baller (to form spherical potato "cells")
- Shortening (or similar solid fat source)
- Cork borer (to form cylindrical potato "cells")
- Additional potatoes

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- Cells are the basic unit of structure and function for biological systems, and cells need to be small to efficiently carry out the functions that sustain life.
- Diffusion of matter and energy occurs along a concentration or temperature gradient.
- Calculating surface area and volume for various geometric shapes: cubes, spheres, cylinders.
- The slope of a line on a graph relates to the rate of a process.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Never eat any materials used in lab activities.
- Use extreme caution when cutting with a knife or scalpel and always cut in a direction away from your body.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Large potatoes

   Each group will need at least one large potato. Potatoes should be at room temperature when students use them for the Initial Investigation.

2. Plastic containers for ice baths

   Plastic disposable containers, such as leftover food containers or large empty yogurt containers, work well. A wider (shallower) container is preferred over a narrow, deep container.

3. Ice for ice baths

   The day of the lab activity, obtain enough ice for the number of ice baths your class will need. Keep the ice in a cooler to prevent melting.

Teacher Tips

Tip 1 – Inserting the temperature probes into the potato cubes

1. It is helpful to set up an example cube with a sensor inserted for students to refer to. Though images are included with the procedure, students may still require assistance.

2. To keep track of which temperature probe is associated with each situation (smaller cube, larger cube, or ice water), students can mark the tape that holds the probe to the toothpick. Each temperature port on the sensor is numbered, "1"–"4", so these numbers can be used as the labels on the piece of tape attached to each probe.

Tip 2 – Immersing the potato cubes in ice water

Since it is difficult to hold two cubes in the water and start or stop data collection, using an automatic stop setting is recommended. If this is not possible with your data collection system, be sure students work in groups of three so one person can manage data collection and the other students can each hold a cube still in the water, ensuring that water does not leak into the hole at the top where the probe is inserted.

NOTE: A small dab of petroleum jelly can be placed around the toothpick and temperature probe to make the hole water resistant.

Tip 3 – Science practices

This lab provides a good opportunity for students to perform numerous trials for each condition of their student-designed experiment and calculate average cooling rates. The runs can be short (approximately 2 minutes), allowing students to run many trials during a single class period.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

1. Put on your safety goggles.

2. Connect the Quad Temperature Sensor to your data collection system. Connect three fast-response temperature probes to the temperature sensor.

3. Build graph displays for each temperature sensor. If your data collection system allows you to set an automatic stop condition, set the stop time for two minutes.

   NOTE: During data collection and analysis, make sure you know which temperature probe is associated with each condition: ice bath, large cube, small cube.

4. Set up an ice bath: Half fill a plastic container with water and add two large handfuls of ice to the water.

5. Cut small and large potato cubes from a large potato. Cut the cubes from the interior of the potato so the cubes are skinless. One cube should measure approximately 1 cm × 1 cm × 1 cm and the other should measure approximately 2 cm × 2 cm × 2 cm. Copy Table 1 into your lab notebook and record the actual dimensions of the cube in the table.

   Table 1: Measurements for the potato cube "model cells"

Potato Cube	Approximate Dimensions *l, w, h* (cm)	Actual Dimensions *l, w, h* (cm)	Surface Area (cm^2)	Volume (cm^3)	SA:V Ratio
Small	1 × 1 × 1	may vary	6	1	6:1
Large	2 × 2 × 2	may vary	24	8	3:1

6. Calculate the surface area, volume, and surface-area-to-volume (SA:V) ratio for each cube. Record these values in Table 1. Which has a greater SA:V ratio, a large cube or a small cube?

   Example response and calculations: The smaller cube has a greater SA:V ratio.

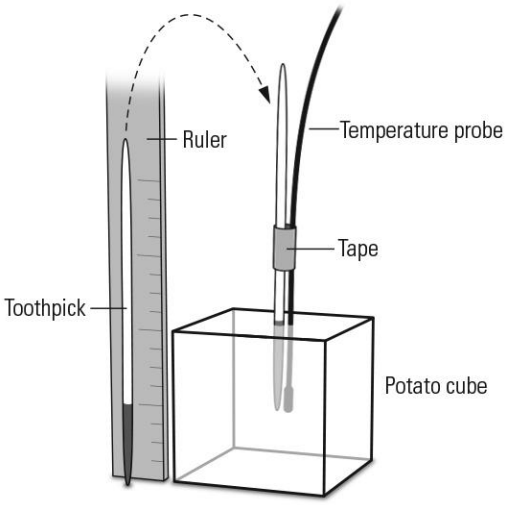
   Surface area = 6*a*, where *a* equals the surface area of one side of the cube (length^2, if the sides are equal)

   Therefore, for the smaller cube, surface area = 6 × 1 cm^2 = 6 cm^2

   Volume = (length)(width)(height). For a cube, these are equal, so for the smaller cube the volume = (1 cm)^3 = 1 cm^3

   The smaller the cube, the greater the SA:V ratio, 6:1 compared to a ratio of 3:1 for the larger cube. (The relationship between cube size and SA:V ratio is an inverse relationship.)

7. Insert a temperature probe into the center of each cube, as follows:



   a. Place a toothpick against a ruler and use a permanent marker to darken the wood of the toothpick from the tip of the toothpick to a height of 1 cm.

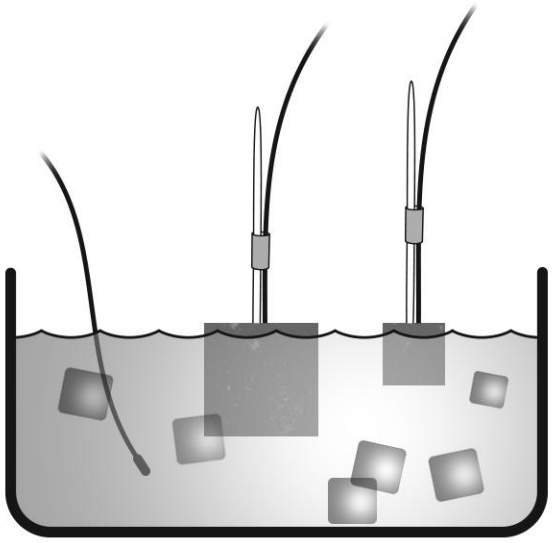
   b. Insert the dark end of the toothpick in the middle of the top surface of the large cube. Gently push the toothpick into the potato just until the black part of the toothpick is no longer showing.

   c. Remove the toothpick and insert a temperature probe into the hole. Reinsert the toothpick into the hole and use tape to secure the wire of the probe to the toothpick.

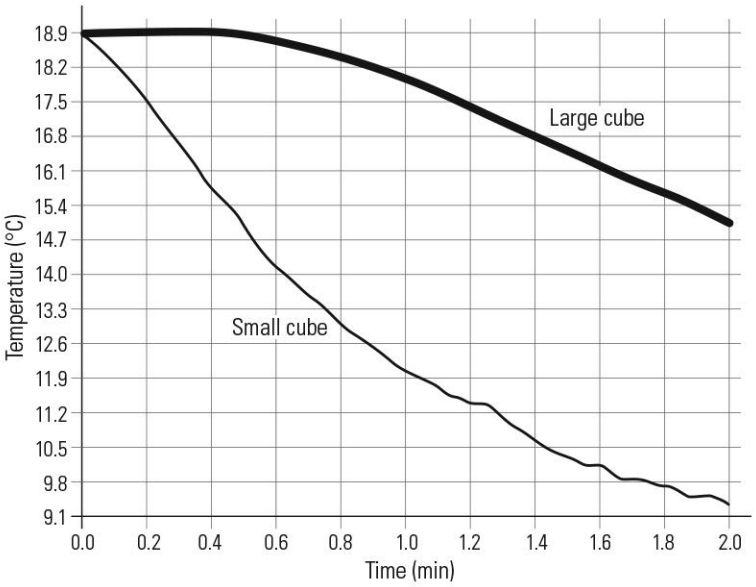
   d. Repeat the process for the small cube, except darken only 0.5 cm of the toothpick before inserting it into the cube.

8. Place the third temperature probe into the ice bath.

9. Immerse the cubes in the ice water bath but avoid submerging the cubes completely. It is important that water does *not* get into the hole with the temperature sensor. Use the toothpicks to hold the cubes; try to hold the cubes still in the water. Begin recording data.



10. After 2 minutes, end data collection and remove the cubes from the ice bath. Draw or print a record of the temperature data.



11. What is the relationship between the cooling rate and the SA:V ratio? Use evidence from the investigation to support your claim.

Cooling rates of different sized cell models

Potato Cube	SA:V Ratio	Determining Rate of Cooling			
		Maximum Temperature (°C)	Minimum Temperature (°C)	Rate of Temperature Change[1] (°C/min)	Normalized Cooling Rate (°C/min)/cm^3
Small	6:1	18.8	9.3	–4.74	–4.74
Large	3:1	18.9	15.1	–2.08	–0.26

[1]The rate was determined using the linear fit tool of the data collection system to find the slope of the line.

The greater the surface-area-to-volume ratio, the greater the cooling rate. The SA:V ratio of the small cell model is two times greater than that of the large cell model, and the small cell model cools down (loses heat to the environment) more than two times faster than the large cell model. If the data is *normalized* to compare the rate at which every cubic centimeter of the cube cooled, the difference in cooling rates is even more significant. The cooling rate per cm^3 of the smaller cube is more than 18 times greater than that of the larger cube.

12. Cells produce wastes that need to be excreted. Do the results of this investigation suggest that cell size impacts the ability of a cell to excrete wastes? Explain your answer.

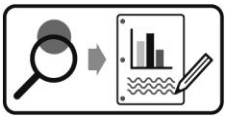
Cell size impacts the ability of a cell to efficiently excrete wastes. Just as the small cube cooled faster than the large cube, a small cell would be able to get rid of wastes faster than a large cell. A small cell has an adequate surface area (cell membrane) to allow wastes to quickly diffuse out of the cytosol enclosed by the cell membrane.

13. The potato cubes are intended to be models for cells; however, cells are rarely cuboidal in shape. Do you think the shape of a cell affects the cell's ability to efficiently exchange substances or heat with its environment? Explain the reasoning for your answer.

Answers will vary. Students are likely to correctly predict that cell shape does affect the efficiency of exchange of matter or energy. Different shapes with the same volume will not have the same surface area. Therefore, the SA:V ratios of similarly sized cells with different shapes will differ. For example, students are likely familiar with the neuron as a cell that is very long relative to other cells, but it is very flat so it can still exchange materials efficiently even as a "large" cell (large in one dimension—length).

Design and Conduct an Experiment

Students can consider variables, in addition to size, that might affect the SA:V ratio of a cell, structure, or body plan of an organism. They can then design potato models that vary in one of these variables and determine if the cooling rate is affected by the chosen variable..



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Do cells of different shapes cool down at different rates?*
- Do cell shapes other than cubes exhibit the same relationship between size, SA:V ratio, and cooling rates?*
- How do indentations or projections of "cellular" surfaces affect cooling rates?
- How efficient is an insulating material, such as fat, at reducing the cooling rate?*

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Do different cell shapes cool down at different rates?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected the cooling rate of the "cells." Does the data support your hypothesis? Justify your claim with evidence from your experiment.

Comparing cooling rates of cylindrical and spherical model cells

Cell Shape	Approximate Dimensions (cm)	Surface Area (cm^2)	Volume (cm^3)	SA:V Ratio	Average Cooling Rate (°C/min)	Normalized Cooling Rate (°C/min)/cm^3
Cylinder	2 cm height × 1 cm radius	18.84	6.28	3:1	3.22	0.51
Sphere	2 cm diameter	12.56	4.19	3:1	2.03	0.48

The data does not support the hypothesis that different shaped cells with the same SA:V ratio will have different cooling rates, that is, that cell shape affects the cooling rate independent of the SA:V ratio. When the cooling rate is adjusted to compare the change in *(°C/min)/cm^3 of volume*, the cylinder and sphere had similar cooling rates.

   b. Based on the evidence you collected, explain why the results occurred.

   Since the SA:V ratio was the same for each cell shape, the surface area through which heat could escape from the cell's volume was proportionately equal in each case. Each cell had 3 times as much surface area as volume, or 3 cm^2 of surface for every cm^3 of volume within the cell, through which heat could be lost.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   There is no evidence of experimental error or uncontrolled variables. Each trial showed the same trend: cylinders and spheres cool down at similar rates (if different volumes are accounted for). The results are repeatable and therefore reliable.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. The following table provides the radii of five spheres.

Table 2: Surface-area-to-volume ratios of different sized spheres

Sphere	Radius (cm)	Surface Area (cm^2)	Volume (cm^3)	SA:V Ratio
A	0.5 cm	3.14	0.52	6:1
B	1 cm	12.56	4.19	3:1
C	2 cm	50.27	33.51	1.5:1
D	4 cm	201.06	268.08	0.75:1
E	8 cm	804.25	2144.66	0.38:1

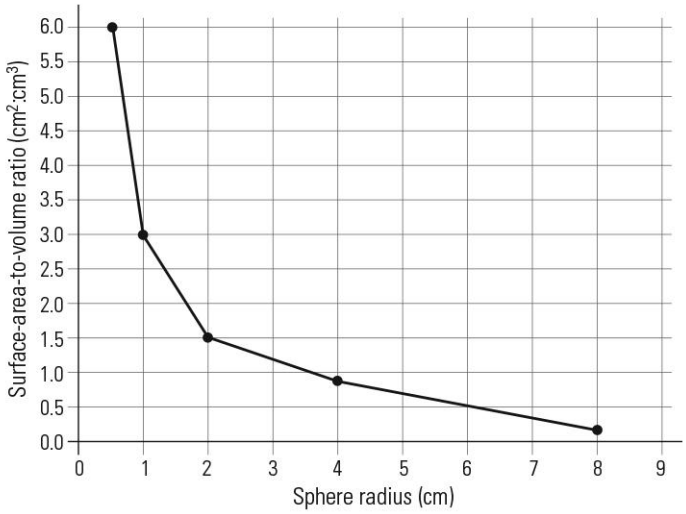
a. Calculate the surface-area-to-volume ratio for each sphere. Then create an appropriately labeled graph to illustrate the relationship between the SA:V ratio and sphere size.

Sample calculations for Sphere A:

Surface area: $4\pi r^2 = 4 \times 3.14 \times 0.5^2 = 3.14\ cm^2$

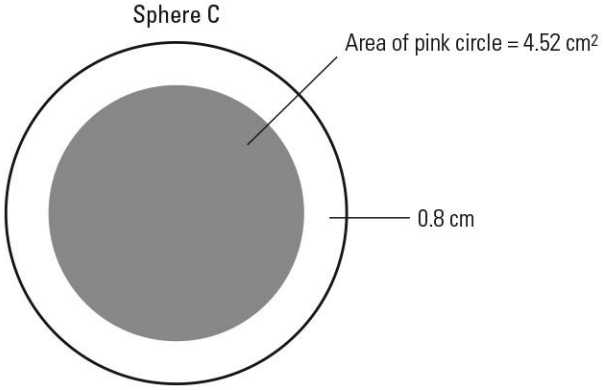
Volume: $\frac{4}{3}\pi r^3 = \frac{4}{3} \times 3.14 \times 0.5^3 = 0.52\ cm^3$

SA:V ratio: $\frac{3.14}{0.52} = \frac{6}{1}$



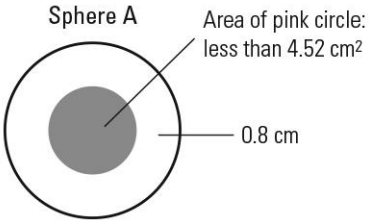
b. A student performed a diffusion experiment to investigate the diffusion of acid through different sized spheres made of agar (a gelatin-like solid). The agar contained an acid–base indicator that caused it to be bright pink. The indicator turns white in an acid.

When agar spheres were submerged in an acidic solution, diffusion of acid into the agar caused the color to change from pink to white. The diagram below shows the results obtained when Sphere C was soaked in a cup of vinegar for five minutes and removed. The sphere was cut in half and the student measured the depth of white and the area of pink in the cross-section of the cut sphere.



Predict the results of soaking Sphere A in vinegar for 5 minutes. Sketch a diagram to illustrate your prediction and use evidence from the graph to help explain your predication.

Students would correctly predict that since Sphere A has a larger SA:V ratio, it would have a proportionally larger white section than Sphere C.



The graph shows that the relationship between sphere size and SA:V ratio is an inverse relationship: the larger the sphere, the lower its SA:V ratio. A cell with a lower SA:V ratio is not as efficient at moving materials into or out of the cell as a cell with a higher SA:V ratio. Therefore, Sphere C, with a ratio of 1.5:1, has a large area of pink in its cross-section, while Sphere A, with a ratio of 6:1, has more white and less pink, as predicted, due to more efficient diffusion of acid into the smaller sphere.

2. Surface-area-to-volume ratio relates not only to cells but also to the bodies of animals. Animals have adaptations that either maximize or minimize SA:V ratio.

a. The largest penguin on earth is the Emperor penguin with an average height of 1.1 m and a body mass of 27–41 kg. Emperor penguins live in the very cold climate of Antarctica. Galapagos penguins live in a much warmer climate and average 0.5 m in height, and 1.7–2.6 kg in body mass. Based on their body size and the relationship between SA:V ratio and cooling, explain why a Galapagos penguin is ill-adapted to live in the frigid weather of Antarctica.

The much smaller body of the Galapagos penguin has a higher SA:V ratio than the large body of the Emperor penguin. Heat dissipates more quickly from a body with a high SA:V ratio, so in frigid Antarctica the Galapagos penguin would experience rapid heat loss and would be unable to maintain a suitable body temperature; it would not survive. Additionally, the smaller body mass of the Galapagos penguin would not generate enough heat through metabolism to counteract the rapid heat loss from its small body.

b. African elephants have much larger ears than Asian elephants. African elephants are adapted to the hot savannah while Asian elephants live in cool forests. Explain the advantage of larger ears in animals living in hot biomes.

Ears on animals are not like cubes or spheres; that is, larger ears are not larger in all dimensions. Rather, ears are relatively flat structures and increasing ear size results in much more surface area per cm^3 and therefore a higher SA:V ratio.

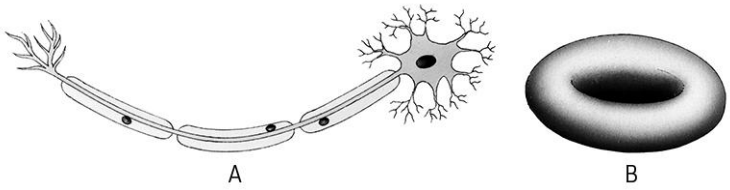
Blood flows through vessels in the ears and the close proximity of the blood to the environment results in heat loss from the body. The greater the SA:V ratio of the ears, the more rapid the heat loss, so elephants in hot environments evolved large ears to help them maintain a proper body temperature and not become overheated.

3. Surface-area-to-volume ratio (SA:V) is important to living things at many levels: from the sub-cellular to the cellular to the system level.

a. Identify one organelle present in eukaryotic cells that has a structure with a high surface-area-to-volume ratio and explain how the organelle's SA:V ratio facilitates the function carried out by the organelle.

The mitochondrion is an example of a eukaryotic organelle with a high SA:V ratio. The mitochondrion has two membranes and the inner membrane is folded many times, increasing the surface area of the membrane without affecting the volume of the organelle. The reactions of the Krebs cycle and of oxidative phosphorylation (aerobic respiration) take place in the mitochondrion. Having a large surface area within the organelles allows for a large number of electron transport systems to fit into a very small structure and increases the number of reactions that can take place within the organelle.

b. Identify each of the cells pictured below. For each cell, describe the cell's function and explain how the SA:V ratio of the cell relates to the efficiency of its function.



Cell A is a neuron. It is a long, flat cell with extensions of the cell membrane at its ends (dendrites and axon terminals). Neurons are the basic component of the nervous system and are cells that receive and send messages, allowing organisms to detect and respond to stimuli in their environment. The high SA:V ratio of their flat shape allows the cells to be long, increasing the distance that messages can travel before being passed to additional cells. (The message travels faster within a cell than between cells.) Additionally, neurons depend on a rapid flow of ions into the cell to generate an action potential (the “message”). Ions can diffuse quickly into the flat-shaped cells.

Cell B is a red blood cell. It is disc-shaped with a concave center, and its SA:V ratio is much greater than a spherical or cube shaped cell. Red blood cells deliver oxygen to the cells of the body and take carbon dioxide from the cells to the lungs where it can be excreted. The high SA:V ratio of these cells allow gases into and out of the cell faster by diffusion.

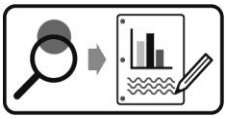
c. The respiratory, circulatory, digestive, and excretory systems of mammals all contain specialized structures that are highly branched to maximize their membrane surface area relative to their volume. Describe two examples of highly branched structures in these systems and explain how the SA:V ratio of these structures facilitates their functions.

The highly branched structures within these body systems include: alveoli (and bronchioles), capillaries, microvilli, and nephrons. All of these structures have a high SA:V ratio which gives the structure a high capacity for facilitating the exchange of substances needed to sustain homeostasis.

For example, microvilli are finger-like projections of the cells in the small intestine and this "folding" of the membrane makes nutrient absorption very efficient. Gas exchange occurs in the lungs when oxygen is brought into the body and carbon dioxide is removed, and the branching of the bronchioles into alveoli provides a large surface area through which these gases diffuse. In fact, in the five-liter volume of each human lung is a membrane surface area equivalent to the area of a tennis court! Likewise, branching of blood vessels into tiny capillary networks provides 60 km of vessels within the human body. This means that each of the trillions of cells in the body has a constant flow of blood nearby to acquire oxygen and nutrients from, and to release wastes to.

Design and Conduct an Experiment Key

Consider variables, in addition to size, that might affect the SA:V ratio of a cell, structure, or body plan of an organism. Design potato models that vary in one of these variables and determine if the cooling rate is affected by the chosen variable.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of the relationship between the SA:V ratio and cooling, what variables could affect the rate of cooling in organisms?

   Variables include cell shape, body shape, the presence or absence of fat, fur, or other insulating materials, indentations or extensions on cells, and size of body structures (such as ears).

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   Do cells of different shapes cool down at different rates?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   There are many variations in cell shape, even within the body of a single organism. For example, within human tissues some cells are cuboidal, others spherical, and others are very flat. Investigating the SA:V ratios and cooling rates in differently shaped cell models might help us understand how the shape of a cell relates to the ability of the cell to carry out a specific function for the organism. In addition, spheres and cylinders are rough approximations of different body plans of animals, so investigating the relationship between shape and cooling may be a way to model cooling rates of different animals.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable of the experiment is the shape of the cell models. The cooling rate of cylindrical potato pieces will be compared to that of spherical potato pieces. A cork borer will be used to make potato cylinders and a melon baller will be used to make potato spheres. An online math calculator will be used to help determine the dimensions of each shape so that the SA:V ratio will be the same for these differently shaped models.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable will be the rate of cooling for each model. Temperature probes will be inserted into the center of each model as was done in the Initial Investigation, and temperature data will be collected for at least 2 minutes of immersion in an ice water bath. The tools of the data collection system will be used to find the rate of cooling (slope of the line) in each trial.

6. Write a testable hypothesis (If…then…).

   If cell shape affects cooling rate, as a variable independent of SA:V ratio, then differently shaped cells will cool at different rates even if the SA:V ratio is the same for these cells.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   The same type of potato (russet potatoes) will be used to create each cell model. The cell models will initially be at room temperature and they will be placed into the same ice bath for the same period of time. The temperature probe will be inserted into the center of each shape. The SA:V ratio will be the same, 3:1, for all of the models tested.

8. How many trials will be run for each experimental group? Justify your choice.

   At least three trials will be run for each shape. Three cylindrical potato cores will be created and three potato spheres will be created. Since the trials are relatively short, approximately 2 minutes, there is enough time in one class period to run multiple trials for each shape and average the results.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The rate of cooling per cubic centimeter will be compared for each shape. First, the cooling rate will be determined for each of the three potato cylinders by applying a linear fit to the temperature data. The average cooling rate will be calculated from the data acquired from the three trials. This average cooling rate will be divided by the volume of the cylinder shape to calculate the rate of cooling per cm^3. The same data processing will be performed for the potato spheres.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    A cork borer is an effective tool that cuts uniform cylindrical shapes from potato tissue. However, uniform spheres are more difficult to obtain, even with a melon baller. It may be difficult to get three identically sized potato spheres.

    Difficulty in cutting out different shapes from potato tissue may result in some cell models being handled more than others. This may affect the integrity of the tissue, or even its initial temperature, which may in turn affect the results.

    Potatoes may have natural variations in water potential or density of the tissue and these variables may affect the rate at which heat dissipates from the cell models.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

    a. Determine the dimensions needed to compare cylinders and spheres that have the same SA:V ratio.

       The cork borer has a diameter of 2 cm, so the cylinders will have a radius of 1 cm. I'll make spheres that also have a radius of 1 cm. This size sphere has a SA:V ratio of 3:1. Using an online calculator and plugging in the desired 3:1 SA:V ratio for a cylinder with a radius of 1 cm, the necessary height of the cylinder will be obtained.

    b. Use a cork borer and melon baller to create three of each cell model. Use a knife or scalpel to shave the spheres to the necessary size (2 cm diameter). Use a knife or scalpel to cut the potato cylinders to a height of 2 cm.

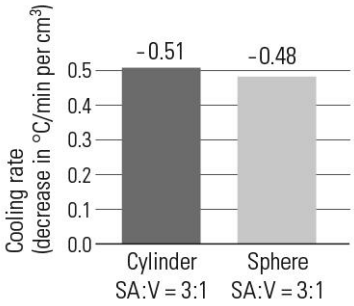
    c. Prepare an ice bath and insert temperature probes into the center of one of the cylinders and one of the spheres.

    d. Immerse the potato shapes in the ice water while collecting temperature data for 2 minutes or longer.

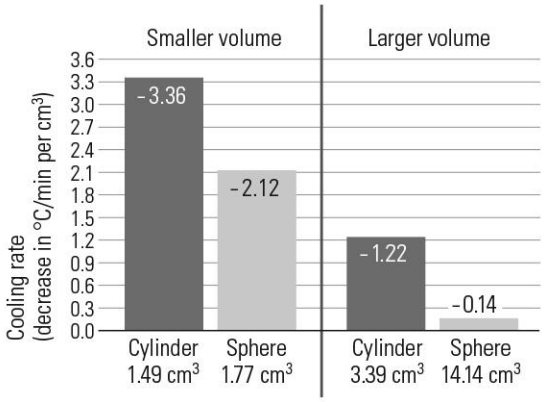
    e. Repeat data collection for 2 additional trials.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

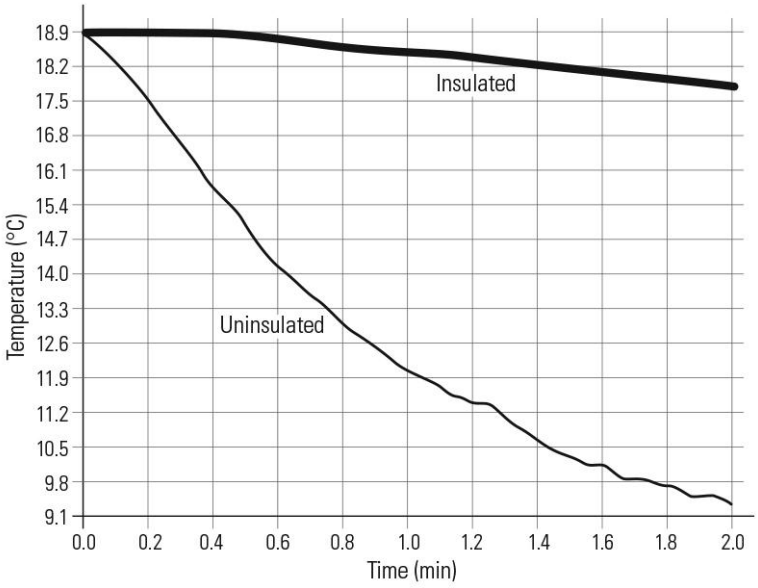
Sample Data



The results shown above are from an inquiry comparing the cooling rates of different shaped "cells" that have equal SA:V ratios.



The results shown above are from an inquiry testing the hypothesis that the relationship between cell size and cooling rate holds true for all cells, regardless of shape: bigger cells will cool slower than smaller cells.



The results shown above are from an inquiry testing the effect of insulation on cooling rate. Two cubes of equal size were placed in ice water, but the insulated cube had shortening spread onto its surface.

6. HOMEOSTASIS

Lab Overview

Students investigate the body's ability to maintain homeostasis with regard to body temperature by testing the body's response to a cold stimulus. They use multiple temperature probes to simultaneously measure the surface temperature of the skin at two locations and relate the results to thermoregulation, which is controlled by the hypothalamus—the hypothalamus receives information from nerves which detect a stimulus in the external environment, interprets information, and then responds by sending signals via efferent nerves to multiple organ systems. Students should find that in response to the cold stimulus, the body adjusts blood flow to maintain homeostasis, keeping the body's core temperature near 37 °C.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	15 min
Initial Investigation	25 min

Student-Designed Experiment	
Experiment Design	20 min
Experiment	40 min
Data Analysis	20 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	3.D.2, 3.E.2, 4.A.4
Science Practices	1.4, 2.2, 3.1, 4.1–4.3, 5.1, 5.3, 6.1, 6.2, 6.4, 7.2
Learning Objectives	3.33, 3.35, 3.43, 3.44, 3.45, 3.46, 3.47, 3.48, 3.49. 4.8

Materials and Equipment

For Each Student Station

- Data collection system
- PASCO Quad Temperature Sensor
- Fast-response temperature probes[1] (2)
- Large shallow bowl or pan[2] (for submerging a hand in ice water)
- Ice
- Water
- Adhesive bandages or medical tape for securing temperature probes to the skin (2 pieces)
- Paper towel

[1]Included with the PASCO Quad Temperature Sensor

[2]Disposable foil pans work well for this activity.

Additional equipment recommended for the student-designed experiment:

- Non-latex disposable gloves
- Physiology sensor(s) such as a hand-grip heart rate sensor, EKG sensor, spirometer sensor, and blood pressure sensor and cuff

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- Basic nervous system structure and function, as well as the function of the cerebrum, brain stem, and cerebellum
- Homeostasis is the interplay between outside factors and interior regulator mechanisms that keep biological systems stable within a very narrow range from a set point.
- Negative feedback loops as an example of a regulating mechanism
- Thermoregulation strategies including ectothermy and endothermy

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times
- If you experience severe discomfort during the immersion, remove your hand from the ice bath. Immersion of the hand in ice water will cause discomfort, however, most students can tolerate the cold water for 60 seconds without issue.
- Do not submerge your hand in ice water for more than 60 seconds. The risk of frostbite is minimal, but prolonged numbness in the hand could occur if you leave your hand in the ice bath for too long.
- If you design an experiment requiring the use of gloves, use non-latex gloves to eliminate any risk posed from allergies to latex gloves.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Shallow pans or bowls for ice baths

   Disposable pans, such as foil cake pans or casserole pans, work well. A wider (shallower) container is preferred over a narrow, deep container.

2. Ice for ice baths

   The day of the lab activity, obtain enough ice for the number of ice baths your class will need. Keep the ice in a cooler to prevent it from melting.

Teacher Tips

Tip 1 – Variability in data

When working with human subjects, the data is rarely as "clean" as when working with enzymes, germinating seeds, or other biological samples. Expect results to vary among students and groups; however, all students and groups should see temperature changes that relate to a response to the ice water stimulus. Variability in data can lead to rich classroom discussions that focus students on graph analysis and using evidence to support their claims.

For student-designed experiments, encourage students to use a "large" sample size of test subjects and to acquire data from multiple trials that can be averaged.

Tip 2 – Using additional sensors in student-designed experiments

Because the hypothalamus regulates not only body temperature but heart rate, blood pressure, and other physiological parameters, students may expand this lab activity to include the use of physiology sensors. Refer to the sensor manuals for additional information about each one.

The Initial Investigation is similar to a common physiological test called the *cold pressor test*. While the cold pressor test specifically measures blood pressure and heart rate, which are factors not measured in the Initial Investigation of this lab, measurement of these factors can be encouraged in the student-designed experiments. To learn more about the cold pressor test and to read recommendations for incorporating the test into a classroom activity, refer to the following article: http://advan.physiology.org/content/37/1/93/.[9]

[9] Silverthorn, D.U.; Michael, J. Cold stress and the cold pressor test. *Advances in Physiology Education.* 37(2013): 93–96. DOI: 10.1152/advan.00002.2013

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

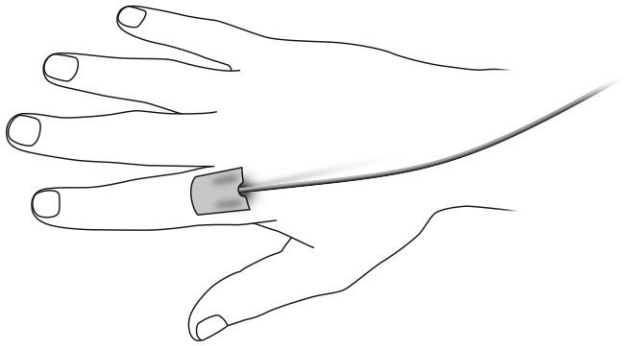
1. Put on your safety goggles.

2. Connect the Quad Temperature Sensor to your data collection system, and connect two fast-response temperature probes to ports 1 and 2 on the sensor.

3. Fill a shallow bowl or pan with water to a depth of approximately 3 cm. Add ice to the water and monitor the temperature of the ice-water bath. The temperature should be between 4 °C and 8 °C for data collection; add or remove ice as needed. Remove the temperature probe from the ice bath.

4. Determine which person in your group will be the *test subject*. Prepare the test subject for data collection:

   a. Using a small adhesive bandage or piece of medical tape, attach the temperature probe from port 1 to the pointer finger of the right hand, as pictured.

   b. Secure the 2nd temperature sensor to the pointer finger on the left hand.

   c. Have the test subject sit comfortably in a chair and relax with both hands resting on the surface of the table or lab bench.

5. Build graph displays for each temperature sensor.

6. Begin recording data. After thirty seconds, end data collection. Record the average skin temperature of each hand.

   For the sample data, the average temperature for the left hand was 30.5 °C and the average temperature for the right hand was 31.5 °C.

7. What is the core body temperature typically reported as in degrees Fahrenheit? What is this temperature in degrees Celsius?

   Core body temperature is generally 98.6 °F, which is 37 °C.

8. Is the skin temperature of the hand the same as the body's core temperature? If not, explain why not.

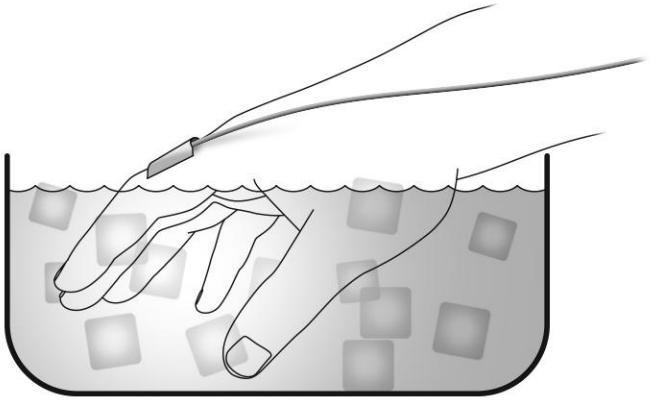
   The skin surface temperature of the hand is only 30–32 °C, quite a bit lower than the 37 °C that is the body's core temperature. As blood flows through the extremities, such as the arms, heat is lost to the environment so the surface temperature of the hand is less than the body's core temperature.

9. Follow the steps below to test how the body responds to the stimulus of a hand being placed in ice water.

   a. The test subject should sit relaxed with both hands resting on the table surface. Begin recording data.

   b. After approximately 10–20 seconds, instruct the test subject to place their right hand in the ice bath. The hand should be submerged up to the first knuckle of each digit, and the palm should be flat on the surface of the water. Leave the left hand relaxed on the table.

      NOTE: The temperature probe should NOT be submerged in the ice bath.



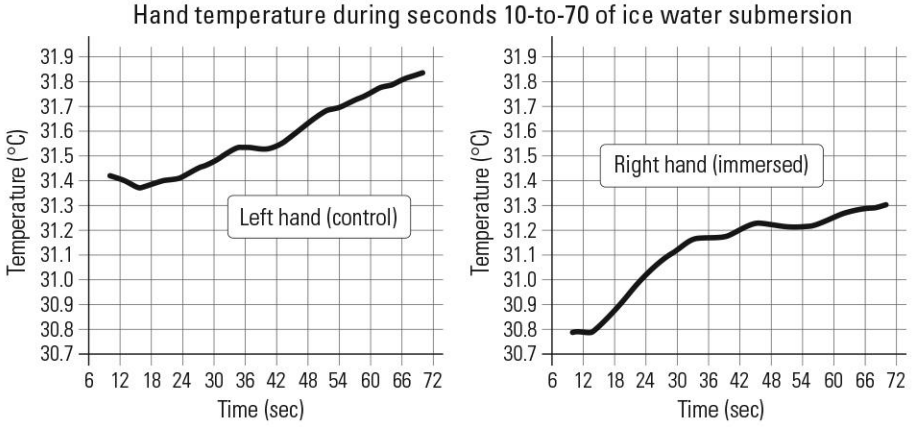
   c. Keep the right hand immersed in the ice bath for 60 seconds.

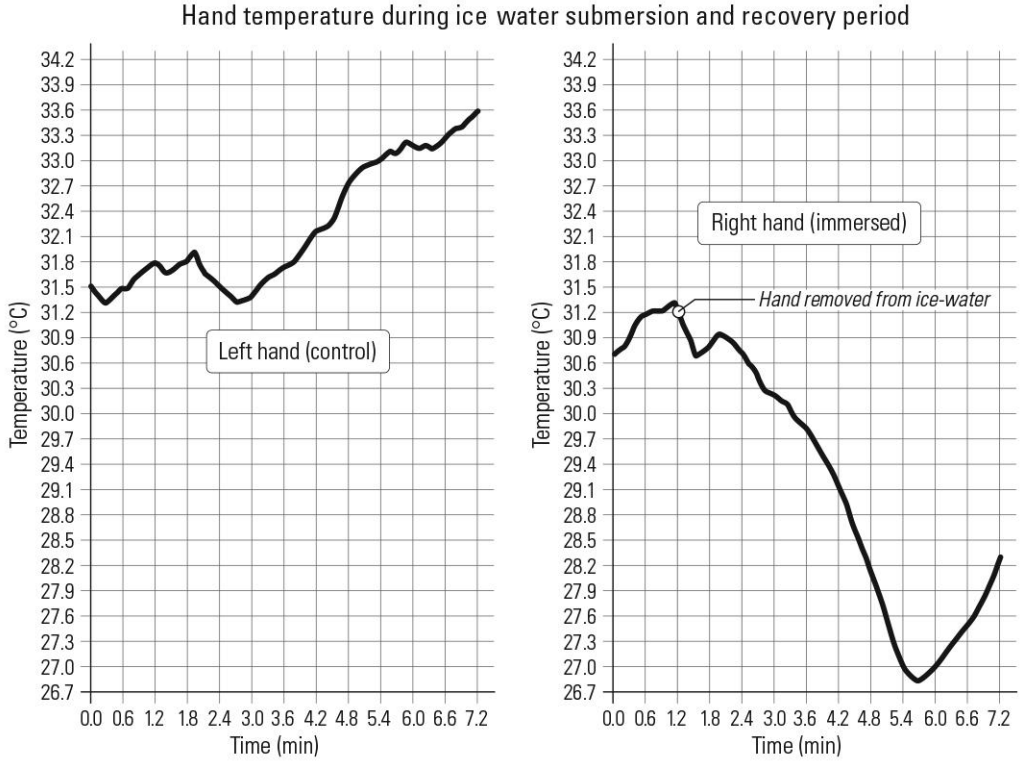
      NOTE: It is expected that the test subject will experience discomfort. However, if the cold becomes too painful, the subject may withdraw their hand and continue with the next step.

   d. After 60 seconds, remove the right hand from the ice water. Gently and quickly blot the hand dry, taking care not to disturb the temperature probe, and then place the hand on the surface of the table.

   e. Continue data collection for five or more minutes—the recovery period—after removing the hand from the ice water.

10. Draw or print a record of the temperature data. Analyze the data for temperature changes that occurred during the time of ice water immersion (from approximately 20 seconds to 80 seconds) and during the five or more minutes following immersion (the recovery period).





11. Did the temperature of the left hand (the control) change when the opposite hand was in ice water? If yes, describe the change that occurred. What purpose does the left hand serve in this experiment?

According to the sample data the left hand was initially 31.50 °C. During the time of ice water immersion, the temperature of the control hand increased slightly to 31.84 °C.

The left hand serves as a reference, or control, in this experiment. This hand does not receive the stimulus and temperature changes to the left hand can be compared to the temperature changes of the hand receiving stimulus to analyze how the body responds directly to the stimulus and how it responds in body parts away from the stimulus.

12. Did the temperature of the right (experimental) hand change when it was submerged in ice water? If yes, describe the change that occurred.

The temperature of the right hand changed less than 1 degree C while it was submerged. The right hand temperature was initially 30.67 °C prior to contact with the ice water. During the ice water immersion period, the temperature of the hand actually warmed slightly to 31.30 °C.

13. Describe any trends in the temperature data collected during the recovery period.

Right hand: After removing the hand from ice water there was a decrease in temperature of approximately 0.5 °C, followed by a small increase in temperature. At about 2 minutes, less than one minute into the recovery period, the temperature of the right hand began to decrease significantly. Over the next three minutes the temperature decreased from 30.94 °C to 26.84 °C. Approximately 4.5 minutes after removing the hand from ice water, the temperature of the hand began to increase, rising to 28.2 °C after the hand had been back in room temperature conditions for 6 minutes.

Left hand: The reference hand also changed in temperature during the recovery period. In the minute following removal of the right hand from ice water, the left hand temperature increased slightly then decreased slightly, before starting an upward trend starting at 2.7 minutes (a little more than a minute after removing the right hand from the stimulus). The temperature of the left hand increased to 33.66 °C, more than 2 °C warmer than its initial temperature.

14. In response to a hot or cold stimulus that threatens homeostasis, the body can alter blood flow by dilating or constricting certain blood vessels (*vasodilation* and *vasoconstriction*), notably blood vessels that supply blood to the skin. Is there any evidence from this investigation that the ice water immersion caused vasodilation or vasoconstriction in the right hand? Use evidence to support your claim.

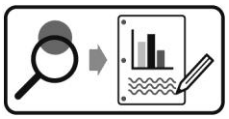
Heat in the body's extremities, such as the hands, comes from blood flowing from the body's core. When blood flow increases, the temperature in the extremities increases. Since the temperature of the immersed hand increased when it was in ice water, the cold stimulus (the initial “plunge”) likely caused vasodilation in the hand's blood vessels. However, the prolonged exposure to cold resulted in decreased blood flow to the area (vasoconstriction), decreasing the temperature of the affected hand significantly. So even when the stimulus was removed, the right hand's temperature continued to decrease due to limited blood flow through the hand.

By constricting blood vessels in the hand, more blood is shunted back to the core and the temperature of the blood is increased. Several minutes into the recovery period there was vasodilation in the hand, widening the vessels to allow warm blood to flow into the affected hand, increasing its temperature. The increase in temperature in the left hand (the control) may have been a result of vasodilation or it may be evidence of the increase in temperature of the blood resulting from vasoconstriction in the opposite hand.

Design and Conduct an Experiment

In addition to temperature, other parameters are carefully regulated within the body, such as blood pressure and heart rate. The hypothalamus plays an important role in maintaining homeostasis for all of these parameters.

For this extension, students can consider testing additional variables related to thermoregulation or they can plan and carry out an experiment to investigate homeostasis with regard to other physiological parameters.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Is a person's heart rate (or blood pressure) dependent on body position?*
- Do warm environments stimulate vasodilation?
- Does the temperature of a location away from the stimulus and near the core, such as the neck or armpit, change when a hand is immersed in ice water?
- Does vasoconstriction occur in all fingers simultaneously if only one or two fingers are placed in an ice water bath?* Does vasoconstriction occur in the feet?*
- How does an insulating layer affect the results—in other words, are the results different if the procedure of the Initial Investigation is repeated with a person wearing a non-latex glove on the immersed hand?
- Does a person's breathing rate change when their hand is placed in ice water?*
- Does the ice water stimulus affect a person's heart rate?*
- Does body temperature change during aerobic exercise?
- What is the effect of aerobic exercise on heart rate? How long is the recovery period before the heart rate returns to the at-rest value? Do the results change if the person lies down, sits, or stands while resting?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Is a person's heart rate dependent on body position?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected the dependent variable of your experiment. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

      The hypothesis that body position affects heart rate (HR) is supported by the data. The average HR in the supine position was 72 bpm (beats per minute) and the average HR in the standing position was 89 bpm. When the means are graphed with errors bars indicating a range of ±2 SEM (standard error of the mean), the HR range for the supine position does not overlap the range for the standing position. When this is the case, it is a strong indicator that the difference in data for these conditions is significant.

   b. Based on the evidence you collected, explain why the results occurred.

      When students lie down, more blood flows into the carotid arteries, therefore blood pressure increases and baroreceptors located in the carotid sinuses sense this increase in pressure. These baroreceptors send signals to the brain, which responds by increasing vasodilation in the arteries and veins, which lowers blood pressure. When students stand up, blood pressure drops suddenly and the baroreceptors send signals to the brain that result in increasing heart rate and vasoconstriction.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   There is no evidence in the data that experimental error or other uncontrolled variables affected the results. The data is reliable.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

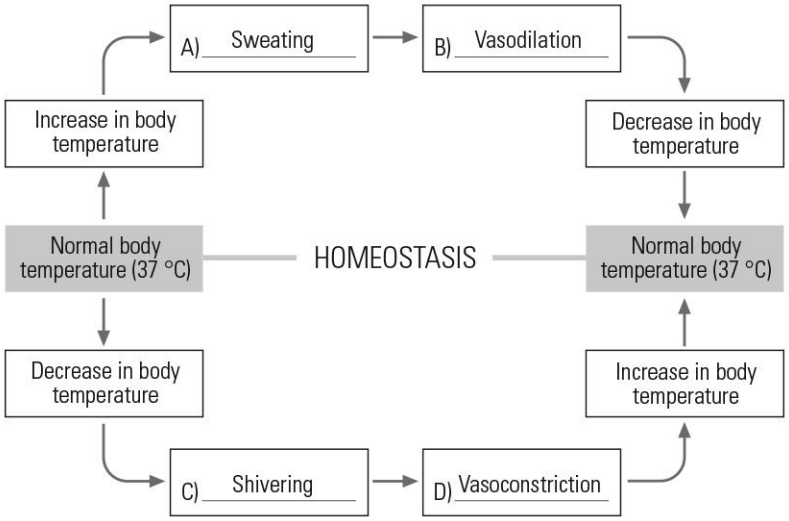
Synthesis Questions

1. If someone is exposed to cold weather for extended periods of time, where are they most likely to get frostbite? Use the results of the Initial Investigation to support your answer.

   A person is most likely to get frostbite on their extremities, specifically the nose, the tips of the fingers, and toes. During exposure to cold weather, the hypothalamus shunts blood back to the body core to conserve warmth. This allows the person to survive but may lead to frostbite because the fingers, toes, and nose do not receive oxygen and nutrients. This leads to necrosis of these tissues.

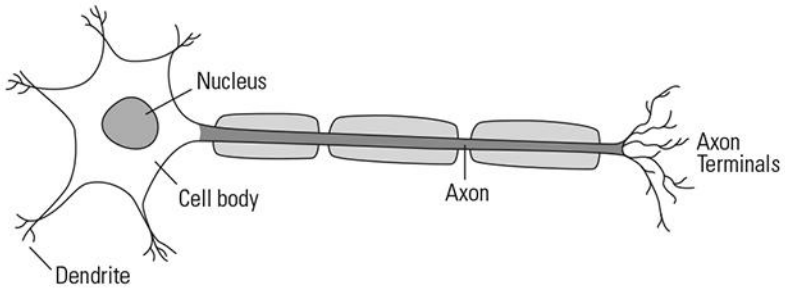
   The sharp decrease in temperature of the right hand following the exposure to ice water indicates blood flow to this hand was limited and the body was not able to replace the heat the hand was losing to the environment. If the exposure time was increased, tissue damage (or frostbite) to the right hand may have occurred if the lack of blood flow to the area was prolonged.

2. Below is a diagram of thermoregulation in the human body. The body detects a change from normal body temperature and responds to maintain homeostasis. Copy and complete the diagram by identifying A, B, C, and D. In other words, what responses can help bring body temperature back to normal?



3. The nervous system plays a critical role in maintaining homeostasis for an organism. The system detects external stimuli, transmits and integrates information about the stimuli, and produces one or more responses.

   a. Describe the basic structure of the neurons that compose the nervous system and explain how neurons detect stimuli and transmit information to various parts of the body.



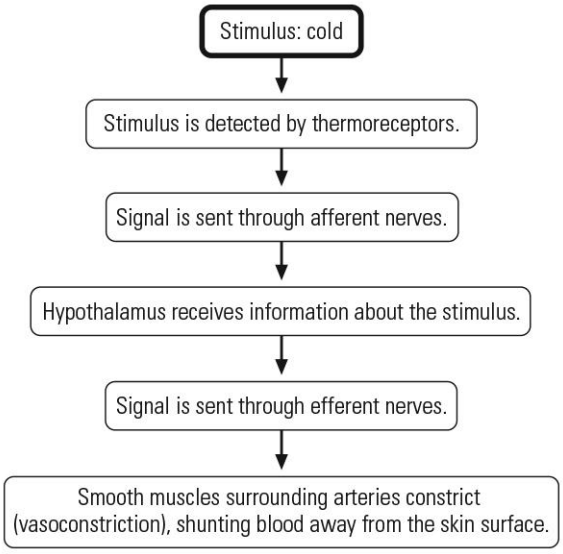
Neurons are cells with four main parts: dendrites, cell body, axon, and axon terminals. These cells are extremely long relative to other cells and they are very thin. This thinness creates a very large surface-area-to-volume ratio, making the diffusion of ions into and out of the cell rapid and giving the cell the ability to conduct an electrical impulse (action potential) across long distances.

Sensory neurons are associated with receptors, such as thermoreceptors in the skin. The dendrites of the neurons detect the stimulus and conduct impulses back to the brain where the information can be processed. The nerve impulses are initiated in the dendrites and propagate across the cell body and along a single axon. The impulse is propagated by changes in the polarity of the membrane.

At rest, a neuron's interior is negatively charged relative to the extracellular fluid. A neuron spends a lot of energy in its "at rest" stage to build up a gradient of positively charged sodium ions outside of the cell (the Na^+/K^+ pump requires ATP and is an example of active transport). A stimulus causes ion channels in the neuron's cell membrane to open, which in turn causes the sodium ions to rush into the cell and reverse the polarity.

Axons have numerous branches at their terminus (axon terminals). Each one is in close proximity to a neighboring neuron, or to a muscle or gland cell. From an axon terminal, the impulse is transmitted chemically by neurotransmitters that diffuse across a tiny gap called a synapse. If the signal is strong enough, the post-synaptic neuron will continue transmitting the impulse, or the muscle will contract, or the gland will respond. Neurons in the brain can activate motor neurons that transmit impulses from the central nervous system to the regions of the body that will carry out the response to the stimulus. For example, a motor neuron may cause smooth muscles around an artery to contract, causing vasoconstriction.

b. Draw a diagram to illustrate the connection between the following structures during the body's response to a cold stimulus: hypothalamus, efferent and afferent nerves, smooth muscles that surround arteries, and thermoreceptors.



c. Vasoconstriction occurs when the smooth muscles surrounding arteries contract. How do nerves cause muscle contraction?

Axon terminals release chemical messengers called *neurotransmitters*. The neurotransmitter acetylcholine binds to receptors on muscle cells and initiates changes, such as an increase in calcium ion concentration, that lead to muscle contraction.

4. Vertebrates have evolved a variety of strategies to deal with thermoregulation: the ability to maintain homeostasis with regard to body temperature.

   a. Ectothermy and endothermy are two different approaches to thermoregulation. Define each approach and describe the benefits and costs associated with each one.

   Different approaches to thermoregulation

Thermoregulatory Strategy	Benefits	Costs
Endothermy: Body temperature is controlled independently by the animal	• Virtually unlimited geographic distribution • Decreased restriction on activity level • Physiological processes can continue despite changes in external temperature	Increased metabolic costs, therefore: • Decreased size for many endotherms (for example, birds and small mammals) • Decreased reproductive output
Ectothermy: Body temperature is controlled by the temperature of the environment	• Less energy spent on internal thermoregulation • Increased reproductive output • Surviving individuals can continually increase in size throughout life (for example, fishes, turtles, and crustaceans)	• Limited geographic distribution • Limited activity levels when external temperatures are hot or cold, often associated with decreased aerobic capacity

   b. Mammals are endotherms and have evolved a wide variety of adaptations to deal with the different challenges to thermoregulation in the world's biomes. Identify three biomes with distinctly different climates. For each biome, name a mammal that lives there and list at least two adaptations each mammal has that relate to thermoregulation.

   Adaptations to different biomes

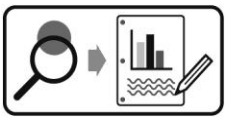
Biome	Mammal	Thermoregulatory Adaptation
Alpine	Marmot	Thick fur, hibernation
Desert	Black-tailed jackrabbit	Long limbs and large ears, mostly nocturnal
Temperate grassland	Bison	Thick fur surrounding head, neck, and vital organs, but no fur or less hair on pelvic girdle and posterior limbs. They shed fur during summer and re-grow a thick coat during winter. Large body size

   c. Smaller mammals have higher basal metabolic rates (BMR) than larger mammals. Explain the relationship between body size, BMR, and thermoregulation.

   Smaller mammals have a much higher surface area relative to their volume and therefore lose heat to their environment at a greater rate than larger mammals. Also, smaller mammals have less body mass and therefore generate less heat through metabolism compared to larger mammals, so for a small mammal to maintain a warm body temperature, the small mammal must have a high metabolic rate to generate the heat needed for thermoregulation.

Design and Conduct an Experiment Key

In addition to temperature, other parameters are carefully regulated within the body, such as blood pressure and heart rate. The hypothalamus plays an important role in maintaining homeostasis for all of these parameters. Consider additional variables to test related to thermoregulation, or plan and carry out an experiment to investigate homeostasis with regards to other physiological parameters.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of homeostasis, what environmental factors (abiotic or biotic) could affect homeostasis in the human body?

   Factors that could affect homeostasis include: ambient temperature or humidity of the environment, food and liquid intake (changes in hydration or blood sugar level), body position, stress, and exercise.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   Is a person's heart rate dependent on body position?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   Heart rate (HR) is the dependent variable in a number of studies. If body position affects HR, then investigators should be sure to take HR measurements the same way for each participant and each condition when they design experiments with HR as a dependent variable.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable of the experiment is body position. Average resting heart rate will be determined for each person in a supine (lying face upward) position and in a standing position.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable of the experiment is heart rate (beats per minute, bpm). A hand-grip heart rate sensor will be used to collect data for 30 seconds while the person is lying down and then while standing up. The data collection system's analysis tools will be used to determine the average heart rate for each body position for each participant.

6. Write a testable hypothesis (If…then…).

   If body position affects heart rate, then a person's resting heart rate measured in the supine position will differ significantly from their resting heart rate measured when they are standing.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   All participants will be teens, aged 16–18 years old. Participants will be healthy with no known physiological problems. The same heart rate sensor will be used for all trials. Participants will first relax in the supine position for 2 minutes before heart rate is measured. After the heart rate is measured for the supine position, participants will stand up and their heart rate will be measured five seconds after standing.

8. How many trials will be run for each experimental group? Justify your choice.

   The experimental group will consist of 10 teens. Two trials will be performed for each participant. The number of participants is a large enough sample size to account for variation in a population, and two trials will illuminate if there is variation in data for any one individual when conditions are held constant.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The two trials for each person will be averaged. For example, the two supine resting HR measurements for participant 1 will be averaged. Then the 10 supine resting HR averages will be used to acquire the value of the average supine HR for the "population." Similarly, the average standing HR will be acquired by averaging the standing HR average of each of the 10 participants. The results will be graphed with error bars indicating ±2 SEM to determine if any difference in HR is significant.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    Both genders will be represented in the experimental group; there may be differences in the average resting heart rates of males and females.

    Some participants may have a difficult time relaxing and the stress of being a test subject may affect their HR measurements.

    The physical effort of standing up may affect each participant's heart rate differently, even though measurements won't begin until five seconds after they stand.

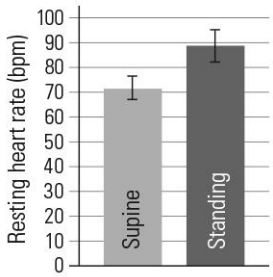
11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

    Enlist 10 other students in class to participate in the investigation. For each participant, collect data in the following way:

    1. Have the participant lie on a table surface or lab bench and relax. Have the participant hold the hand-grip heart rate handles and wait for the system to show that heart rate is being acquired and beats per minute are being detected by the sensor.
    2. After 2 minutes of relaxation, begin recording data. After 30 seconds, end data collection.
    3. Have the person stand up and after standing 5 seconds, collect heart rate data for 30 seconds.
    4. Repeat the data collection for the supine and standing positions for the same person a second time.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data



The results shown above are from an inquiry that compares the resting heart rate of subjects, 4 males and 7 females between the ages of 16 and 18, in different body positions. Subjects lay in the supine position for 120 seconds prior to obtaining resting heart rate, recorded for 30 seconds. Then the subject stood up, waited 5 seconds, and the heart rate was recorded for 30 seconds. Error bars indicate ±2 SEM (standard error of the mean).

Determining if the temperature of all fingers is affected when only two are in ice water

Finger	Average Temperature Prior to Incubation (°C)	Temperature Change during Incubation (°C)	Temperature Change following Incubation (°C)
Forefinger[1]	24.0	–2.3	1.0
Middle finger	26.1	–0.1	0.6
Ring finger	26.1	0.0	0.1
Little finger	25.6	–0.1	0.2

[1]Of the two fingers immersed in the ice water bath (the thumb and forefinger), only the temperature of the forefinger was measured.

The results shown above are from an inquiry investigating the temperature change of four fingers of one hand when only the forefinger and thumb were immersed in ice water.

Temperature response of feet when one is submerged in ice water

Interval	Control Foot			Experimental Foot		
	Initial Temperature (°C)	Final Temperature (°C)	Temperature Change (°C)	Initial Temperature (°C)	Final Temperature (°C)	Temperature Change (°C)
Pre-incubation (0–60 seconds)	26.75	27.09	0.34	27.25	27.56	0.31
Submersion in ice (61–120 seconds)	27.08	26.68	–0.4	27.54	25.58	–1.92
Recovery (121–240 seconds)	26.59	27.04	0.45	26.51	26.71	0.2

The results shown above are from an inquiry similar to the Initial Investigation but recording the response times for feet, instead of hands, subjected to partial immersion in ice water.

Heart rate and lung volume response to a hand immersed in ice water

Conditions	Average Heart Rate (bpm)	Lung Volume (L)
Before immersion	64.5	0.53
During immersion	69.0	0.46

The results shown above are from an inquiry measuring the heart rate, using an EKG sensor, and lung volume, using a spirometer sensor, before immersing a hand in ice water and then while the hand was immersed in ice water.

7. CELLULAR RESPIRATION

Lab Overview

Students investigate the rate of cellular respiration of germinating seeds using a carbon dioxide gas sensor to measure one of the products of this process.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{Energy (36 ATP)}$$

Students design and conduct an experiment to investigate a factor that can affect the cellular respiration of germinating seeds or to measure respiration in other organisms, such as crickets. Investigations that vary the species, germination time, pH, temperature, and salinity work well.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	20 min
Initial Investigation	20 min

Student-Designed Experiment	
Experiment Design	20 min
Experiment	60 min
Data Analysis	20 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	1.B.1, 2.A.1, 2.A.2, 2.B.3, 4.A.2, 4.A.6
Science Practices	1.4, 2.2, 3.1, 6.1, 7.2
Learning Objectives	1.14, 1.15, 2.1, 2.2, 2.4, 2.13, 4.5, 4.14

Materials and Equipment

For Each Student Station

- Data collection system
- Carbon dioxide gas sensor
- Sensor extension cable[1]
- Sample bottle, 250 mL[1]
- Balance, readability: 0.01 g
- Paper towel
- Germinating pinto beans (50)[2]

[1]Included with the carbon dioxide sensor

[2]Refer to the instructions in the Lab Preparation section for material preparation.

Additional equipment recommended for the student-designed experiment:

- Fast-response temperature sensor
- Oxygen gas sensor
- Solutions of different pH or salinity levels
- Additional germinating pinto beans
- Germinating and dormant seeds of other species, or small animals

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- Mitochondria structure and the role of mitochondria in aerobic (cellular) respiration
- The overall process (reactants and products) of cellular respiration and an introductory understanding of the aerobic respiration pathways (glycolysis, Krebs cycle, and oxidative phosphorylation)
- The diffusion of oxygen and carbon dioxide across cell membranes
- The role of enzymes in biochemical pathways, and variables that can affect enzyme-catalyzed reactions

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Handle living organisms with care.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Germinating seeds

   The Initial Investigation uses pinto beans (*Phaseolus vulgaris*), available at most supermarkets and through science suppliers. Prior to using them in the lab, germinate the seeds for 24 hours by placing them in a tray on a wet paper towel. The percentage of viable seeds can vary widely from source to source. If the viability is not stated, germinate 50–100 seeds and make sure at least 70% are viable to improve students' results.

2. Recommended small animals

   For the student-designed experiment, students may choose to study respiration in animals instead of seeds. Crickets, mealworms, earthworms, cockroaches, and other animals that fit easily into the sample bottle can be used and are available at many pet stores. Rough handling and carbon dioxide levels above 50,000 ppm are stressful to most animals and should be prohibited.

Teacher Tips

Tip 1 – Using the carbon dioxide gas sensor

Review the sensor manual before using the sensor and be sure to calibrate it before starting the lab.

Be sure students empty the sample bottle between runs. If they do not, the organisms will continue to produce carbon dioxide in the bottle, changing the initial value for each run.

The sensor can be affected by changes in temperature and ambient infrared radiation from incandescent lighting. For best results, use the sensor in a stable environment.

Tip 2 – Using the oxygen gas sensor

NOTE: The carbon dioxide sensor typically provides better data from small-scale studies with seeds. The oxygen sensor can be used in these studies, but PASCO trials showed that longer time periods are needed (15 minutes or more) for significant data. The oxygen sensor is more appropriate for investigations that involve organisms with higher metabolic rates and can be used in student-designed experiments.

Review the sensor manual before using the sensor and be sure to calibrate it before starting the lab. The sensor reports oxygen concentration in parts per million (ppm) or in percent (a 1% concentration equals 10,000 ppm). It may be beneficial to review parts per million with students before starting the lab.

Although the sensor reports oxygen concentration to a high resolution, its accuracy is ±1%, or 10,000 ppm. If students are measuring very small changes in oxygen concentration over a short period of time, they may see no change, or even a trend in the wrong direction. Often the change is insignificant despite the appearance of a trend on the data collection system. Students can try switching to a carbon dioxide gas sensor, measuring for a longer period of time, or increasing the size or number of organisms they are sampling to create a more observable change.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

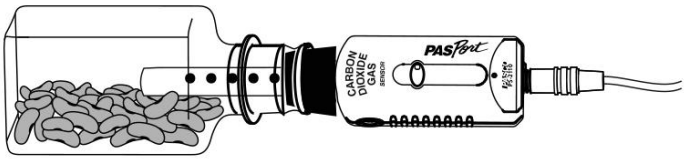
From the student handout:

1. Put on your safety goggles.

2. Connect and calibrate the carbon dioxide gas sensor.

3. Create a display of the sensor measurement in ppm versus time in minutes, and adjust the sample rate to one sample every 15 seconds.

4. Select 50 germinating seeds. Dry the seeds with a paper towel and record their mass.



Setup with CO_2 gas sensor

5. Place the seeds in the sample bottle with the sensor and lay it horizontally on your table as shown.

6. Wait for 1 minute and then start data collection; record data for 5 minutes.

7. The cells within germinating seeds carry out cellular respiration to acquire adenosine triphosphate (ATP).

   a. Identify the organelle in which cellular respiration occurs in eukaryotic cells and describe the structure of this organelle.

   Cellular respiration occurs within mitochondria. These organelles are bean-shaped and have two membranes. The inner membrane is repeatedly folded (cristae). The innermost compartment of the organelle is called the *matrix*.

   b. Summarize how ATP is produced within this organelle and describe the importance of ATP for the germinating seeds.

   ATP is produced within mitochondria primarily through oxidative phosphorylation. The Krebs cycle produces a small amount of ATP, but the majority is produced through the activity of the electron transport chains embedded in the inner membrane of the mitochondria. The energy of electrons from NADH and $FADH_2$ generated in earlier respiration pathways is used to build a gradient of hydrogen ions (H^+) in the intermembrane space. The diffusion of these hydrogen ions back into the matrix, through channels formed by ATP synthase, provides energy to generate ATP from ADP and inorganic phosphate. Oxygen is the final electron acceptor of the chain and keeps the process going.

   Germinating seeds are undergoing rapid growth and development as they change into seedlings. ATP provides the energy for cell activities, including cell division and DNA replication.

   c. Explain why carbon dioxide is produced during the process of cell respiration.

   Carbon dioxide is produced by the breakdown of carbohydrates, lipids, and proteins for the production of ATP. It is a byproduct of the reaction.

8. After 5 minutes, compare your data for germinating seeds to the data for dormant (dry, non-germinating) seeds in Table 1.

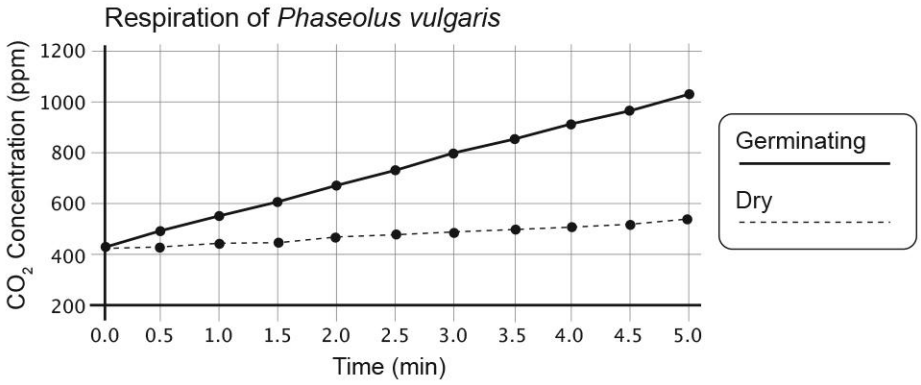
NOTE: To make the comparison, you will first need to normalize *the data by finding the respiration rate per gram.*

Table 1: Dormant seed respiration data

Condition	Seed Quantity	Seed Mass (g)	Seed Respiration Rate (ppm CO_2/min)	Normalized Respiration Rate [(ppm CO_2/min)/g]
Dormant	50	19.01	22.8	1.20
Germinating (24 hr)	50	28.44	118	4.15

* Dormant seed respiration data was collected using *Phaseolus vulgaris* in a 250-ml sample bottle over 6 hours. The rate was determined from a linear regression of the data. Given the difficulty of measuring the low rate of respiration in dormant seeds, a research grade respirometer was used.

Students' results may differ slightly but the germinating seeds should respire at a significantly higher rate than non-germinating seeds. In testing, germinating seeds respired at two to fifty times the rate of non-germinating seeds.



Sample data from the initial investigation of dormant seeds and germinating seeds after 24 hours

9. How do you explain the difference in the rate of respiration between germinating seeds and dormant seeds?

Dormant seeds are not growing and need very little ATP to sustain the low level of activity within the cells, so they have a very low respiration rate. The growth and development taking place in germinating seeds requires a large amount of ATP. Therefore, germinating seeds carry out cellular respiration at a much higher rate and produce more carbon dioxide per minute per gram than dormant seeds.

10. What other ways could the data be normalized to enable us to make some comparisons across trial groups? What are the limitations and assumptions of each approach?

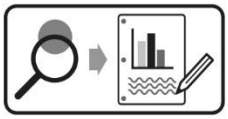
The data could be normalized using the number of seeds or the volume instead of mass. However, if the seeds being compared have different shapes, volume may not be appropriate. For example, a lima bean has a lower SA:V ratio than a kidney bean, facilitating greater gas exchange. Using the number of seeds has a similar limitation, as the seed size can vary dramatically, even within a species.

11. If a similar experimental protocol was repeated after the seed had sprouted and matured into a seedling with several leaves, how would you expect the results to change?

Once the plant has leaves, it would start to conduct photosynthesis in the light. This would decrease observable respiration since CO_2 would be consumed in the photosynthetic processes even as it was being produced through cell respiration.

Design and Conduct an Experiment

Based on the data, analysis, and discussion of the Initial Investigation, students should be able to identify factors that might change the rate of respiration in seeds or develop a related question using another model organism.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Work follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Does the respiration rate change as germination proceeds?*
- Does the temperature of germinating seeds affect the rate of respiration?*
- Do different varieties or species of seeds respire at different rates?*
- Do different animals respire at different rates when at rest?
- Do monocot and dicot seeds respire at different rates?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Does the respiration rate change as germination proceeds?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

    a. Describe how the independent variable you manipulated affected the rate of respiration. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

    The independent variable in this investigation was germination time. Thirty pinto beans germinated for the following intervals: 0, 3, 6, 12, 24, and 48 hours. The data indicates a direct relationship between the germination time and respiration rate. At each data collection interval, the rate increased between 30% and 70%, with increases becoming less dramatic as germination progressed. The data supports the hypothesis that as germination progresses, the plant will consume more energy—cells become metabolically active and additional cells are added through cell division during growth.

b. Explain why the results occurred.

To initiate germination, the seeds were placed on a moist paper towel. As the seeds undergo imbibition, the enzymes hydrate and become active in the cells and the rate of respiration increases. Once the process is started, the cells will use energy stored in the endosperm to replicate. As the number of cells increases and the roots and stem begin to grow, the rate of respiration increases further.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

There is no indication from the data that uncontrolled variables affected the results. The data is reliable enough to use and supports my hypothesis.

3. Identify any new questions that have arisen as a result of your research.

Answers will vary based on the student's knowledge, experience, and results.

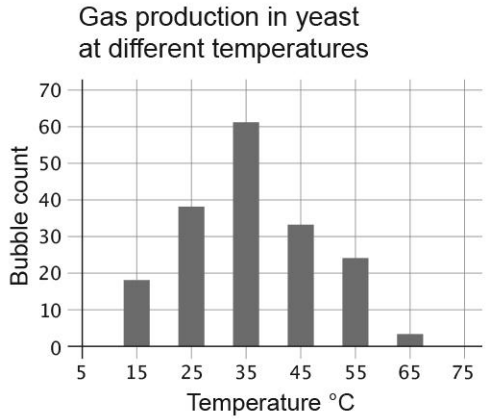
Synthesis Questions

1. A yeast culture is placed into a flask attached to an apparatus that detects bubbles released by the solution. Twenty grams of glucose are added to the culture and the temperature is incrementally increased and monitored by a sensor. The results are shown below.

Table 2: Counting bubbles to measure the effect of temperature on yeast respiration

Temperature °C	5	15	25	35	45	55	65	75
Five Minute Bubble Count	0	18	38	61	33	24	3	0

a. Draw a graph showing the effect of temperature change on the rate of respiration in yeast cells.



b. Using your knowledge of enzymes and the data provided, explain the results of the experiment.

Cellular respiration is a series of enzyme-catalyzed reactions that transfer energy from sugars like glucose to adenosine triphosphate (ATP), a compound that makes the stored chemical energy accessible to cells. Enzymes are complex proteins that are folded chains of amino acids. The folded structure (the tertiary or quaternary level of protein structure) is determined by the amino acid sequence and interactions between the acids.

These interactions are controlled by hydrogen bonding, R-group interactions, and hydrophobic and hydrophilic regions. Changes in temperature (and pH) can alter the bonding and change the shape of the enzyme, a process known as *denaturing*.

In the data provided, activity steadily increased as the temperature increased to 35 °C; the increased kinetic energy of the molecules leads to more frequent enzyme–substrate collisions. As the temperature increased to 45 °C, enzyme activity declined. Above that temperature, enzyme structure was likely altered, rendering the metabolic enzymes unable to catalyze the reactions of respiration.

2. The breakdown of sugars to carbon dioxide and water during respiration releases energy. Much of this energy is captured by the cells to generate ATP through oxidative phosphorylation, but some energy is lost as heat.

a. Describe a procedure to use temperature to measure metabolism in a human.

Student answers will vary, but should include a way to measure the temperature of a closed system. Their design may include an insulated chamber or a water bath with a person immersed. If the starting temperature of the system is known and heat cannot escape, any increase in temperature can be attributed to metabolic processes.

b. Explain how the laws of thermodynamics apply to cellular respiration in this example.

The laws of thermodynamics state that energy is conserved but that entropy can never decrease in a closed system. In the case of cellular respiration, as energy is transferred from glucose to ATP, some energy is lost as heat. Because heat energy is less useful, the entropy of the system has increased during the transformation.

3. An experiment was carried out to compare the effect of temperature on respiration rate in crickets and in mice. The experiment showed that at cold temperatures the respiration rate in crickets decreased. However, in mice the respiration rate increased at colder temperatures. Do you think the results of the experiment are valid? Explain your position.

The results of the experiment are valid. Crickets have no internal temperature control mechanism, while mice can regulate their body temperature. Organisms that can regulate their internal temperature (endotherms) tend to increase metabolic activity at colder temperatures; this increased metabolism generates heat which helps counteract the effect of a cold ambient temperature. Ectotherms, such as crickets, have decreased metabolism at colder temperatures. The cold slows down enzyme activity, and thus respiration.

4. Free energy *G* is an important aspect of understanding how organisms obtain, use, and transform energy to maintain their complex levels of organization and grow and develop. Table 1 shows the change in free energy that accompanies two processes that take place in cells.

Table 1: Free energy changes

Reaction	ΔG
ADP + P_i → ATP	7.3 kcal/mol
Glucose → 2 Pyruvic acid	–32.1 kcal/mol

a. Which reaction is more energetically favorable? How do you know?

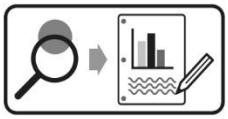
The conversion of glucose to pyruvic acid is more favorable because its ΔG value is negative, meaning the reaction occurs spontaneously.

b. In cells, a number of reactions are "coupled." What purpose does coupling reactions serve?

Cells carry out many reactions that require an input of energy (endergonic, anabolic reactions, having a positive ΔG). If one of these reactions is coupled with a reaction that has a sufficiently negative ΔG, the reaction with a positive ΔG acquires the energy needed for the reaction. For example, energy released in the conversion of glucose to pyruvic acid can be used to provide energy needed to make ATP from ADP and inorganic phosphate.

Design and Conduct an Experiment Key

Cellular respiration is critical to utilizing stored energy for cells and organisms. It is a process that can be affected by a number of factors. Identify factors that might change the rate of respiration in seeds or develop a related question using another model organism.



Develop and conduct your experiment using the following guide.

1. For the reaction shown below, which reactants or products can you measure with the available equipment? Explain which sensor, procedure, or equipment can be used and what variable would be measured.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{Energy}\,(36\ \text{ATP})$$

   If an oxygen gas sensor is available, it can be used to measure the consumption of oxygen and the carbon dioxide gas sensor can measure carbon dioxide production. Both sensors can measure the rate of the reaction. The sugar, water, or energy cannot be measured directly using this equipment.

2. Based on your knowledge of cellular respiration and biological systems, what environmental factors (abiotic or biotic) could affect this process?

   Because bioenergetic reactions are governed by enzymes, the rate of reaction would depend on environmental factors such as pH and temperature. The reaction rate will also depend on the availability of oxygen and the biological demand, since many organisms and cells can regulate gas exchange.

3. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   How does the respiration rate change as germination proceeds?

4. What is the justification for your question, that is, why is it biologically significant, relevant, or interesting?

   Seeds need to consume their internal energy stores to provide energy for growth until the plant sprouts and is able to conduct photosynthesis. The rate at which the seeds consume energy is important because if the stores are consumed before the leaves develop, it will die.

5. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable is the length of time since germination began (at the point when seeds came in contact with water).

6. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   Carbon dioxide production is the dependent variable. A CO_2 gas sensor will be used to measure the carbon dioxide production for 5 minutes at set intervals (12, 24, and 48 hour) after germination begins. This data can be used to calculate the normalized respiration rate, which can be compared to determine if the rate changes as germination proceeds.

7. Write a testable hypothesis (If…then…).

   Once germination is started, the cells will use energy stored in the endosperm to replicate. As the number of cells increases and the roots and stem begin to grow, the rate of respiration will further increase.

8. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   The sample bottle size must remain constant, since the sensor measures concentration, which depends on volume. The CO_2 gas sensor must be properly calibrated before each trial and the temperature of the seeds should also be kept constant as they germinate.

9. How many trials will be run for each experimental group? Justify your choice.

   Two trials for 2 groups of 20 beans each will be run at each interval. This should minimize the effect of variations within the seed population that could skew the results of the experiment. (More trials for each group of beans or using more groups of beans would minimize the effect of variations even more, but isn't practical in the classroom.)

10. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

    A normalized rate [(ppm/min)/g] will be calculated for each interval: The results will be averaged for the two trials of each group of beans and then averaged for the two groups. Then the normalized respiration rate at each interval will be compared to determine if the rate changes as germination proceeds, with error calculated based on the accuracy of the sensor.

11. Describe at least 3 potential sources of error that could prevent you from gathering accurate and reliable data.

    The carbon dioxide gas sensor produces noisy data if moved during data collection; our setup will remain flat on the table and undisturbed during each run. Temperature can affect metabolic reaction rates so germinating seeds will be placed in an insulated container during germination to keep them at a constant temperature.

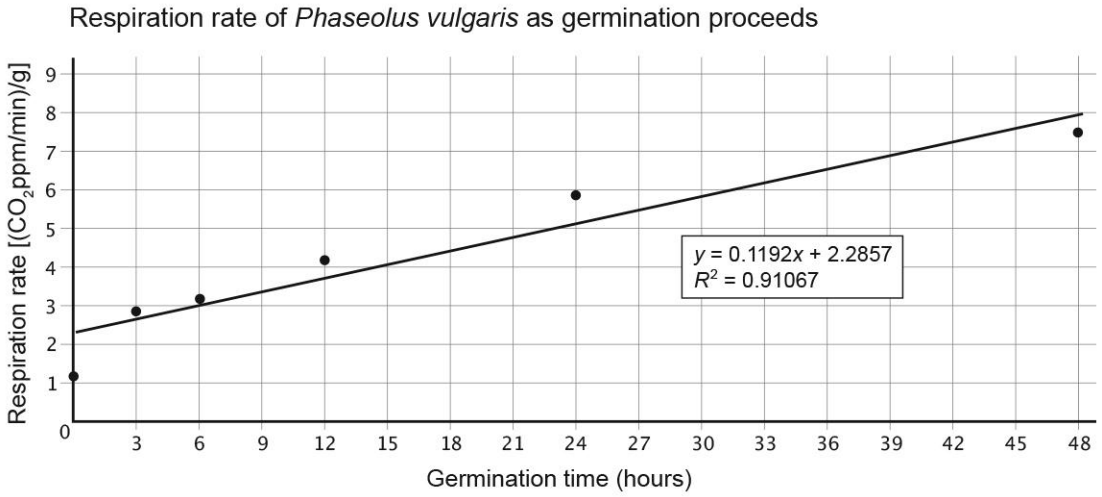
    Finally, even within a given seed sample there is variation in seed size which could affect the rate of gas exchange and therefore respiration. Using multiple seeds in each trial group and normalizing the data by finding the respiration rate per gram should mitigate the effect of this error.

12. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

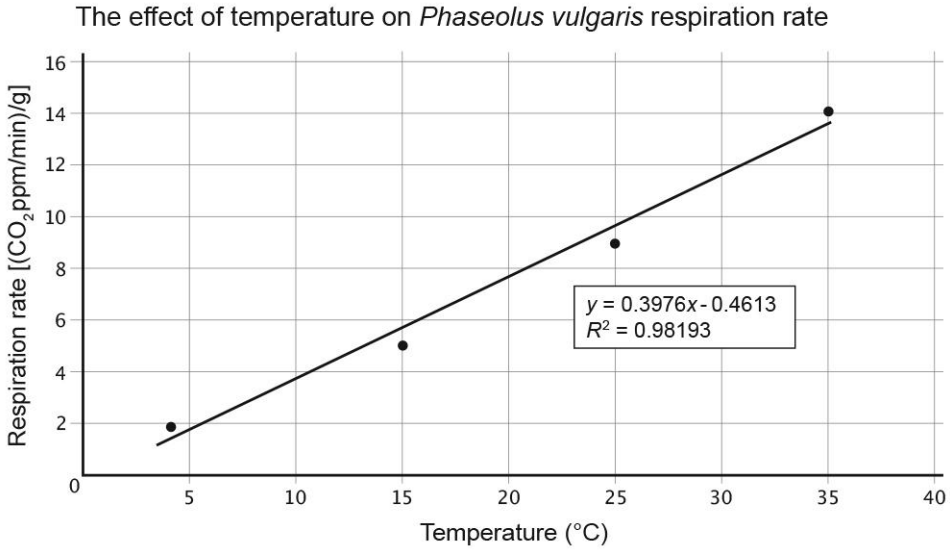
    1. Divide 60 pinto bean seeds into three trial groups of 20 seeds each and obtain the mass of each group.
    2. Place each group into a sample bottle and collect respiration data for five minutes using the CO_2 sensor.
    3. Return the seeds to a tray or large beaker lined with a moist paper towel.
    4. Allow the seeds to germinate for 3 hours and then repeat steps 1 through 3.
    5. Repeat the procedure at 6, 12, 24, and 48 hour intervals from the time the seeds started germination.
    6. Find the average rate [(ppm/min)/g] for each trial group at each time interval and determine if the results support the hypothesis.

13. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

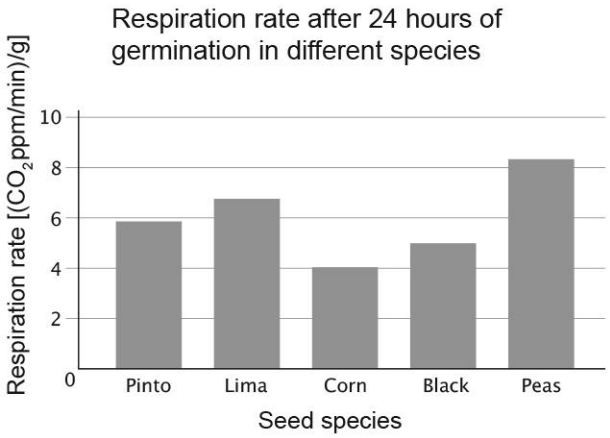
Sample Data



The results shown above are from an inquiry measuring the respiration rate of pinto beans at 3, 6, 12, 24, and 48 hrs. Data was averaged for each experimental group (which consisted of five groups of 20 beans each). The final data was graphed on a scatter plot and a linear fit applied. The temperature was maintained between 24–26 °C throughout the duration of the experiment.



The results shown above are from an inquiry using pinto beans at different temperatures. Beans were soaked in a water bath for 15 minutes at one of the following temperatures: 4 °C, 15 °C, 25 °C, and 35 °C. The raw data was used to calculate the respiration rate in ppm/min and then normalized by dividing the rate by the mass of the seeds used. The final data was graphed on a scatter plot and a linear fit was applied.



This inquiry experiment compared the respiration rate of different species after germinating for a 24 hours. Twenty seeds were used in each group and 5 runs of data were collected and then averaged for each set of seeds.

8. FERMENTATION

Lab Overview

Students determine the ability of yeast to use different types of carbohydrates—sucrose and starch—for fermentation. Yeast are facultative aerobes, carrying out both aerobic respiration and fermentation, depending on whether oxygen is readily available. When yeast ferment sugar, they produce ethanol and carbon dioxide and obtain ATP from glycolysis.

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

Since carbon dioxide is a product of both aerobic respiration and fermentation, this lab makes use of an ethanol sensor to measure a product formed only during fermentation. (Both processes can occur simultaneously, so measuring carbon dioxide concentration is not a direct measurement of the rate of fermentation.)

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	20 min
Initial Investigation	30 min

Student-Designed Experiment	
Experiment Design	15 min
Experiment	45–60 min
Data Analysis	15 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.A.1, 2.A.2, 2.D.1, 4.A.2
Science Practices	2.2, 4.2–4.3, 5.1, 5.3, 6.1–6.2, 7.2
Learning Objectives	2.1, 2.5, 2.8

Materials and Equipment

For Each Student Station

- Data collection system
- Ethanol sensor
- Graduated cylinders (2), 50-mL
- Sampling bottle[1] or glass flask (125-mL or 250-mL)
- Plastic pipet
- Small beaker
- Magnetic stir plate and stir bar
- Rod stand and 3-finger clamp *(optional)*
- 1% Ethanol (for calibration)[2]
- Yeast suspension, 40–60 mL[3]
- 2% Sucrose solution, 30 mL[2]
- 2% Starch solution, approximately 150 mL[2]
- Iodine indicator (IKI), 5–10 drops
- Water from germinating seeds, 5 mL[2]
- 2% Starch solution mixed with amylase, 30 mL[2] *(optional)*

[1]Included with PASCO gas sensors (oxygen and carbon dioxide), or available for purchase

[2]To formulate or prepare the sample, refer to the Lab Preparation section.

[3]To prepare the yeast suspension using active dry yeast and warm water, refer to the Lab Preparation section.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Additional equipment recommended for the student-designed experiment:

- Oxygen gas sensor
- pH sensor
- EcoChamber™ Container
- Additional yeast suspension
- Different types of yeast
- Additional energy sources: glucose, fructose, lactose, artificial sweeteners

Prerequisites

Students should be familiar with the following concepts:

- Fermentation pathways, the purpose of anaerobic respiration, and the conditions under which fermentation takes place
- Of the two main types of fermentation, lactic acid fermentation and ethyl alcohol fermentation, yeast carry out ethyl alcohol fermentation.
- Carbohydrates are a main energy source for cells. There are different types of carbohydrates, such as monosaccharides, disaccharides, and polysaccharides. Sucrose is a disaccharide and starch is a polysaccharide.
- To metabolize a particular molecule, a cell must contain enzymes specific to that molecule. For example, the cell needs sucrase to digest sucrose into glucose and fructose.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Do not get the end of the ethanol sensor wet; the sensor is a gas sensor and should never be submerged in a liquid.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Prepare the 1% ethanol solution needed to calibrate the ethanol sensors.

   a. Add 1 mL of anhydrous ethanol (ethyl alcohol) to 99 mL of distilled water and mix.

   b. Divide the volume of solution between four 25-mL beakers. (The calibration process is quick and student groups can share the solutions.)

      NOTE: If the solutions will not be used immediately, cover the beakers to prevent evaporation.

2. Prepare the 2% sucrose and starch solutions.

   To prepare 500 mL of each solution, a volume that should be sufficient for 1 or 2 class periods, dissolve 10 g of the solid into 490 mL of water. The sucrose solution should be clear; the starch solution will remain cloudy and will need to be stirred before each sample is taken.

3. Prepare the yeast suspension for students at least 30 minutes before students need to use it.

   Add 2 packets (14 g) of dry, active yeast to 500 mL of warm water (40–45 °C). Let the yeast settle in the water and then stir to mix well.

4. Prepare the water from germinating seeds.

   a. Obtain beans (pinto, lima, or black beans) from a grocery store.

   b. Spread the beans on the bottom of a shallow dish or pan. Cover the beans with water and leave them in a dark environment overnight.

5. (*Optional*) Prepare a mixture of starch and amylase if you anticipate that time will be available at the end of the Initial Investigation to carry out the fermentation of amylase-treated starch.

   a. Prepare 500 mL of a 2% starch solution. (Refer to the preparation of the starch solution above.)

   b. Add 25 mL of water from around the germinating seeds to the starch solution and mix it well. (Be sure the seeds show visible signs of germination before taking a sample of the water.)

      NOTE: Unused "seed-water" can be poured into a container and stored in the refrigerator for many months. The amylase enzymes will remain active and can be used in future investigations.

Teacher Tips

Tip 1 – Use fresh yeast

Best results are obtained using active dry yeast purchased in packets, as opposed to a jar of active yeast. Be sure the expiration date for the yeast has not passed.

Tip 2 – Connect the ethanol sensors to the data collection systems at least 5–10 minutes before their use.

The ethanol sensor probe contains a heating element. It can take up to 10 minutes for the sensor's temperature to stabilize. The "warm-up" period should occur prior to calibration. (Refer to the product manual for calibration procedures.)

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

Part 1

1. Put on your safety goggles.

2. Connect the ethanol sensor to your data collection system. (If possible, connect the data collection system to a power source during data collection, rather than relying on the system's battery power.) Allow the sensor to "warm up" for at least 5 minutes. After the warm-up period, calibrate the sensor with a 1% ethanol solution.

3. Set up the data collection system to measure percent ethanol over time.

4. Add 30 mL of 2% sucrose solution to a clean, empty container (sampling bottle or flask). Place a magnetic stir bar into the bottle and place it on a magnetic stir plate.

5. Add 20 mL of yeast suspension to the sampling bottle and set the stir speed to a low–medium setting.

   NOTE: Be sure the bottle and sensor do not tip over. If possible, use a rod stand and 3-finger clamp to secure the bottle. The sensor is a gas sensor and should NOT be immersed in a liquid.

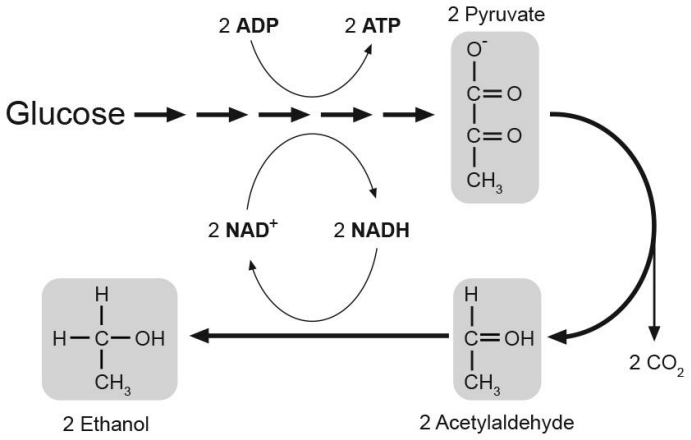
6. Seal the sampling bottle with the ethanol sensor. The seal between the stopper of the sensor and the bottle's opening should be air tight. Leave the system undisturbed for 1–2 minutes before starting data collection.

7. Begin data collection. Collect data for at least 15 minutes. Answer the following questions as you wait for data collection to end.

8. Describe how ethanol is formed during fermentation.

Yeast (and other organisms) use glucose as the main reactant for glycolysis. Glycolysis converts glucose into pyruvic acid and nets the cell 2 ATP in the process. During fermentation, the pyruvic acid produced during glycolysis is converted to carbon dioxide and ethanol.

First, the pyruvic acid is converted to acetylaldehyde, producing two molecules of carbon dioxide in the process. Then, the acetylaldehyde is converted to ethanol by adding two hydrogen atoms (from NADH molecules). This regenerates the NAD^+ needed to keep glycolysis running.



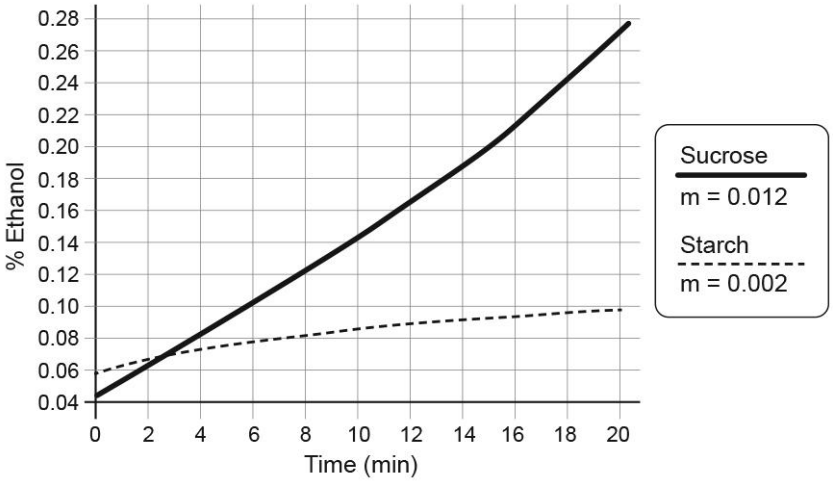
9. What type of molecule is sucrose? What would yeast have to do with sucrose in order to utilize it as an energy source for fermentation?

Sucrose is a carbohydrate, specifically a disaccharide (glucose + fructose). Since fermentation begins with glycolysis, which requires glucose, yeast would have to first digest sucrose to acquire glucose.

10. After at least 15 minutes has transpired, stop data collection. Thoroughly rinse the sampling bottle. Then add 30 mL of 2% starch solution and 20 mL of yeast suspension to the bottle.

11. Measure the rate of ethanol production over the course of 15 minutes (or longer).

12. Sketch or print a record of your data. Which is a better energy source for yeast, sucrose or starch? Describe evidence that supports your answer.



Comparing ethanol production by yeast provided with sucrose and starch

Comparing fermentation of sucrose and starch by yeast

Substance	Initial Ethanol Concentration	Final Ethanol Concentration	*Increase of Ethanol Concentration*	Rate of Ethanol Production*
Sucrose	0.04%	0.27%	0.23%	0.012% per minute
Starch	0.06%	0.10%	0.04%	0.002% per minute

*The rate was determined by using the system's software tools to obtain a linear fit to the data.

Sucrose is a better energy source for yeast. When yeast were provided with sucrose, the cells produced ethanol, a sign that the yeast were able to use the sucrose to carry out fermentation. Over the course of the experiment, the ethanol concentration increased by 0.23%.

When yeast were provided with starch, very little ethanol was produced (the ethanol concentration increased by only 0.04%) which indicates that the yeast were not able to use the starch for fermentation. The rate of ethanol production for yeast with starch was almost zero (0.002%/min), whereas the rate of fermentation for yeast with sucrose was 0.01%/min.

Part 2

13. Fill a small beaker halfway with starch solution. Add 5–10 drops of iodine indicator (IKI) to the beaker and mix. Record the color of the mixture.

    The starch–IKI mixture is dark blue.

14. Add 5 mL of water from germinating seeds to the starch/IKI solution. Slowly swirl the beaker until you observe a change. Record your observations.

    Within a minute of swirling, the blue color begins to disappear. Eventually, the solution becomes amber-colored (the color of IKI). Some blue-colored particles are suspended within the solution.

15. What effect did the water from germinating seeds have on the starch solution? How do you know this?

    The water from germinating seeds digested the starch; it caused the starch to break down into smaller molecules. Starch and IKI form a blue product when they combine, which is why the initial solution was blue. Since the solution lost its blue color over time, the starch disappeared from the solution. It must have been broken down into other molecules.

16. What enzyme is present in germinating seeds that helps explain the color change that occurred when seed-water was added?

    Germinating seeds contain amylase, an enzyme that breaks down stored starch to provide cells with the glucose they need for cellular respiration. The seed-water contains this enzyme, which explains the disappearance of the blue color—the enzymes were digesting the starch molecules so there were fewer starch–IKI associations.

17. If you added seed-water to a starch solution and then provided this solution to yeast for fermentation, how do you think the results would compare to the original yeast-starch results? Explain your prediction.

    Student answers will vary. Ideally, students will predict that the seed-water will help digest starch, making more sugar available to yeast for fermentation.

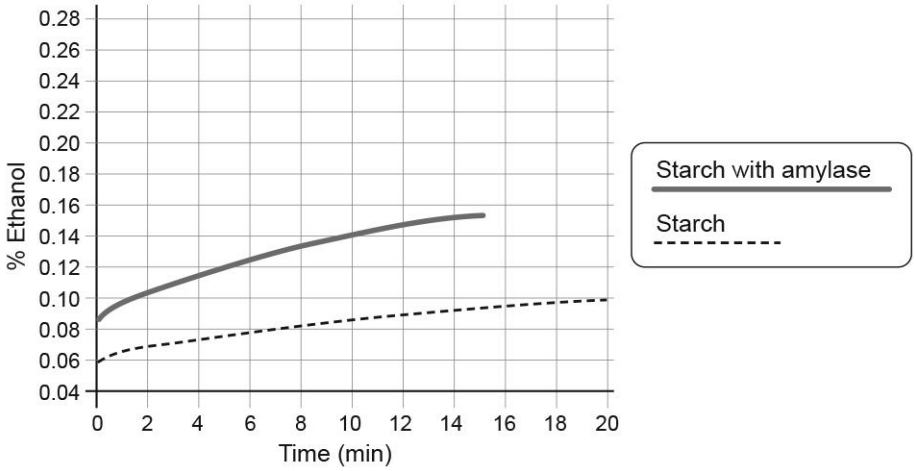
18. Depending on the time available, your teacher may have you carry out this experiment or may simply provide data for you to compare to your prediction. Based on the data collected or provided, how do the results compare to your prediction?

If students perform this experiment, they essentially repeat the initial procedure but use 30 mL of starch-amylase solution as the energy source for 20 mL of yeast. It is recommended that you prepare the starch-amylase solution in advance (refer to the Lab Preparation section). The data in the table below provides sample data for the experiment. If time is limited, you may share this data with students. Alternatively, you may wish to carry out this part of the investigation yourself as a demonstration for students, or concurrently with students as they work on the investigation, and display your data for students to analyze.

Student answers will vary. If students predicted that adding seed-water to starch would increase the fermentation rate, compared to using only starch, the students' prediction is supported by the data. While the rate of fermentation is still lower than with sucrose, the presence of the amylase enzyme in the seed-water makes some sugars available for yeast to ferment. The rate of ethanol production is two times greater when amylase is present in the starch solution.

Fermentation of amylase-treated starch compared to non-treated starch

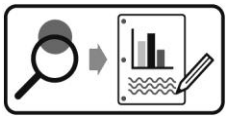
Substance	Initial Ethanol Concentration	Final Ethanol Concentration	Increase in Ethanol Concentration	Rate of Ethanol Production
Starch	0.06%	0.10%	0.04%	0.002% per minute
Starch + amylase	0.08%	0.15%	0.07%	0.004% per minute



Design and Conduct an Experiment

From the initial investigation, students can conclude that yeast are able to use sucrose more readily than starch for their energy needs. In addition to the types of compounds present in the environment, there are myriad factors that may affect the efficiency of fermentation in yeast. Many industries depend on optimum fermentation rates to produce products.

Students can design experiments to test the ability of yeast to use other common carbohydrates or other substances. They may also adjust yeast or sugar concentration, pH, or other factors to determine if these variables affect fermentation rate.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Do yeast utilize monosaccharides in fermentation?*
- Can other disaccharides be used by yeast as an energy source?*
- Can artificial sugars be used by yeast as a reactant for fermentation?*
- Do yeast carry out fermentation and aerobic respiration at the same time?*
- How does the rate of fermentation of monosaccharides compare to the rate of fermentation of disaccharides?
- Are the fermentation rates of different types of yeast similar?
- Does increasing the sugar concentration increase the rate of fermentation?
- Does the concentration of ethanol eventually inhibit further fermentation? (In other words, does the toxicity of ethanol affect yeast cells?)
- How much does pH change during fermentation (due to carbon dioxide production)? Does the decrease in pH eventually inhibit further fermentation?
- Industry makes use of very large vats for fermentation. Does increasing the size of the system—using a large volume of yeast and sugar—affect the fermentation rate?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Do yeast ferment monosaccharides?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected the rate of fermentation. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

      The independent variable was the type of sugar provided to yeast. Two monosaccharides were compared to each other, to sucrose, and to a control with no sugar added. Both monosaccharides (glucose and fructose) were fermentable by yeast; the ethanol concentration in the container increased over time. The results supported my hypothesis that yeast can utilize monosaccharides.

      In the presence of glucose, yeast increased the concentration of ethanol by 0.27% and in the presence of fructose, it increased by 0.26% during 20-minute trials. These values were much greater than the increase in the concentration of ethanol in the control group (0.004%). Additionally, the rate of fermentation for each monosaccharide was slightly higher than the rate observed when yeast were provided sucrose as the energy source. The rate of fermentation with sucrose was 0.012% per minute but for glucose and fructose the rates were 0.014% per minute and 0.013% per minute, respectively.

   b. Based on the evidence you collected, explain why the results occurred.

      Because ethanol concentration increased with either sugar, the yeast must have been able to use both glucose and fructose for fermentation. Glucose enters the glycolysis pathway directly, as a reactant in the first step of the pathway. However, yeast must be able to convert fructose into a molecule that can also enter the glycolysis pathway, or else they have a metabolic pathway similar to glycolysis that converts fructose to acetylaldehyde (a precursor to ethanol), or both.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   There is no evidence in the data or from observations that indicates experimental error or uncontrolled variables affected the results. The data was consistent between trials. The data is reliable enough to support the hypothesis.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. Yeast are used to ferment sugars as part of the production process of many human-made products including biofuels—fuels made from organic "feedstocks" such as corn or sugarcane.

   a. These feedstocks contain a large amount of sugar, but also contain starch and cellulose. What is the purpose of using enzymes such as amylase or cellulase in the biofuel production process?

      Polysaccharides such as starch and cellulose are not easily used by yeast for fermentation. If these polysaccharides are digested by amylase and cellulase before providing the feedstocks to yeast, the yeast will have plenty of simple sugars (the products of starch and cellulose digestion) to ferment.

b. What is the role of genetic engineering in biotechnological methods that efficiently produce products such as ethanol?

Genetic engineering creates genetically modified organisms with traits ideal for producing a particular product. For example, a bacterial gene that codes for amylase can be added to yeast so they are then able to digest starch. This reduces the need for enzymes to pre-treat the feedstocks and is more cost effective, since purified enzymes are no longer needed. Additional genes can be inserted to enable the yeast to grow in conditions optimal for ethanol production.

2. Compare and contrast lactic acid fermentation and alcohol fermentation. What determines the type of fermentation carried out by an organism?

Lactic acid fermentation and alcohol fermentation both convert the product of glycolysis—pyruvic acid—into other molecules, producing NAD^+ from NADH in the process. The regeneration of NAD^+ keeps the glycolysis pathway running, thereby providing microorganisms with a small amount of ATP per glucose molecule. In lactic acid fermentation, lactic acid is the only product of the pathway, whereas alcoholic fermentation produces carbon dioxide in addition to ethyl alcohol. The difference in the pathways depends on which fermentation enzyme is present. If the cell has the enzyme *lactate dehydrogenase*, pyruvate is converted to lactic acid. If the cell has the enzyme *alcohol dehydrogenase*, pyruvate is converted to ethanol.

3. Free energy *G* is an important aspect of understanding how organisms obtain, use, and transform energy to maintain their complex levels of organization and grow and develop. Table 1 shows the change in free energy that accompanies two processes that take place in cells.

Table 1: Free energy changes

Reaction	ΔG
Glucose + Oxygen → Carbon dioxide + water	–2870 kJ/mol
Glucose → Ethanol + Carbon dioxide	–285 kJ/mol

a. Explain the relationship between the data provided in Table 1 and the difference in ATP production for aerobic respiration compared to fermentation.

Aerobic respiration produces a much greater number of ATP molecules for a cell than fermentation does. The complete oxidation of glucose releases more energy than the partial oxidation that occurs during fermentation. Essentially, fermentation only provides a net of 2 ATP molecules for a cell (produced during glycolysis) compared to a net of 36 ATP that are produced from aerobic respiration (glycolysis, Krebs cycles, and oxidative phosphorylation in the electron transport chain).

b. How does the information above help explain why fermentation is limited to microorganisms or to brief periods of time in certain cells of multicellular organisms?

The energy needs of multicellular organisms cannot be met with the small number of ATP produced in anaerobic fermentation. When energy levels are low, such as occurs in muscle cells during strenuous exercise, cells may temporarily carry out fermentation; but this is not a viable pathway for the organism as a whole. Microorganisms require a lot less energy and fermentation is sufficient to meet all of their energy needs, although it is far less efficient than aerobic respiration.

4. Metabolic pathways are closely regulated to maintain homeostasis within organisms.

a. Describe two ways in which the activity of metabolic enzymes can be altered, thereby providing a mechanism for a cell to control a biochemical pathway?

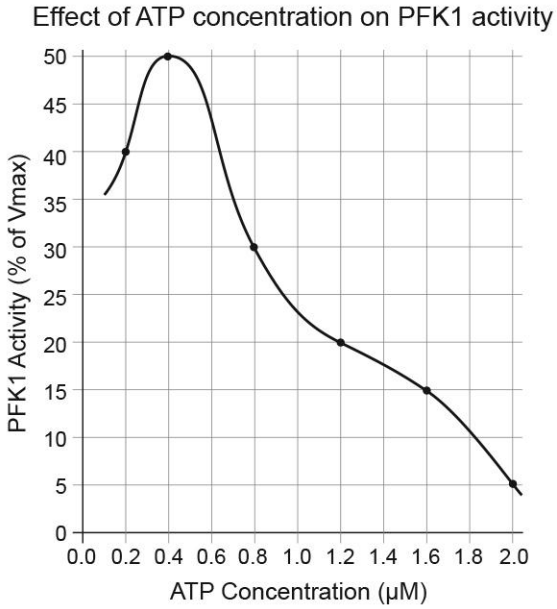
The activity of an enzyme can be altered by the presence of an inhibitor. Some inhibitors bind to the enzyme's active site, reducing the enzyme's function. These are "competitive" inhibitors.

Other molecules bind to a site on an enzyme that is not the active site. These might be "non-competitive" inhibitors, or they may be molecules that activate enzymes. These molecules cause the shape of the enzyme to change—into either a more functional or less functional form. This regulation isknown as *allosteric regulation*. Often the end product of a biochemical pathway is involved in allosteric regulation, helping regulate an enzyme that catalyzes a step early in the pathway (negative feedback).

b. Phosphofructokinase 1 (PFK1) is an enzyme which catalyzes a step of glycolysis that is highly regulated by the cell. If the enzyme is active, glycolysis takes place. If the enzyme is inhibited, glycolysis is inhibited. Use the data below to draw a graph of PFK1 activity versus ATP concentration. Explain the shape of the curve from 0.4 µM to 2 µM. Also explain why the activity of one enzyme in a pathway would affect the entire pathway, not just the step it catalyzes.

Table 2: PFK1 activity versus intracellular ATP concentration

PFK1 Activity (% of V_{max})	ATP Concentration (µM)
40	0.2
50	0.4
30	0.8
20	1.2
15	1.6
5	2.0

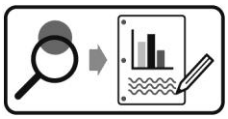


As ATP concentration increases between 0.4 µM and 2 µM, PFK1 activity decreases. There is negative feedback between ATP and PFK1. When ATP concentrations are high, the cell does not need to carry out glycolysis to acquire more ATP, so the ATP molecules in the cell inhibit activity of PFK1 to inhibit glycolysis.

A biochemical pathway uses the products of one step as the reactants for the next step. If one step is inhibited, the subsequent steps will be inhibited as well, since the reactants of those steps will be missing or in very low concentrations.

Design and Conduct an Experiment Key

In addition to the types of compounds present in the environment, there are myriad factors that may affect the efficiency of fermentation in yeast. Many industries depend on optimum fermentation rates to produce products. Consider a factor that you think affects ethanol production by yeast and design an experiment to test that factor.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of fermentation, what environmental factors (abiotic or biotic) could affect this process?

   Environmental factors that may affect fermentation include: availability of oxygen, type and amount of sugars or other compounds present, temperature, population growth of the fermenting organism, possible contamination by bacteria or other organisms, a decrease in pH, and increase in ethanol over time that may create toxic conditions.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   Do yeast utilize monosaccharides in fermentation?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   Knowing which sugars yeast can ferment can help a scientist create ideal conditions for yeast in baking, beer or wine making, or other industrial applications of fermentation.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable of the experiment is the type of sugar provided to yeast. Two monosaccharides will be used for the experimental groups: glucose and fructose. Two controls will be set up: a positive control using sucrose and a negative control using water, to compare to the rate of ethanol production from the fermentation of monosaccharides.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable of the experiment is the percent ethanol present in the sealed system, as measured by an ethanol sensor. The ethanol will be measured over time and graphed. The slope of the line will be used to compare rates of fermentation.

6. Write a testable hypothesis (If…then…).

   If yeast are able to ferment monosaccharides, then the ethanol concentration in the system will increase over time.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   All trials will be carried out at room temperature, using yeast from the same batch and the same amount of yeast. All solutions will be 2% solutions and the same volume of solution will be used in all trials. The mixture will be evenly stirred during each trial.

8. How many trials will be run for each experimental group? Justify your choice.

   Two trials will be run for the experimental groups and one trial each for the control groups. A typical class period provides roughly 60 minutes for data collection, the time needed to collect data for 6 runs.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The rate of fermentation will be determined from the slope of the line. If the line is not linear, the rate will be determined by the overall change in ethanol over time. The two trials for each experimental group will be averaged.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    There may be small differences in yeast concentration in each sample taken from a large batch of yeast. Even though the batch will be stirred before removing a sample, with the intention of making each sample uniform, there may still be differences between samples.

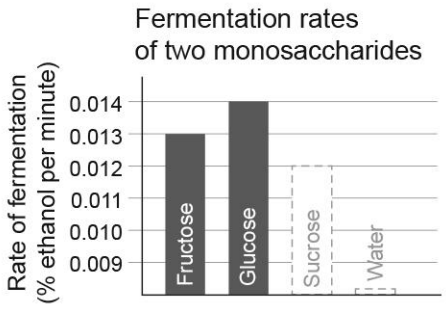
    The ethanol sensor measurements can be affected by changes in temperature. If the yeast is prepared with warm water and the batch cools over time, the ethanol readings may vary due to differences in temperature of the yeast solution rather than fermentation.

    The sugar solutions may not be prepared accurately, which may affect fermentation rates.

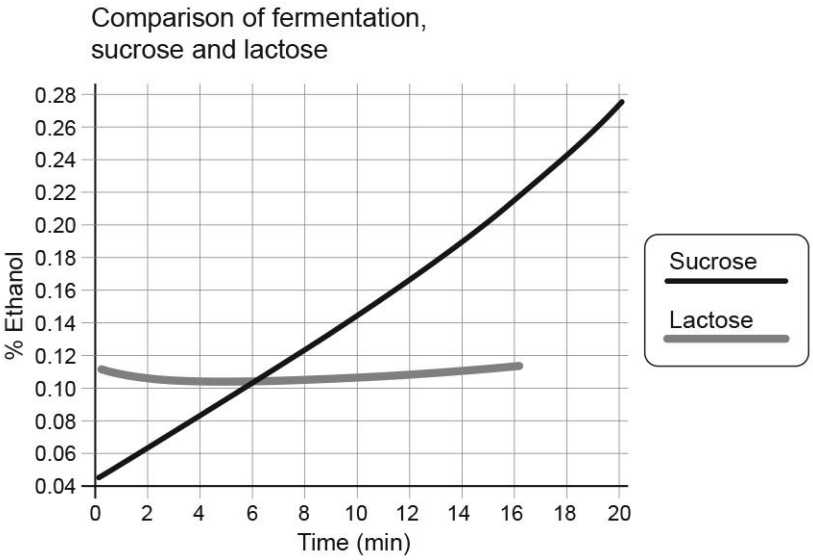
11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)
    a. Prepare 2% solutions of sucrose, glucose, and fructose: Measure 2 g of the sugar and dissolve it in 98 mL of water.
    b. Add 30 mL of sucrose and 20 mL of yeast suspension to a sampling bottle. Place the bottle on a stir plate set at a low to medium speed. Measure the ethanol concentration over time, stopping data collection at 10 minutes, or as long as it takes to get a clear trend of ethanol increasing over time.
    c. Rinse the bottle thoroughly. Repeat the procedure with 30 mL of water and 20 mL of yeast suspension.
    d. Follow the same procedure, using 30 mL of glucose in each of two trials.
    e. For the last two trials, use 30 mL of fructose in the bottle with the yeast suspension.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

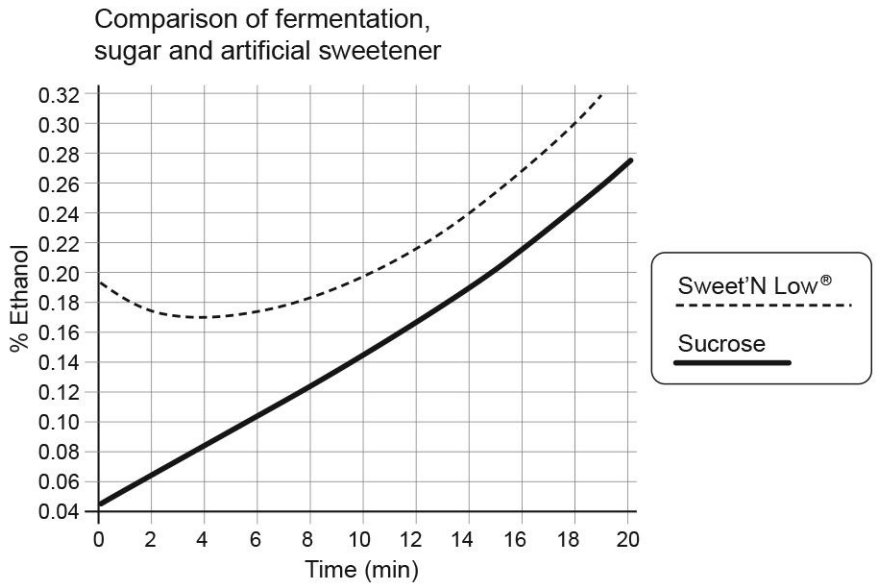
Sample Data



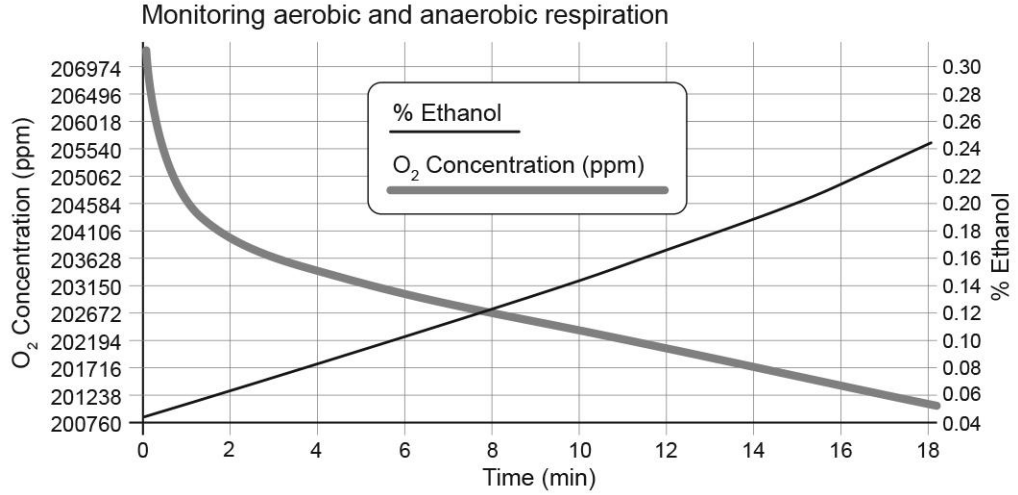
The results shown above are from an inquiry comparing the fermentation rates for two monosaccharides. (Sucrose was included as a positive control and water as a negative control.) The experiment was run at room temperature with equal concentrations of sugar solutions (2%). The same yeast suspension was used for each trial.



The results shown above are from an inquiry comparing the fermentation rate for sucrose and lactose, both disaccharides. The experiment was run at room temperature with equal concentrations of sugar solutions (2%) and the same yeast suspension for each trial.



The results shown above are from an inquiry comparing the fermentation rate of an artificial sweetener (Sweet'N Low) to that of sucrose. Sweet'N Low contains saccharin as well as dextrose. Each solution was prepared as a 2% solution and the same yeast suspension was used for each trial.



The results shown above are from an inquiry that determined whether yeast carry out aerobic respiration at the same time as fermentation. An EcoChamber™ was used to monitor ethanol and oxygen gas concentrations above a suspension of yeast and sucrose.

9. PHOTOSYNTHESIS

Lab Overview

Students test the effect of light on the rate of photosynthesis. Given the equation for photosynthesis, students can determine that either a carbon dioxide gas sensor or an oxygen gas sensor would be appropriate equipment for measuring the photosynthetic rate.

$$6CO_2 + 6H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6O_2$$

For the Initial Investigation, a carbon dioxide gas sensor is used to determine the rate of uptake of CO_2 by spinach leaves. Students measure the change in carbon dioxide concentration caused by photosynthesis occurring in the leaves under red light and compare that to the change due to photosynthesis under green light. They then design an experiment to investigate the effect of a parameter such as the type of light bulb, the distance of the light from the leaves, or the color of the leaves.

Pacing and Length of the Lab

Initial Investigation	
Preparation	10 min
Initial Investigation	25 min

Student-Designed Experiment	
Experiment Design	15 min
Experiment	60 min
Data Analysis	30 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.A.2, 2.D.1, 4.A.6
Science Practices	2.2, 3.1–3.3, 4.2–4.3, 5.1, 6.1–6.2, 7.2
Learning Objectives	2.5, 2.8, 2.9, 2.24, 4.14

Materials and Equipment

For Each Student Station

- Data collection system
- Carbon dioxide gas sensor
- Sensor extension cable[1]
- Sampling bottle, 250-mL[1]
- Box, foil, or cloth for shading the setup
- Light source
- Compact fluorescent light bulb, 60 W equivalent (or higher), red
- Compact fluorescent light bulb, 60 W equivalent (or higher) green
- Fresh spinach leaves[2]
- Forceps or pencil

[1]Included with the carbon dioxide sensor. [2]Refer to the Lab Preparation section.

Additional equipment recommended for the student-designed experiment:

- Oxygen gas sensor[3]
- Variety of leaf types, such as: non-green or less-green, and needles
- EcoChamber (to accommodate larger plants)
- Variety of light sources, such as a grow light and other types of fluorescent bulbs

[3]Best results are obtained using this sensor with the EcoChamber, rather than the sampling bottle.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- Sunlight is a mixture of different wavelengths of light
- Leaves contain pigment molecules that absorb light
- The color of an object is the result of light reflected by pigments in the object
- Chlorophyll is not the only pigment present in leaves
- Plants absorb carbon dioxide as a source of carbon for the sugars and other organic compounds made during photosynthesis

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times
- Allow the light bulb to cool before removing it from the light source

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Replace the standard light bulbs of the light sources with a red or green compact fluorescent bulb.
   - Sixty watt or equivalent light bulbs were adequate in the trials. (The green and red bulbs must have the same intensity ratings.)
   - Bulbs of different colors can be purchased in most hardware stores.
2. Obtain fresh spinach leaves.

   Spinach leaves from the produce section of a grocery store work well. If possible, purchase the spinach in bulk instead of purchasing spinach in a sealed bag.

Teacher Tips/Lab Checkpoints

Tip 1 – Arranging the leaves

Students can use forceps or a pencil to arrange the leaves so they lay flat against one side of the sampling bottle. The leaves should overlap as little as possible.

Tip 2 – Shading the setup from ambient light

To concentrate the colored light on the sampling bottle containing the leaves, it is best to shield the setup from ambient light in the room. The following are some suggestions for how to do this. Choose one based on the materials you are able to obtain easily.

- Use a large box, such as a copy paper box. Place the box over the desk lamp and sampling bottle.
- Use a large piece of aluminum foil. Wrap the foil around the desk lamp shield, creating a cone that shades the sampling bottle and reflects the colored light onto the bottle.
- Use a dark cloth. Drape the cloth over the desk lamp and sampling bottle.

Tip 3 – Carbon dioxide gas sensor

1. Turning on the light source during data collection can cause erroneous readings by the carbon dioxide gas sensor. Be sure students turn on the light source and have it shining on the sampling bottle *before* beginning data collection.

2. The carbon dioxide sensor will give erroneous readings if exposed to infrared light. Avoid using incandescent bulbs (such as halogen bulbs). A heat sink can be placed between the light and the sensor and sampling bottle, but the use of fluorescent light bulbs is ideal.

Tip 4 – Colored light bulbs

Suggestions for the use of colored light bulbs:

- The outcome of the experiment is not affected by which color (green or red) is used first. Student groups can share the light bulbs, with half the class using one color first and then switching with other groups to complete data collection with the other color.
- Some red light bulbs do not provide expected results. We recommend purchasing a few different brands to test before buying a class set of light bulbs, which can be used for many years. To test the bulb, follow the initial investigation procedure. You should observe a notable decrease in carbon dioxide over the course of 2–3 minutes.
- A blue light was used in trials for this lab and did not provide expected results (that is, it did not result in higher rates of photosynthesis, compared to red light or green light). This is likely due to the blue "party bulb" emitting light in the green and yellow wavelengths.
- An alternative to colored light bulbs is colored cellophane. The cellophane can be placed between a white light source and the sampling bottle (containing the spinach leaves), to filter the white light, providing colored light to the leaves.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handouts

1. Put on your safety goggles.

2. Connect the carbon dioxide gas sensor to your data collection system and calibrate the sensor.

3. Display a graph of Carbon dioxide (ppm) on the *y*-axis versus Time in minutes on the *x*-axis. Adjust the sample rate to one sample every 5 seconds.

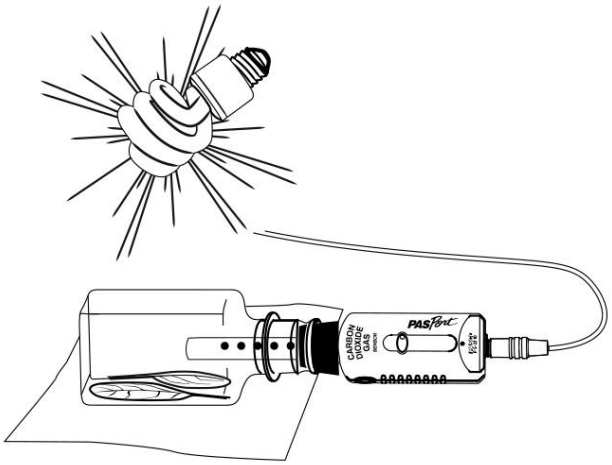
   NOTE: If your data collection system allows you to set an automatic stop condition, set the stop time for 7 minutes.

4. Holding the sampling bottle horizontally, place two fresh leaves into it. The leaves should lay flat and overlap as little as possible.

5. Seal the sampling bottle with the carbon dioxide sensor and stopper. Rest the bottle on its side on a flat surface.

6. Arrange the light source directly above the sampling bottle.

   NOTE: Make sure a colored light bulb (red or green) is in the light source.



7. Shade the bottle from ambient white light in the room.

8. Turn on the light source. Wait approximately 15 seconds and then start collecting data. Adjust the scale of the graph to show all data. Collect data for 7 minutes.

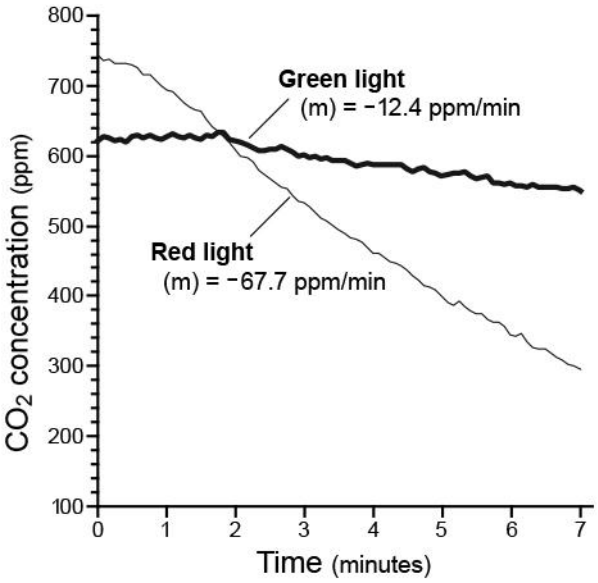
9. After 7 minutes, stop recording data and turn off the light source.

10. Refresh the air in the bottle by waving the bottle through the air a number of times. If necessary, readjust the leaves so they lay flat.

11. Remove the colored bulb from the light source and replace it with a different colored bulb.

12. Repeat the data collection using the other colored bulb.

13. Draw or print a record of the data.



This graph compares the change in concentration of CO_2 in a closed chamber due to photosynthesis in red light and in green light.

14. Describe the apparent trend of the data.

The decrease in CO_2 in the chamber under red light was much greater than the decrease under green light, indicating that in red light, the leaves absorbed more carbon dioxide than in the green light.

15. How can you quantify the results? What type(s) of mathematical analysis would be appropriate?

a. Identify the method(s) chosen for quantification and analysis of results.

Students may suggest calculating the change in CO_2 to determine how much CO_2 was used or calculating the slope of the line to determine the rate of photosynthesis, or both.

b. Apply the method(s) chosen to the data collected in the initial investigation.

Change in CO_2 Concentration (ppm)	
Red light	–434 ppm
Green light	–76 ppm

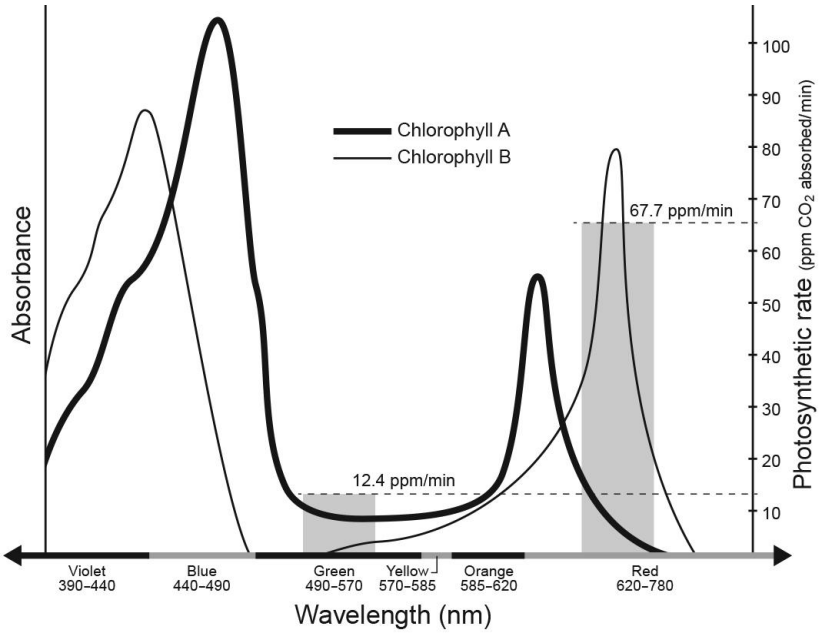
Photosynthetic Rate (ppm/min)	
Red light	67.7 ppm/min
Green light	12.4 ppm/min

Example tables and calculations:

Change in CO_2 concentration (final – initial) for red light: 303 ppm – 737 ppm = –434 ppm

Photosynthetic rate: determined by applying a linear fit to the slope of the curve (expressed as a positive value since the CO_2 is being absorbed by the plant).

16. Create a graph that relates the absorbance spectrum to the action spectrum. The absorbance spectrum is provided in the graph below. For the action spectrum, draw a bar graph indicating the photosynthetic rate at the wavelengths tested in the investigation.



The action spectrum bar graph shows the rate of photosynthesis in the green and red wavelength ranges, the two colors tested in this investigation.

17. Based on a typical absorbance spectrum for leaves, explain the results of this investigation. Your response should provide a clear connection between light absorbance, the reactions of photosynthesis, and evidence from the investigation.

In most leaves, chlorophyll is the dominant pigment; it is present in greater amounts than other pigments. There is little absorbance of green light by chlorophyll, hence a leaf appears green because the green light is reflected. However, chlorophyll absorbs a much greater amount of red light. The energy from the red light absorbed by the chlorophyll molecules within the chloroplasts of the leaf raises electrons to an excited state.

The light-dependent reactions driven by the energy of these excited electrons supply ATP to the Calvin Cycle reactions. Carbon dioxide consumption increases due to the carbon fixation within the Calvin Cycle, causing the concentration of CO_2 in the sampling bottle to decrease at a greater rate than when the plants were under green light.

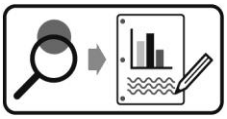
In green light, chlorophyll molecules do not absorb much energy and fewer electrons become excited. The light-dependent reactions occur slowly, resulting in a lower rate of the light-independent reactions. Less carbon fixation occurs, so less CO_2 is absorbed by the leaf. The concentration of CO_2 in the sampling bottle decreases at a much lower rate.

18. What could be done to confirm the relationship observed in the data collected by your group?

Our group could perform more trials, repeating the procedures to test the reliability of the results. Also, since the investigation was carried out by all groups, class data can be collected and compared.

Design and Conduct an Experiment

Photosynthesis is critical for providing energy to organisms in an ecosystem. It is a process that can be affected by a number of factors. Guide students in thinking of possible factors. They should then design an experiment to test one factor.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If students are not ready to create their own testable questions, they can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Do different types of white light bulbs cause different photosynthetic rates?*
- Does the greenness of a leaf affect photosynthetic rate? *
- Does the light intensity (watt equivalent or lumens) affect the rate of photosynthesis? *
- Does distance between the light source and the leaves affect the rate of photosynthesis? *
- Do trees with needles have different rates of photosynthesis than plants with leaves?
- In ambient light, do house plants carry out much photosynthesis?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the Suggested Inquiry Question: "Do different types of white light bulbs cause different photosynthetic rates?"

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data,

   a. Describe how the independent variable you manipulated affected the rate of photosynthesis. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

      The independent variable in this inquiry was the type of white light bulb. The type of bulb did affect the rate of photosynthesis, but more testing is needed to determine if the hypothesis (if the wavelength spectrum is similar for light emitted from different brands of white light bulbs, then plant leaves exposed to the different light bulbs will have the same rate of photosynthesis) is supported or rejected.

      The natural-daylight and bright-white bulbs resulted in photosynthetic rates of 21.6 ppm/min and 23.4 ppm/min, respectively. The compact fluorescent light bulb (CFL) resulted in a lower rate of photosynthesis, 12.6 ppm/min.

   b. Based on the evidence you collected, explain why the results occurred.

      While the three types of bulbs used were "white lights" which emit more than one wavelength, the spectrum of wavelengths emitted by each must be different, especially the spectrum from the CFL bulb. The bulbs which caused the greatest decrease in CO_2 (and therefore the greatest photosynthetic rate) must emit more red or blue light compared to the regular CFL bulb.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   While I attempted to arrange the leaves exactly the same way each time and keep the light source the same distance from the leaves, results did vary from one trial to the next. For example, in one trial with the bright-white light bulb, the rate of photosynthesis was 17.9 ppm/min. In the next trial, the rate was 28.9 ppm/min. This variation indicates that additional trials may be needed.

3. Identify any new questions that have arisen as a result of your research.

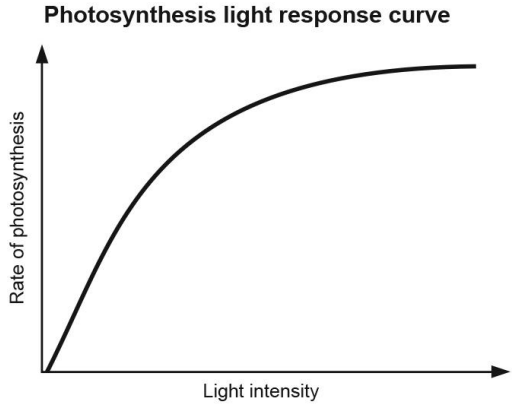
   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. Incandescent light bulbs are inefficient light sources due to a large amount of energy released as heat into the environment. If you carried out a photosynthesis experiment using an incandescent bulb, how would you expect this to affect the results? Explain your reasoning.

   An incandescent light bulb is likely to result in reduced photosynthesis. High temperatures cause the stomata of leaves to close to limit water loss. When the stomata close, carbon dioxide gas is not absorbed and photosynthesis slows or stops. For this reason, a heat sink is often used in photosynthesis experiments if an incandescent bulb is the light source.

2. Refer to the generalized light response curve for photosynthesis to answer the following.



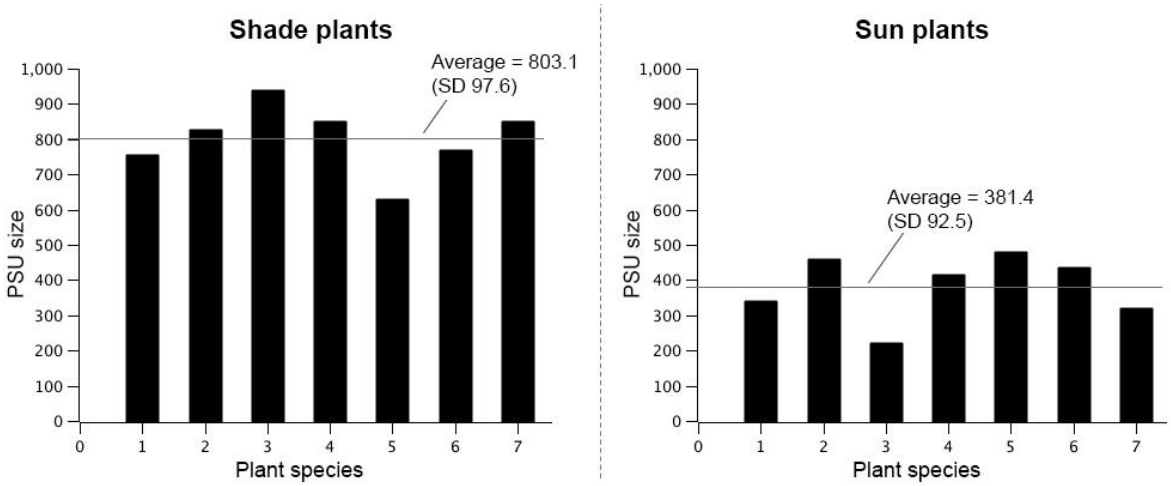
   a. For low to medium light intensity, explain the trend in the data.

      Increasing light intensity from low light levels to medium light levels causes an increase in photosynthesis. The increase is almost linear. With more light available to leaves, more energy is available and the rate of photosynthesis increases.

   b. For higher levels of light intensity, explain the trend in the data.

      At high levels of light intensity, photosynthesis reaches a steady, maximum rate. At these light levels, the pigment molecules in the chloroplasts are saturated; the excitation of electrons by photons of light is occurring at the fastest rate possible so increasing the light intensity further has no effect on the rate of photosynthesis.

3. A "photosynthetic unit" (PSU) is a complex of pigment molecules and one or more reaction centers. The reaction centers are responsible for passing excited electrons to an electron acceptor within a photosystem. Graphs of the data from a study that compared the PSU sizes for a variety of plants are shown below.[10] In the study, scientists measured the PSU size of seven different species of shade plants and seven different species of sun plants.



a. What conclusion can be made from the data acquired in this study?

There is a notable difference in the size of a photosynthetic unit in shade plants compared to sun plants. The mean PSU for shade plants is more than double the mean for sun plants. While there is variation within sun plants or shade plants of PSU size, the PSU size for a shade plant was always larger than that of a sun plant.

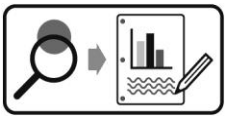
b. Considering the environments of the plants, provide an explanation for the difference in the mean PSU sizes.

Shade plants have less light available to them. Having a large photosynthetic unit increases the probability that energy from light is captured. Shade plants have more pigment molecules so that they can maximize their use of available light. Sun plants have plenty of light available to them and do not need as many pigment molecules, so the mean PSU is smaller in these plants.

[10] Malkin, S.; Fork, D.C. Photosynthetic Units of Sun and Shade Plants. *Plant Physiology* 67 (1981): 580–583.

Design and Conduct an Experiment Key

Photosynthesis is critical for providing energy to organisms in an ecosystem. It is a process that can be affected by a number of factors. Think of possible factors and design an experiment to test one of them.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of photosynthesis, what environmental factors (abiotic or biotic) could affect this process?

   Factors that can affect photosynthesis include: temperature, humidity, light intensity, wavelength of the light, and light availability.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   Do different types of white light bulbs cause different photosynthetic rates?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   Many plants are grown indoors or in greenhouses. The light sources used in these locations may affect the photosynthetic rate of the plants, so it is good to know which light bulbs are best for photosynthesis.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The light source is the independent variable. Different brands of white light bulbs will be used (natural-daylight, bright-white, and regular CFL).

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The change in carbon dioxide gas concentration is the dependent variable. A CO_2 sensor will be used to measure the change over 10 minutes using 2 leaves of spinach in a sampling bottle. Both the total change and the rate of change will be calculated.

6. Write a testable hypothesis (If…then…).

   If the wavelength spectrum is similar for light emitted from different brands of white light bulbs, then plant leaves exposed to the different light bulbs will have the same rate of photosynthesis.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   Conditions to be held constant:

   Light intensity: the different white light bulbs will all be 100 W equivalent light bulbs providing approximately 1600 lumens.

   Distance: The light bulbs will be positioned 10 cm above the sampling bottle.

   Temperature: Trials will be conducted at room temperature using the same type and number of spinach leaves (2).

8. How many trials will be run for each experimental group? Justify your choice.

   Two trials will be conducted with each light bulb. Results are expected to be consistent within trials with the same light bulb. Data will be collected for 10 minutes, allowing time for at least 3 different bulbs to be tested in one class period. The average of the two trials will be used to compare the results between different light bulbs.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The average rate of change of CO_2 concentration will be compared for the leaves exposed to different sources of white light. The rate of change will be determined by finding the slope of the line for each trial. Depending on the results, statistical analysis may need to be used to determine if any differences in results are statistically significant.

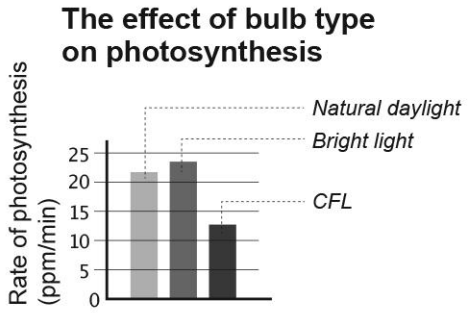
10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

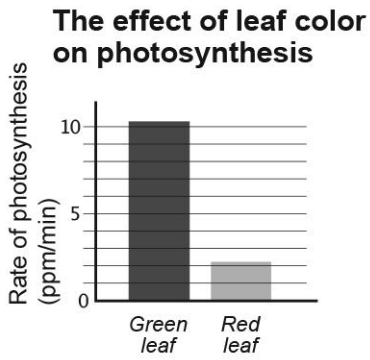
    Error could stem from the fact that it may be difficult to have the spinach leaves arranged exactly the same way in the bottle for each trial. It may not be possible to buy light bulbs of exactly the same number of lumens. The light source may not be positioned above the sampling bottle exactly the same way in each trial.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)
    1. Obtain three different white light bulbs and two spinach leaves.
    2. Set up the sampling bottle with the two spinach leaves and CO_2 sensor and put the lamp (with the first bulb) 10 cm above the bottle.
    3. Turn on the light source and collect data for 10 minutes.
    4. Refresh the air in the sampling bottle and repeat the trial with the same light bulb.
    5. Change the light bulb, refresh the air in the sampling bottle, and obtain two 10-minute trials with this light bulb. Repeat this step with the third light bulb.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

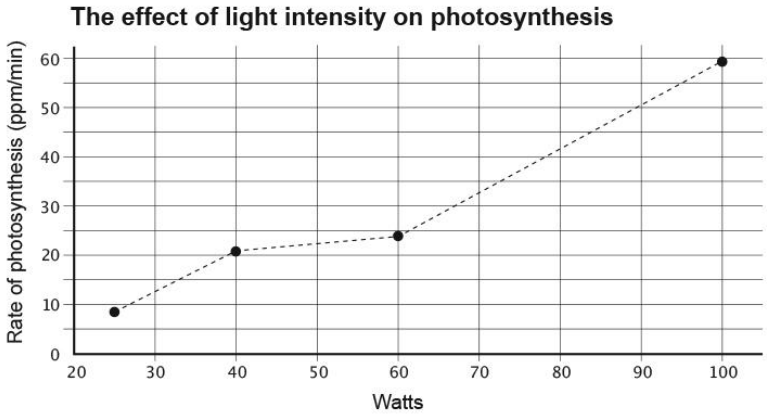
Sample Data



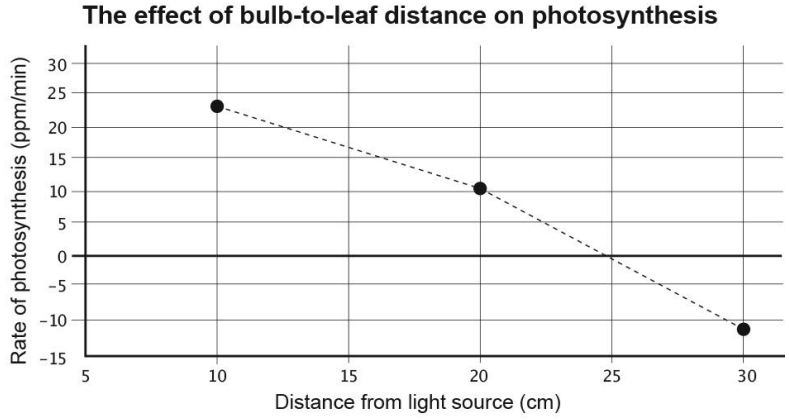


Each white light bulb used was a 15 watt bulb with an output of 800 lumens. In each trial, two spinach leaves were used and the light source was placed 15 cm above the leaves.

Lettuce leaves were placed under a 25 watt light bulb positioned 20 cm above the leaves. The green and red leaf lettuce samples had the same surface area.



Light intensity was varied using soft-white light bulbs with different power ratings: 25 W, 40 W, 60 W, and 100 W, placed 20 cm above the leaves. Two spinach leaves were used in each trial.



The distance between the spinach leaves and the light source was varied while the same light source, a soft-white 25 watt bulb, was used in each trial.

10. PLANT PIGMENTS

Lab Overview

Students extract pigments from spinach leaves for analysis using chromatography and colorimetry. Paper chromatography separates the pigments present in the extract so they can be identified. Analysis of the extract with a colorimeter allows students to determine the relative absorbance of four different colors of light (blue, green, orange, and red). They relate the chromatography results to the colorimeter measurements to refine their understanding of how plants capture light for photosynthesis. If available, a spectrometer allows students to view the full absorbance spectrum for spinach leaves.

The colorimeter is used again in Part 2 to measure photosynthesis using DPIP (2,6-dichlorophenolindophenol), an electron acceptor that experiences a color change if photosynthesis is occurring. For student-designed experiments, students can analyze factors such as the effect of different light sources, distance from a light source, and comparison of pigments in the leaves of different plants using chromatography, DPIP analysis, or both.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	20 min
Part 1: Chromatography	25 min
Part 2: Measuring Photosynthesis with DPIP	25 min

Student-Designed Experiment	
Experiment Design	15 min
Experiment	40–55 min
Data Analysis	15 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.A.2, 2.A.3, 2.B.3, 2.D.1, 4.A.2, 4.B.1, 4.C.1
Science Practices	1.2, 1.4, 3.1, 4.1–4.3, 5.1, 5.3, 6.1, 6.2, 7.1, 7.2
Learning Objectives	2.2–2.5, 2.9, 4.1, 4.5, 4.6, 4.16, 4.18, 4.22

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Materials and Equipment

For Each Student Station

PART 1

- Data collection system
- Colorimeter
- Colorimeter cuvette
- PASCO Wireless Spectrometer and spectrometry software[1]
- Spectrometer cuvette (1-cm glass cuvette)
- Plastic pipets (3), 1-mL
- Capillary tube or eye dropper without a bulb
- Chromatography chamber with solvent[2,3]
- Chromatography paper
- Ethanol, 10 mL
- Colorimeter and spectrometer blanks (cuvettes with ethanol)[3]
- Pigment extract, 10 mL, or if necessary to prepare:[3]
  Spinach leaves (3)
  Ethanol, 5–10 mL
  Beaker, small
  Mortar and pestle
  Cheesecloth or coffee filter paper
- Scissors
- Small stapler or paper clips
- Ruler
- Pencil
- Kimwipes®

PART 2

- Data collection system
- Colorimeter
- Colorimeter cuvettes (3)
- Plastic pipets (4), 1-mL
- Chloroplast suspension, 9 drops[3]
- 0.1 M Phosphate buffer solution, 3 mL[2,3]
- DPIP (2,6-dichlorophenolindophenol) solution, 2 mL[2]
- Lamp with a compact fluorescent (CFL) light bulb
- Kimwipes
- Aluminum foil, to cover a cuvette
- Distilled water, 10 mL

[1] The software is included with the purchase of the spectrometer. Download the software onto the devices to be used by students before the lab activity. Alternatively, the spectrometer procedure (Part 2) can be a teacher demonstration.

[2] We recommend that you purchase a kit from a supply company that contains chromatography solvent, phosphate buffer (KH_2PO_4 and K_2HPO_4), and DPIP solution. The chromatography solvent is typically a solution that contains acetone: either petroleum ether and acetone, or ethanol and acetone. Kits also contain chambers that can be used for many years. An alternative is to use glass mason jars.

[3] Refer to the Lab Preparation section for information on preparing the chromatography chamber, the colorimeter and spectrometer blanks, the pigment extract, 0.5 M sucrose ($C_{12}H_{22}O_{11}$), the chloroplast suspension using spinach leaves and a cold 0.5 M sucrose solution, and the 0.1 M phosphate buffer (KH_2PO_4 and K_2HPO_4).

Additional equipment recommended for the student-designed experiment:

- Different types of leaves
- Different light sources: different colored lightbulbs or lightbulbs of different wattage
- Additional chromatography paper and solvent
- Hot plate
- Frozen or canned spinach

Prerequisites

Students should be familiar with the following concepts:

- The color of a leaf relates to the colors reflected by pigments present in the leaf.
- Pigments are molecules that absorb certain wavelengths of light, thereby providing the energy needed to drive photosynthesis.
- The light reactions of photosynthesis involve electron transport chains that accept "excited" electrons from photosystems and pass these electrons to the electron acceptor $NADP^+$ at the end of the chain.
- Chromatography is a process of separating molecules based on their differential affinity for a substrate and a solvent.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Never use ethanol near a flame; it is highly flammable.
- Work in a well-ventilated area, ideally a fume hood, when carrying out the extraction and chromatography procedures that use ethanol.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

The day before the lab

1. Prepare spinach

   Purchase fresh spinach from a grocery store; for best results buy the loose spinach that you bag yourself. Place the leaves in a shallow tray of water and place the tray under a bright light. Use a fluorescent light source that does not generate much heat.

2. 0.5 M sucrose solution (used to prepare spinach for Part 2, the DPIP investigation)

   A 0.5 M sucrose solution is used to extract chloroplasts from spinach. If not supplied in a kit you've purchased, prepare the solution by dissolving 171 g of sucrose in 1 L of distilled water. Place the solution in a refrigerator so it will be cold when used.

3. Prepare 0.1 M phosphate buffer

   If you've purchased a kit, this buffer is likely one of the components of the kit. It is also likely that this buffer is in the chemistry stockroom of your school.

   If you need to prepare the buffer yourself:

   a. Dissolve 43.5 g of K_2HPO_4 (dibasic phosphate) in 200 mL of distilled water. Adjust the final volume to 250 mL using distilled water.

   b. Dissolve 34 g of KH_2PO_4 (monobasic phosphate) in 200 mL of distilled water. Adjust the final volume to 250 mL using distilled water.

c. In a new container, mix together 171 mL of monobasic buffer and 79 mL of dibasic buffer. This is a 1 M phosphate buffer solution. To dilute it to 0.1 M, add 100 mL of the phosphate buffer to 900 mL of distilled water.

NOTE: Unused buffer solution can be stored in a chemical stock room at room temperature.

On the day of the lab

NOTE: For best results, prepare the chloroplast suspension for students close to the time when students will use it.

4. Pigment extract: If time or equipment is limited, you may want to prepare the pigment extract ahead of time for students to use in the chromatography investigation (Part 1).

   a. Cut at least 10 large spinach leaves into small pieces. Remove any stems from the leaves and discard. Place the cut leaves into a beaker.

   b. Cover the leaves with ethanol and cover the beaker. Let the leaves soak at least one hour in the ethanol. Stir the mixture occasionally.

   c. Transfer the mixture to a mortar and pestle and grind the mixture for a few minutes. Then filter the mixture through cheesecloth.

5. Chloroplast suspension for the DPIP investigation

   a. Pour a small amount of cold 0.5 M sucrose solution (100 to 200 mL) into a blender. Add a large handful of spinach leaves to the blender and lightly pack them down with a spatula.

   b. Add additional sucrose solution if needed so the depth of the liquid is just below the leaves.

   c. Turn on the blender and blend the spinach in a series of three 10-second bursts.

   d. Filter the blended spinach through cheesecloth into a container and place the container on ice until the students use it.

6. Chromatography chamber setup

   Pour approximately 10 mL of chromatography solvent into each chamber and tightly cover each.

7. Ethanol for pigment extraction

   Use either anhydrous ethanol or 70% ethanol in water.

8. Colorimeter and spectrometer blanks, one of each for each lab group

   Fill the requisite number of clean colorimeter and spectrometer cuvettes with the ethanol that will be used for the pigment extraction. Having prepared blanks for the groups will save time during the class period.

   NOTE: Use 1-cm glass cuvettes for the spectrometer. Do not use the plastic cuvettes with organic solvents.

Teacher Tips

Tip 1 – Using the PASCO wireless spectrometer

The wireless spectrometer uses different software than the software on the data collection system used with the colorimeter. This designated software is called "Spectrometry" and is free. Refer to www.pasco.com for additional details on downloading the software for Windows® or Apple® computers, as well as a user guide for the device. For tablets, the app is available in the Apple App Store or from Google Play.

The spectrometer can be connected wirelessly or through a USB connection. After connecting the spectrometer and launching the software, choose the "Analyze Solution" option. A graph is generated showing Absorbance versus Wavelength. For pigment analysis, we are interested in the visible light spectrum (400–700 nm). Clicking and dragging the numbers on the *x*-axis allows you to change the scale of the axis to show only this range. Clicking and dragging the colored area on the graph translates the graph and allows you to center the visible wavelength range in the center of the graph for an optimized view of the wavelengths of interest.

Next to the record button are two calibration buttons, "Calibrate Light" and "Calibrate Dark." To *calibrate light*, place a blank into the spectrometer and press the Calibrate Light button. To *calibrate dark*, place your hand over the cuvette and press the Calibrate Dark button.

After clicking Record, snapshots can be taken of the resulting absorbance spectrum. The snapshots can be saved, printed, or copied and pasted directly from the application into a document.

Tip 2 – Chlorophyll fluorescence

A neat demonstration that fits well with this activity is demonstrating the fluorescence of chlorophyll. Use the spinach blended in sucrose from the DPIP activity as the source of chlorophyll for the demonstration. Place the chlorophyll suspension into a container, darken the room, and shine a black light (UV light) through the solution. The chlorophyll will fluoresce red. This demonstration can be viewed at https://www.youtube.com/watch?v=PhBg2uH8MWE.

The UV light excites electrons in the chloroplasts, but the electrons are not passed along an electron transport chain to NADPH. Rather, the electrons fall back to their lower-energy ground state and release energy in the form of red light.

The spectrometer can be used to measure this fluorescence as well as absorbance and transmittance. Switch the software from "Absorbance/Transmittance" to "Fluorescence at 405 nm" to view the wavelengths of light emitted from the chlorophyll when it fluoresces.

Tip 3 – Absorbance spectra of isolated pigments

A large number of chromatograms need to be created to have sufficient quantities of isolated pigments for analysis. (This is one of the suggested inquiry options.) One option is to have all students contribute their chromatograms to this purpose after they record their observations in the Initial Investigation.

Students should cut carefully along the border lines between pigments to cut strips of chromatography paper that have only one pigment per strip. Discard the sections of paper that do not contain pigments. Place all of the chlorophyll *a* strips into a beaker with ethanol or chromatography solvent. Place all of the chlorophyll *b* strips into a different beaker, and do the same for the carotenoids and xanthophylls. Over time, the pigments will dissolve out of the paper and into the solvent. Cutting the strips into smaller pieces, or crumpling the strips, can accelerate the process.

Place samples from the four beakers into four cuvettes and analyze them using the spectrometer.

Tip 4 - Emission Spectroscopy

An interesting extension to this lab is to use the wireless spectrometer to analyze different light sources, such as different white light bulbs or different colored light bulbs. This activity requires the use of the fiber optics cable sold as an accessory to the wireless spectrometer. Simply insert the fiber optics cable into the cuvette holder of the spectrometer and point the end of the cable at the light source. In the spectroscopy software, view the peaks in the emission spectrum to determine which wavelengths are emitted at higher intensity from the light source.

Students can see whether red light bulbs actually emit red light, or they can see that some white bulbs emit more blue light than other white light bulbs. They could combine emission spectroscopy data with their results of DPIP experiments to help explain differences in photosynthetic rates with different light sources.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

Part 1 — Pigment Chromatography and Absorbance of Light

1. Put on your safety goggles.

2. If your teacher has prepared the pigment extract using ethanol, continue to the next step. Otherwise, follow the procedure below for preparing this extract yourself.

   a. Cut three large spinach leaves into small pieces. Discard the stem of the leaves. Place the leaves into a mortar and add approximately 5–10 mL of ethanol to soak the leaves.

   b. Use a pestle to grind the spinach to help the ethanol dissolve the pigments in the leaves. Continue grinding for at least 3–5 minutes.

   c. Place a piece of cheesecloth or a coffee filter over the top of a beaker. Filter the spinach mixture from the mortar, taking care to prevent the cheesecloth or filter paper from falling into the beaker.

      NOTE: You can squeeze the cheesecloth to help the liquid pass through.

3. Use a pencil to draw a line across a square of chromatography paper 2 cm above the bottom edge of the paper.

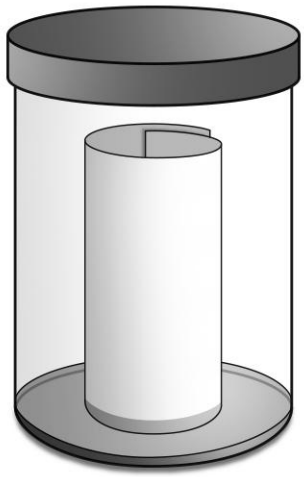
4. Make a green line of pigment extract on or slightly above the pencil line as follows:

   a. Place a capillary tube or the tip of an eye dropper into the pigment extract. Place your finger over the top of the tube or dropper to prevent the liquid from falling out when you remove it from the beaker.

   b. Place the capillary tube or pipet tip at the edge of the chromatography paper and release your finger slightly from the top of the tube, allowing a small amount of the green extract to absorb into the paper while moving the capillary tube or dropper across the paper. As needed, acquire more extract in the capillary tube or pipet tip. Be sure to keep the extract at or above the line.

   c. Let the extract dry on the paper and then repeat the process once.

5. Roll the paper into a cylinder and staple or paper clip the paper where the edges meet to prevent it from unrolling. Place the paper into a chromatography chamber that contains a small volume of solvent at the bottom. It is important that the pencil line be above the solvent.



6. Seal the chromatography chamber and observe the movement of the solvent up the paper. Leave the chamber undisturbed until the solvent moves most of the way up the paper. While you wait, continue with the questions and procedures that follow.

   NOTE: Do not allow the solvent to reach the top of the paper.

7. For some chromatography investigations, water is used as the solvent. In this investigation, the solvent is a solution that contains acetone (C_3H_6O). Why is an organic solvent used in this chromatography experiment rather than water?

   The plant pigments are large organic molecules that are mostly nonpolar and do not dissolve in water, which is polar. For the pigments to move up the paper with the solvent, they must be attracted to the solvent. Organic solvents such as petroleum ether and acetone are nonpolar and will attract the pigments.

8. Describe the location of pigments within plant leaf cells. Be specific.

   Pigments are located within chloroplasts, specifically, they are embedded in the thylakoid membranes of chloroplasts. The pigments are in groups and are the molecules that comprise photosystems.

9. Connect the colorimeter to your data collection system and calibrate the sensor with a blank that contains ethanol.

10. Fill a clean colorimeter cuvette about three-quarters full with ethanol. Add 10–15 drops of pigment extract to the ethanol, cover, and invert the cuvette to mix. The mixture should appear light green and transparent. Place the cuvette into the calibrated colorimeter.

11. Record the *absorbance* of light for each of the four colors the colorimeter detects.

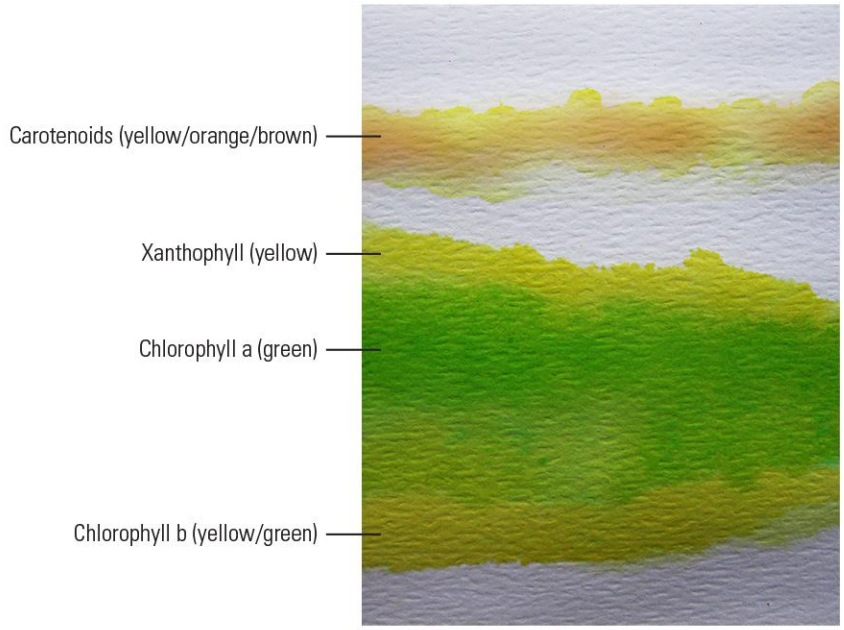
Absorbance of light in pigments from spinach leaves

Absorbance			
Blue Light	**Orange Light**	**Red Light**	**Green Light**
1.504	0.515	0.813	0.464

12. For the pigment extract, which color of light has greatest absorbance? Which color of light has the lowest absorbance in the sample? Provide an explanation for these results.

    Blue light has the greatest absorbance, 1.579, and green light has the lowest absorbance, 0.688. Because the leaves are green, green light is not absorbed by the leaf pigments but is reflected. Pigments, such as chlorophyll, absorb blue light the best.

13. When the paper chromatography is complete, remove the paper from the chromatography chamber and sketch the results in your lab notebook. Lay the paper flat to dry.



14. How many different types of pigments are present in spinach leaves? Provide evidence to support your claim.

Answers may vary; typically students will observe at least 4 pigments. When chromatography is complete, there are four distinct color bands on the paper, indicating the presence of four different pigments.

15. The absorbance of green light is low for a sample extracted from green leaves. However, the absorbance is not zero. Which pigments absorb green light in the spinach leaves?

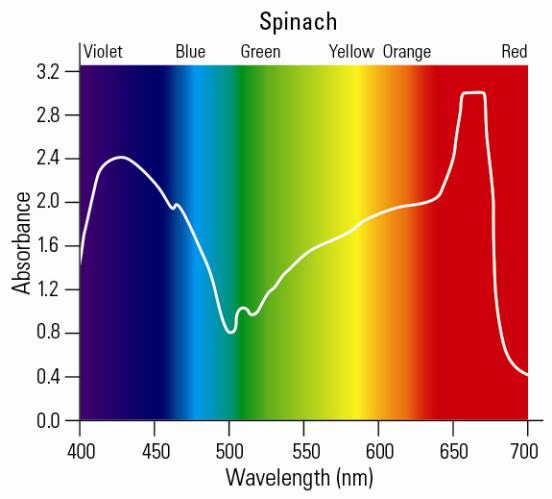
The non-green pigments such as carotenoids (which appear orange, orange-brown, or orange-yellow on the paper) and xanthophylls (which appear yellow on the paper) absorb green light.

16. Use a PASCO Wireless Spectrometer and corresponding software to observe the full absorbance spectrum for the pigment extract:

    a. Connect the spectrometer to the PASCO spectrometer application using the Bluetooth® or USB connection. Conduct the light calibration using the cuvette blank—a glass cuvette that contains ethanol.

    NOTE: Do not use the plastic cuvettes with organic solvents.

b. Use the same diluted extract as before (from the cuvette three-quarters full with ethanol plus 10–15 drops of pigment extract) and transfer the solution to a clean spectrometer cuvette. Draw a sketch or print a record of the spectrometer data.



Part 2 — Measuring Photosynthetic Activity with DPIP

NOTE: For this part of the investigation, use the spinach chloroplasts your teacher prepared by blending spinach with an ice-cold sucrose solution. Keep the sample on ice for the DPIP procedure.

17. DPIP (2, 6-dichlorophenolindophenol) is a blue-colored compound. When reduced, it turns colorless.

    DPIP (blue) + $2H^+$ + $2e^-$ → Reduced DPIP (colorless)

    In the following procedure, DPIP acts as an electron acceptor for excited electrons.

    a. Given what you know about the light-dependent reactions of photosynthesis, describe how electrons in chloroplasts become *excited*.

    Electrons become excited in chloroplasts when pigments (in photosystem I or II) absorb photons (light).

    b. In this investigation, excited electrons will be transferred to DPIP. In a typical photosynthesis reaction, what happens to these excited electrons?

    The electrons are transferred to other molecules that comprise the electron transport chains in the thylakoid membrane and are eventually transferred to the electron acceptor $NADP^+$, forming NADPH.

18. Connect the colorimeter to your data collection system so you can measure the change in DPIP color under different conditions.

19. Label the caps of three colorimeter cuvettes "1," "2," and "3." Use two clean pipets to add phosphate buffer and distilled water to the cuvettes in the volumes specified in Table 1.

Table 1: Set up for the DPIP photosynthesis experiment

Cuvette Contents	Cuvette 1 (blank)	Cuvette 2 (placed in light)	Cuvette 3 (placed in the dark)
Phosphate buffer	1 mL	1 mL	1 mL
Distilled water	4 mL	3 mL	3 mL
Chloroplast suspension	3 drops	3 drops	3 drops
DPIP	none	1 mL	1 mL

20. Cuvette 1 is a blank, a cuvette that contains the same contents as the other cuvettes but lacks the colored DPIP. Add 3 drops of chloroplast suspension to Cuvette 1 and invert it a few times to mix the contents. Place the cuvette into the colorimeter and calibrate the sensor.

22. Add 3 drops of chloroplast suspension to Cuvette 2 and 3 drops to Cuvette 3.

23. Use a clean pipet to add DPIP to Cuvette 2 and invert the cuvette to mix the contents. Place Cuvette 2 into the colorimeter. Record the initial transmittance or absorbance for the suspension. Repeat the process for Cuvette 3.

    NOTE: Whether you record transmittance or absorbance, and for one color or more, are decisions left up to your group. Create a data table to organize the results of this part of the investigation.

Change in light absorbance and transmission for a chloroplast suspension that contains DPIP

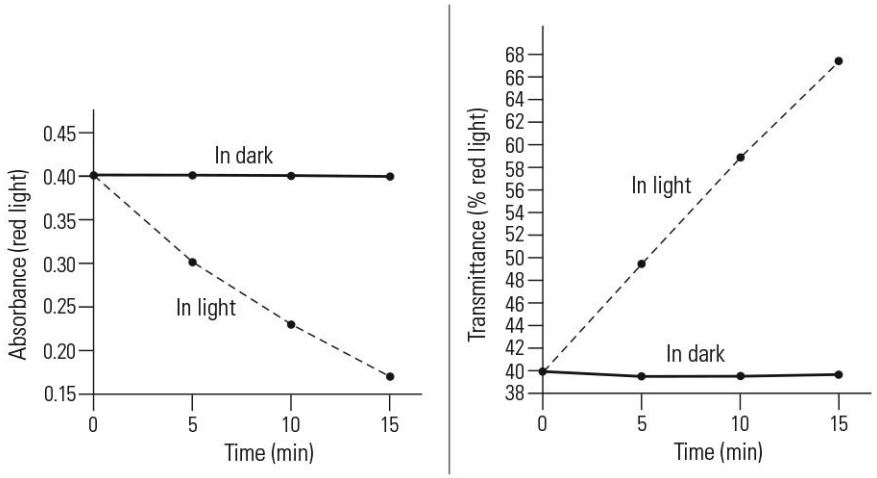
Cuvette and Conditions	Colorimeter Measurements							
	Absorbance (red light)				Transmittance (% red light)			
	Initial	5 min	10 min	15 min	Initial	5 min	10 min	15 min
#2 (in light)	0.401	0.305	0.232	0.171	39.8	49.5	58.7	67.4
#3 (in dark)	0.402	0.405	0.404	0.403	39.7	39.4	39.5	39.6

24. Place Cuvette 2 near a bright light source. Wrap Cuvette 3 completely in foil and place it next to Cuvette 2.

25. After 5 minutes, 10 minutes, and 15 minutes, record the transmittance or absorbance of light for Cuvettes 2 and 3. Be sure to invert the cuvettes to mix the contents well before recording measurements. Also, unwrap Cuvette 3 each time, make the measurement, and then re-wrap it.

    NOTE: It is a good science practice to place the blank in the colorimeter each time to ensure the transmittance of the blank continues to read 100% (or the absorbance reads 0.00).

26. Create an appropriately labeled graph to illustrate the results of the DPIP investigation.

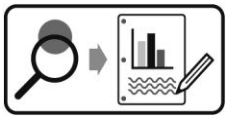


27. Explain any difference in results for Cuvettes 2 and 3.

The absorbance or transmittance of light changes significantly in Cuvette 2 but remains the same for Cuvette 3. The change in color in Cuvette 2 indicates that electrons were excited within the chloroplasts and transferred to DPIP, turning the compound colorless. The excited electrons were generated due to light absorption by pigments. Cuvette 3 was in the dark and did not generate excited electrons, so DPIP remained blue.

Design and Conduct an Experiment

The Initial Investigation provides students with a comprehensive study of plant pigments and their role in photosynthesis. Student-designed experiments may focus on the use of one or more of the techniques utilized in the Initial Investigation—chromatography, colorimetry, spectrometry, or DPIP reduction—to investigate differences between different leaves or different pigments, or explore factors that affect photosynthesis.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Is the rate of photosynthesis affected by the distance between the chloroplast suspension and the light source?*
- How do the chromatograms and absorption spectra for other leaves compare to spinach leaves?*
- Can active chloroplasts be obtained from frozen spinach or cooked spinach?
- How does the rate of photosynthesis, as measured by the relative amount of DPIP reduced, compare for chloroplast suspensions prepared from different leaf types?
- Is the rate of photosynthesis affected by the wattage of the light bulb?
- Can chromatography be used to determine the different pigments in flowers?
- Will photosynthesis occur under a UV light?
- What are the absorption spectra for each type of pigment (chlorophyll *a*, chlorophyll *b*, carotenoids, and xanthophylls)?
- Can accessory pigments pass electrons to DPIP or does chlorophyll have to be present?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Is the rate of photosynthesis affected by the distance between the chloroplast suspension and the light source?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected the rate of respiration. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

      The data supports the hypothesis that lower light levels result in a slower rate of photosynthesis. The absorbance of red light decreased at a slower rate for the cuvettes placed 60 cm from the light source than the cuvettes placed 40 cm and 20 cm from the light source. The cuvettes closest to the light source experienced a decrease in absorbance from 0.401 to 0.193 over the course of 15 minutes and the blue color of the solution was noticeably lighter after 15 minutes. The cuvettes placed 60 cm away from the light source lightened only slightly in color and the absorbance decreased from 0.402 to 0.344.

   b. Based on the evidence you collected, explain why the results occurred.

      All of the cuvettes contained chloroplasts that had the ability to carry out photosynthesis. When exposed to light, electrons in chlorophyll and other pigments in the chloroplasts become excited and the energy of the electrons is used to drive the light reactions of photosynthesis. DPIP accepts these electrons and changes from blue to colorless. The greater the photosynthetic activity of the chloroplasts, the more DPIP molecules are reduced by available electrons. The cuvettes closest to the light source had the most active chloroplasts, due to increased light exposure; in other words, there were more excited electrons generated in the chloroplasts inside of the cuvettes placed at 20 cm from the light source.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

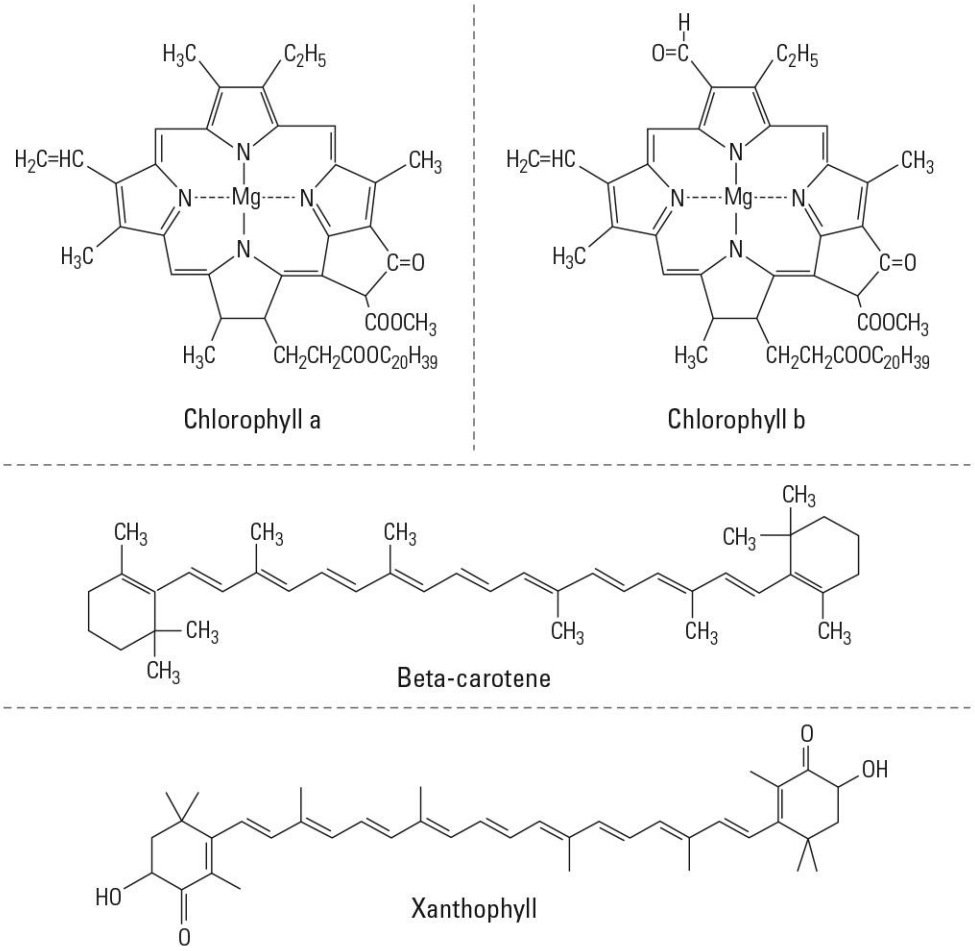
   There is no evidence that experimental error or uncontrolled variables affected the results. The data for the 3 replicates set at each distance was similar, indicating that variables were well controlled during data collection.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. Refer to the diagrams below illustrating the structures of four common plant pigments.



a. Which pigment is more polar, chlorophyll *a* or chlorophyll *b*? Justify your answer by describing aspects of the pigment structure that relate to its polarity.

Chlorophyll *b* is more polar than chlorophyll a due to the presence of the functional aldehyde group (–CHO) on chlorophyll *b* where chlorophyll *a* has a methyl group ($–CH_3$). The aldehyde on chlorophyll *b* attracts water and the unequal sharing of electrons between oxygen and hydrogen makes that part of the pigment molecule polar.

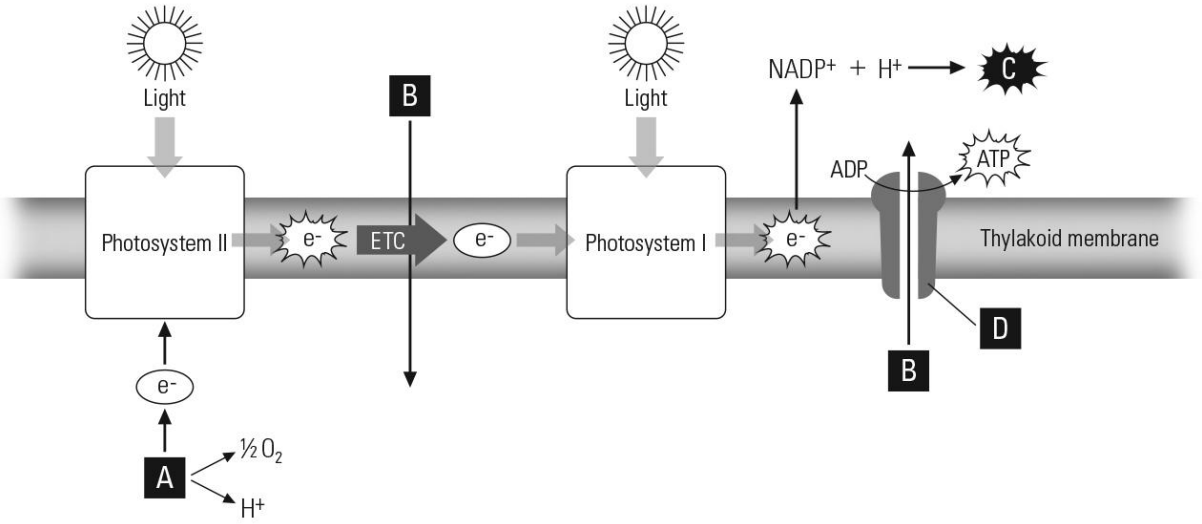
b. Which pigment is least polar, beta carotene or xanthophyll? Explain your reasoning for your choice.

Because beta carotene has no hydroxide groups (–OH) it is the least polar molecule. While xanthophyll is long and mostly nonpolar, its two hydroxide groups and the oxygen make it more polar than beta carotene.

c. How do the structures of these pigments relate to the process of paper chromatography?

The different levels of polarity in these structures result in some of the molecules being more attracted to the chromatography solvent than others. The molecule most attracted to the solvent moves up the paper the farthest and the others travel less far. Since each pigment molecule's structure and polarity is different, each one ends up at different locations on the paper.

2. The diagram below shows events of the light reactions of photosynthesis.



a. Identify the items labeled A, B, C, and D.

"A" is a molecule of water, "B" is a hydrogen ion, "C" is a molecule of NADPH, and "D" is the structure ATP synthase.

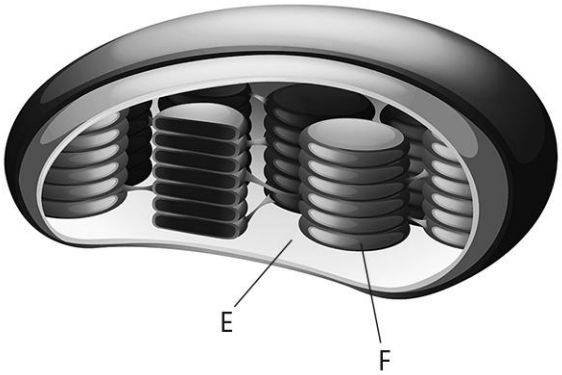
b. Of what importance is B to the function of D?

As hydrogen ions (B) are transported across the thylakoid membrane by the energy released as electrons move down the electron transport chain, they become concentrated in the thylakoid space. They then diffuse through a channel within ATP synthase, crossing the membrane into the stroma. This diffusion of hydrogen ions provides the energy to the ATP synthase complex to generate ATP from ADP.

c. Of what importance are C and D to the reactions of the Calvin cycle—the series of reactions that follow the light reactions?

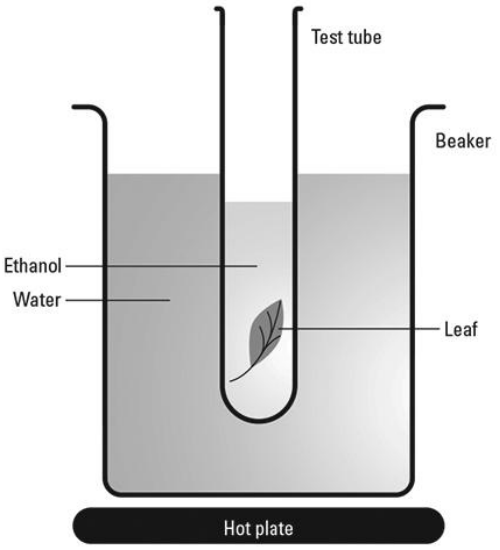
Production of carbohydrates from carbon dioxide in the Calvin cycle requires energy from both NADPH and ATP. So molecule C, NADPH, participates directly in the Calvin cycle, giving up the electrons it accepted in the light reactions. Structure D produces ATP molecules that are used in reactions of the Calvin cycle, providing energy to drive endergonic reactions.

d. Identify labels E and F on the diagram of a chloroplast. Describe the processes that occur in each area.



Label E points to the stroma, the space between the outer membrane of the chloroplast and the stacks of thylakoids. F points to one of these thylakoids. The light reactions of photosynthesis take place along the membrane of the thylakoids, where the photosystems and electron transport chains are embedded. The reactions of the Calvin cycle take place in the stroma.

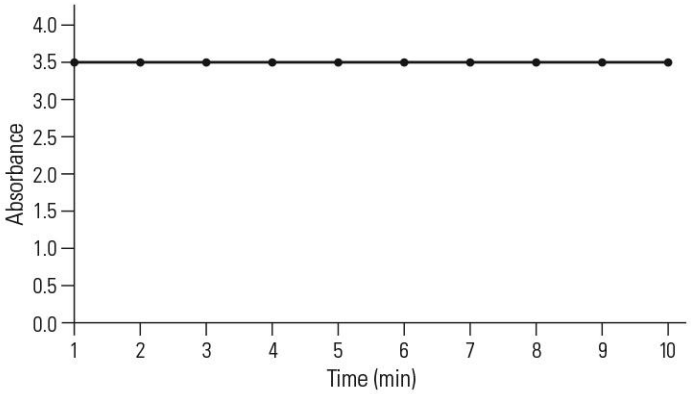
3. A student researches various methods of extracting chlorophyll from leaves. One method describes boiling leaves in ethanol, as illustrated:



The boiling process causes the ethanol to turn green and "bleaches" the leaf.

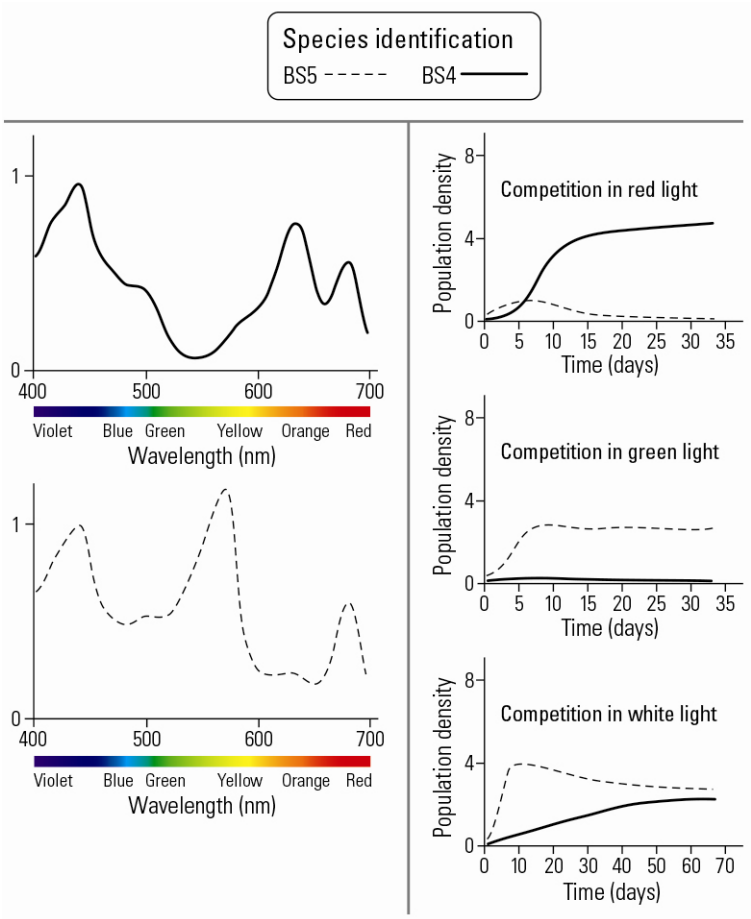
The student reads about this method in an investigation that tests leaves for the presence of starch and wonders whether this method might be used for a DPIP investigation similar to the Initial Investigation. He sets up a cuvette with the following contents: phosphate buffer, distilled water, DPIP, and 3 drops of the green-colored ethanol obtained after boiling the leaf in ethanol in a test tube, as described in the investigation. The cuvette is placed under a bright light source and the student measures light absorbance in the solution at 1 minute intervals for 10 minutes.

Copy the graph below and sketch a line on the graph to illustrate what you predict will happen to the absorbance of the solution in the cuvette the student set up. Explain the reasoning for your prediction.



The method of bleaching a leaf to extract chlorophyll will not result in active chloroplasts in the ethanol the leaves are boiled in. One reason is that the chloroplasts will likely remain in the leaf cells, since the leaves are not being blended or crushed. Secondly, boiling the leaves will destroy the structure of chlorophyll as well as the enzymes of the electron transport system and Calvin cycle, so photosynthesis will not be able to take place even in chloroplasts that end up in the ethanol. There will be no change in the color of DPIP if there are no active chloroplasts in the cuvette.

4. In the Baltic Sea, two similar species of photosynthetic picocyanobacteria were found to occupy water at a similar depth. One species, BS4, is blue-green in color and the other species, BS5, is red. Both species contain chlorophyll *a* but differ in the presence of certain accessory pigments: phycocyanin is found only in BS4 and phycoerythrin is found only in BS5. The absorbance spectra for the pigments in these species are shown below. Also shown are the results of experiments in which the species were grown together under different light conditions.[11]



a. Explain why the picocyanobacterium BS5 is red in color.

The least absorbance is in the red part of the spectrum, indicating that most red light is reflected by the pigments in BS5, so this is the color the organism appears.

b. Describe evidence from the absorbance spectra that both of the species contain chlorophyll *a*.

Both species' absorbance spectra have peaks in the blue and red ends of the spectrum. These are the colors of light that chlorophyll *a* absorbs.

c. Describe the results of growing BS4 and BS5 together in green light and provide a biological explanation for the results.

In green light, BS4 did not survive; the population density was zero or very close to zero. The population density of BS5, however, increased during the first 10 days or so and then leveled off as the carrying capacity was reached. The absorbance spectrum for BS5 indicates that the species contains a pigment that absorbs green and yellow wavelengths, the wavelengths of light emitted from a green light source. Therefore, BS5 was able to carry out photosynthesis and had the energy needed for reproduction (population growth). BS4 does not absorb this color of light and was not able to photosynthesize.

[11] Stomp, M. et al. Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature* (Impact Factor: 38.6). 12/2004; 432(7013):104–7. DOI: 10.1038/nature03044

d. Describe the results of growing BS4 and BS5 together in white light and provide a biological explanation for the results.

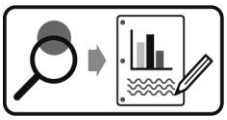
In white light, both species survived and experienced an increase in population density. The population of BS5 increased rapidly in the first 10 days or so and then leveled off. The population of BS4 increased steadily over the 65 days of the investigation. By Day 60, the populations of each species were nearly equal in density. The results indicate that these species can successfully coexist in the same habitat. The ability of each species to use wavelengths of light not used by the other species allows each to acquire enough energy to carry out photosynthesis.

e. If paper chromatography was performed using pigments extracted from BS5, what color or colors would you expect to see on the paper after chromatography is complete? Explain the reasoning for your choice(s).

There would be a red colored pigment on the paper, the same red pigment that makes the cells appear red in color. There would also be a green pigment on the paper, since the species also has chlorophyll *a* in its cells.

Design and Conduct an Experiment Key

Use one of the tools or techniques from the Initial Investigation to explore a question of your own related to plant leaves and pigments, or to photosynthesis.



Develop and conduct your experiment using the following guide.

1. Create a driving question: choose a factor that can be controlled in the lab and develop a testable question for your experiment.

   How is the rate of photosynthesis, as measured by DPIP, affected by the distance between the chloroplast suspension and the light source?

2. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   In their natural habitats, plants may be shaded by other plants, causing a reduction of the light available for photosynthesis. Placing the cuvettes at different distances from the light source will simulate providing plants with different amounts of light and will allow me to determine if plants can continue to carry out photosynthesis even in low-light conditions.

3. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable is the distance between the cuvettes and the light source. Cuvettes containing chloroplasts and DPIP will be placed at three different distances from the lamp: 20 cm, 40 cm, and 60 cm.

4. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable of the experiment is the change in light absorbance over time. Each suspension will be measured for initial absorbance of red light before being placed in light, and then the absorbance will be measured at 5 minute intervals. There will be 3 replicates at each distance and the change in absorbance for each distance will be determined by calculating an average change for the 3 cuvettes.

5. Write a testable hypothesis (If…then…).

   If amount of light affects photosynthesis, then the cuvettes placed 60 cm from the light source will experience a slower rate of change in absorbance over time than the cuvettes placed 20 cm from the light source.

6. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   The source of chloroplasts for the cuvettes will be the same for all cuvettes and each cuvette will contain 3 drops of the chloroplast suspension. The cuvettes will contain the same volume and concentration of phosphate buffer and the same volume and concentration of DPIP. Trials will be run at room temperature using a CFL light source that does not generate heat. Before each measurement is taken, cuvettes will be inverted to mix the contents well. The time interval between measurements will be kept constant for all cuvettes.

7. How many trials will be run for each experimental group? Justify your choice.

   There will be three replicates set up for each experimental group. The cuvettes are small and three of them can fit side by side directly in front of the light source. Collecting data for three trials simultaneously reduces the time needed for the investigation.

8. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The average change in absorbance of red light will be calculated for 20 cm, 40 cm, and 60 cm over the course of 15 minutes of light exposure. The change in absorbance will be graphed and the slopes of the lines will be compared to compare the rates of photosynthesis at each distance.

9. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

   The chloroplast suspension will be mixed well before taking a sample, but there may still be some variation in the number of chloroplasts contained in the 3 drops added to each cuvette.

   The time taken to place the cuvettes into the colorimeter to obtain measurements may vary, resulting in some cuvettes being in light for slightly more or less time than others.

   If the cuvettes are not wiped with a Kimwipe just before placement into the colorimeter, the absorbance measurement could be affected.

10. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

    Turn on a light source that is positioned to emit light at the sides of the cuvettes (instead of a top-down light source position).

    Put pieces of tape on the table surface at 20 cm, 40 cm, and 60 cm from the light source.

    Create a blank cuvette that contains all of the contents below except DPIP to calibrate the colorimeter.

    Add the following contents to 9 cuvettes:

    - 1 mL phosphate buffer
    - 3 mL distilled water
    - 3 drops chloroplast suspension
    - 1 mL of DPIP

    Work quickly after adding DPIP to measure the initial absorbance of red light for the 9 cuvettes.

    Put 3 cuvettes side-by-side on top of the first piece of tape. Place 3 cuvettes on the 40 cm piece of tape and at 60 cm.

    After 5 minutes of light exposure, remove the cuvettes one at a time and quickly measure the absorbance of light. Return the cuvettes to the proper position in front of the light after taking measurements.

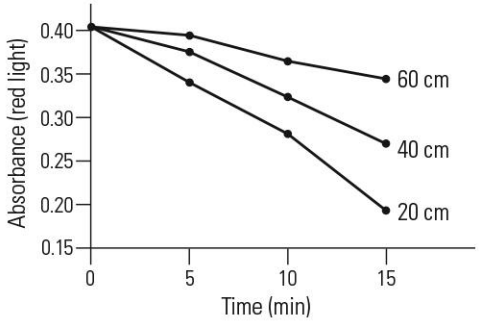
    Repeat data collection at 10 minutes and 15 minutes.

11. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

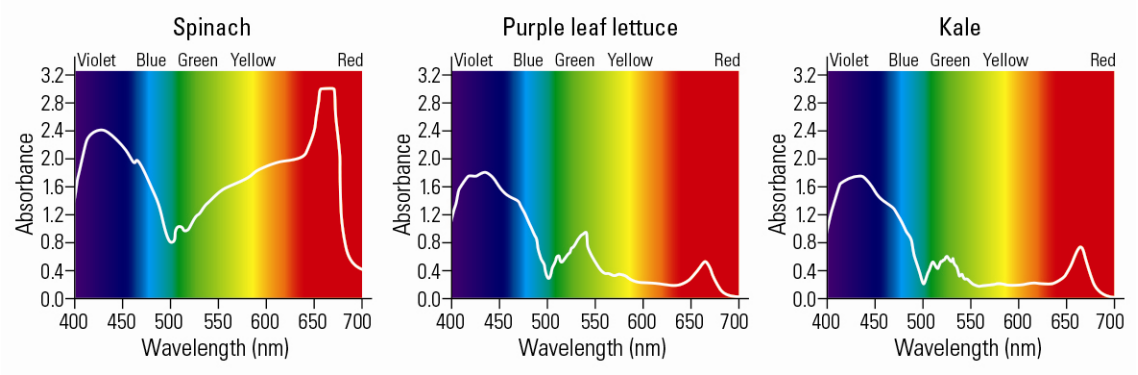
Light absorbance for a chloroplast suspension with DPIP at different distances from the light source

Distance from Light Source (cm)	Colorimeter Measurements			
	Absorbance (Red Light)			
	Initial	5 min	10 min	15 min
20	0.401	0.340	0.258	0.193
40	0.402	0.373	0.324	0.277
60	0.402	0.393	0.366	0.344



Absorbance of light of four colors measured by a colorimeter

Leaf Sample	Chromatogram Observations	Absorbance			
		Blue Light	Orange Light	Red Light	Green Light
Spinach	There are four pigments present: chlorophyll *a* and *b*, xanthophylls, and carotenoids	1.504	0.515	0.813	0.464
Purple leaf lettuce	Both chlorophylls are present as well as xanthophylls, but the carotenoids seem to be missing. There seems to be an additional pigment below chlorophyll *a*.	1.578	0.197	0.266	0.188
Kale	Contains the same pigments as spinach.	1.594	0.166	0.334	0.078



11. TRANSPIRATION

Lab Overview

Students investigate the rate of transpiration in plants under normal and humid conditions and have an opportunity to conduct inquiry experiments of their own design to test additional factors. In the Initial Investigation, students create a potometer using a pressure sensor and they monitor microclimate conditions using a weather sensor. The potometer measures transpiration by detecting the change in pressure due to the evaporation of water from the leaves of a plant sample.

The Initial Investigation provides students with sample data from a "whole-plant" transpiration experiment to introduce students to this alternate method of measuring transpiration. For experiments of their own design, students can use either the potometer method or whole-plant method to measure how certain factors affect the rate of transpiration.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	25 min
Initial Investigation	45 min

Student-Designed Experiment	
Experiment Design	15 min
Experiment w/potometer, 1 day Experiment w/whole-plant, 2–3 days	45 min total
Data Analysis	30 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.A.3, 2.B.2, 2.C.2, 2.D.1, 2.D.3, 4.A.6
Science Practices	4.2, 5.1, 5.3, 6.1, 6.2, 7.1
Learning Objectives	2.9, 2.11, 2.12, 2.15, 2.21, 2.28, 4.14

Materials and Equipment

For Each Student Station

- Data collection system
- Low pressure sensor (barometer)
- Weather sensor
- Sensor extension cables (2)
- Quick-release connector[1]
- Clear plastic tubing, 40–50 cm[1], with a one-hole rubber stopper on one end
- Large tub or bucket (for water)
- Paraffin film or petroleum jelly *(if available)*
- Plant sample containing numerous leaves[2]
- Base and support rod
- 3-finger clamps (2)
- Test tube clamp
- Clear plastic bag, 1 gallon
- Spray bottle with water
- Electronic balance, centigram
- Small syringe, 60-mL or larger, without needle
- Pipet
- Metric ruler
- Large scissors or small pruning shears

[1]Included with most PASCO pressure sensors.

[2]The plant sample should have a woody stem that fits tightly in the narrow diameter of the clear tubing. Suggestions include ornamental pear, oleander, hydrangea, and gardenia.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Additional equipment recommended for the student-designed experiment:

- EcoChamber™ containers (at least 2), including stoppers
- Weather sensor for each EcoChamber container
- Carbon dioxide gas sensor
- Small plants that fit in the EcoChamber container[1]
- Clear plastic bags and twist-ties to cover the root ball (or quart or gallon zip-close bags)
- Additional plant samples (different species) that fit in the tubing of the potometer[2]
- Electronic balance, centigram
- Small fan
- Lamp with incandescent or UV bulb that provides heat
- Lamp with a CFL bulb that remains cool

[1]Plants recommended for the EcoChamber container and whole-plant transpiration investigations include pansy, marigold, and impatiens.

[2]Encourage students to look for plant samples on the school campus and choose plants that appear to be adapted to different environments.

Prerequisites

Students should be familiar with the following concepts:

- Properties of water: cohesion, adhesion, and hydrogen bonding.
- The relationship between volume and pressure is an inverse relationship. If the volume of a gas increases, molecules move about within a greater space and the pressure decreases due to fewer molecular collisions.
- Water potential is the driving force behind transpiration; a water potential gradient moves water through a plant from the roots to the leaves, and eventually into the air (the region of lowest water potential). Solute concentration and pressure are two components of water potential.
- Plants have vascular structures specialized for water transport.
- Plants must balance their requirements for water and carbon dioxide with the risk of excessive water loss through evaporation. Gas exchange is regulated by the opening and closing of stomata.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Handle sharp objects carefully.
- Avoid contact with eyes or skin when handling plant materials. Wash hands thoroughly after touching plants.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Acquire plant samples

    - For potometer method: Select plants with woody stems the size of the inside diameter of the plastic tubing. The stems need to be sturdy enough to insert into the tubing without breaking. Cuttings from trees or shrubs will work and students can find their own samples from plants on campus. Alternatively, you can bring in samples from home or purchase a few plants from a nursery. Examples include ornamental pear, oleander, hydrangea, and gardenia.

    - For whole-plant transpiration method (this method may be chosen by students for their own experiments): Purchase 6-packs of small plants from a local nursery. Examples include pansies, primroses, and impatiens.

2. Fill several large tubs or buckets with water.

3. Assemble the base and support rod setup for each group, attaching a test tube clamp and two 3-finger clamps to each support rod (refer to the diagram in the Initial Investigation).

   NOTE: If you wish to reduce the time needed by students for the Initial Investigation, you can also attach the sensors to each base and rod setup. The barometer (low pressure) sensor and a weather sensor should be attached to the support rod using the 3-finger clamps.

4. Prepare the tubing for the potometers by inserting one end of the tubing through a one-hole stopper (#6 or similar size) and extending the tubing 3–5 cm beyond the top of the stopper.

Teacher Tips

Tip 1 – The potometer

NOTE: We recommend that you practice the potometer assembly steps so you are able to help students troubleshoot this procedure.

TUBING ASSEMBLY

- In order for transpiration to occur, it is critical that there is an unbroken water column that extends from the water in the tubing to the water in the xylem of the plant. Any air bubbles in the tubing or at the cut surface of the stem will affect the data. This is why the tubing and plant stem must remain submerged in the tub of water during the setup.
- The assembly is difficult for one person to coordinate. Students should work in pairs to complete the procedures.
- The stem of the plant sample must fit tightly in the opening of the tubing. If students collect plant samples from the schoolyard or home, they should take the tubing outside with them to test for a good fit of the stem in the tubing. When using plants obtained this way, students do not need to immerse the plant samples in water in the field. They can cut the sample from a plant in the schoolyard and then cut the stem again (as specified) during the procedure of the Initial Investigation.
- To ensure an airtight seal, a small piece of paraffin film can be wrapped around the tubing edge where the plant stem is inserted. Alternatively, a dab of petroleum jelly can be applied at the edge of the tubing. However, the paraffin film or jelly is not sufficient to provide a seal if there is a gap between the stem and tubing. *Avoid getting jelly in the tubing as it is difficult to remove.*

ATTACHING THE BAROMETER SENSOR

- The sensor measures the air pressure in the small gap between the water in the tubing and the quick release connector of the sensor. Here is an explanation that may help students understand why the pressure changes when transpiration occurs:

  > As the plant loses water through its leaves—transpiration—the plant draws water from the tubing into its xylem. Thus the volume of water in the tubing decreases slightly, increasing the volume of the air gap near the barometer. The barometer detects the resulting decrease in pressure; the larger air gap, with no change in the number of molecules, means there are fewer molecular collisions between the trapped air molecules and their container.

- The quick release connector needs to fit tightly in the tubing but it is not necessary to insert it completely. It just needs to be tight enough that the connector doesn't easily come out.

Tip 2 – Time management

- Although 10 minutes is recommended as the length of time for data collection, potometer runs may be shortened to 7 minutes if a clear trend is observed in this time.
- For the whole-plant method, sufficient data could be collected in 48 hours rather than 72 hours. While 72 hours is recommended, if time is limited, the opportunity for student-designed inquiry should not be abandoned.

Tip 3 – Whole-plant transpiration method

The Initial Investigation provides students with data from a whole-plant transpiration experiment. Students may choose this method for their student-designed experiments. The following procedure was used in the PASCO trial to collect the sample data provided.

a. Saturate the soil of the plant with water the day before beginning the experiment. If flowers are present, remove them as well as any dry leaves.

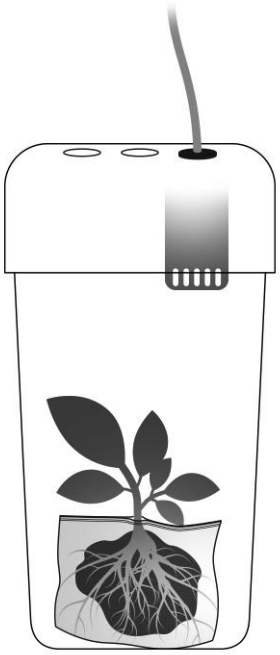
b. Using the sensor extension cable and stoppers, suspend the weather sensor in the EcoChamber container.

c. Create a display of one or more of the relevant weather sensor measurements and reduce the sample rate. One sample every 30 minutes or once per hour will provide sufficient data.

d. Remove the plant sample from its packaging and place the root ball (including soil) of the plant into a plastic bag (or use plastic wrap). Seal the bag or plastic wrap around the stem of the plant so that the leaves remain uncovered.

e. Measure and record the initial mass of the plant, including the plastic.

f. Place the plant inside the EcoChamber container and secure the lid as shown.



g. For control conditions, the holes of the EcoChamber container were left open. For the humid condition, water was added to the bottom of the chamber to a depth of 1 cm, and all holes of the chamber were sealed with rubber stoppers.

   NOTE: For other conditions tested in PASCO trials (such as the room temperature versus warm temperature trial) the holes of the chamber were left open.

h. Record the final mass of the plant and plastic bag together after 48 or 72 hours. Calculate the percent change in mass to normalize any difference in starting mass between the plants.

i. Determine the total leaf surface area for each plant and compare the percent change in mass per cm^2 for each plant and condition to determine relative rates of transpiration.

Tip 4 – Leaf surface area

The Initial Investigation uses the same plant sample for the control and humid conditions, eliminating the need to "normalize" data by determining the surface area of the leaves. However, the various student-designed experiments will likely need to take into account leaf surface area to provide a valid comparison between experimental groups. In the PASCO trials, leaf surface area was determined by mass, following the procedure below. A leaf tracing method could also be used, but is not described here.

a. Remove all of the leaves from the plant sample and find the collective mass of the leaves.

b. Cut five 1-cm^2 pieces from the leaves and find the collective mass of the five pieces using a centigram balance. Obtain the average mass/cm^2.

c. Knowing the mass per cm^2 of the leaves and the total mass of all the leaves, calculate the total surface area for the plant:

$$\frac{\text{total mass}}{\text{mass/cm}^2} = \text{total surface area (cm}^2\text{)}$$

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

1. Put on your safety goggles.

2. Use the two 3-finger clamps to attach the barometer (low-pressure sensor) and weather sensor to the base and rod stand. Connect the sensors to your data collection system using extension cables.

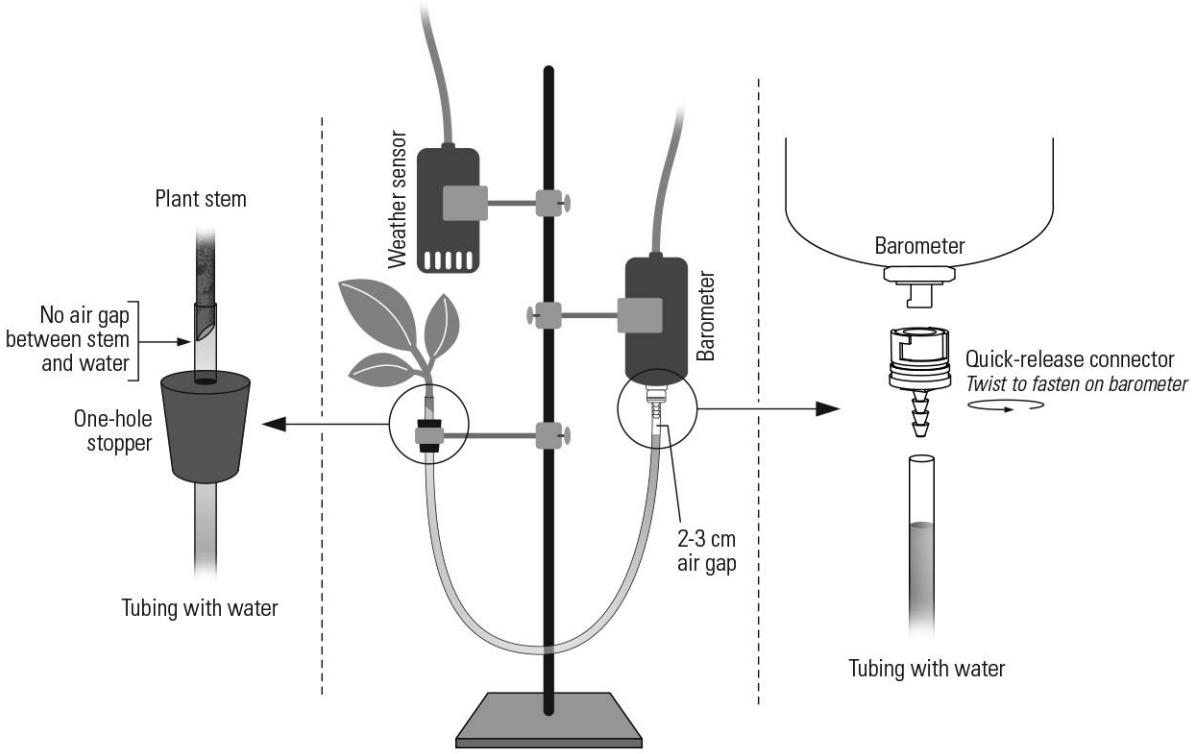
3. Create a graph display of Barometric pressure versus Time. If possible, set the sampling rate to one sample every 30 seconds. If your data collection system allows for an automatic stop condition, set the stop time for 10 minutes.

   NOTE: The units for barometric pressure default to inHg (inch of mercury) on the data collection system. These units can be changed within the system to the SI unit kPa (kilopascals).

4. Create one or more displays for Relative humidity (%) and Temperature (°C); these measurements are detected by the weather sensor and provide information about the microclimate surrounding the plant sample in the investigation.

SET UP THE POTOMETER

The diagram below illustrates the *potometer*, the apparatus that will detect transpiration in the plant sample.



5. To set up the potometer, perform the following steps using a large bucket or tub of water.

   a. Check that one end of the plastic tubing extends 3–5 cm past the stopper. Submerge the entire length of tubing in the tub of water.

      NOTE: It is important to keep the plastic tubing submerged in a tub or sink while preparing the plant and tubing of the potometer.

   b. Fill the syringe with water and attach it to one end of the tubing. Push the plunger on the syringe to fill the tubing with water. Watch the open end of the tubing—the tubing is filled with water when air bubbles no longer exit the tubing. Keep the tubing submerged in the water.

   c. Holding the stem of the plant sample under water, use sharp scissors to cut the plant stem at a 45° angle. Immediately insert the cut end of the stem into the short section of the tubing that extends past the rubber stopper. The stem should fit tightly in the opening of the tubing.

      NOTE: If paraffin film or petroleum jelly is available, it can be used to help obtain an airtight seal. Use petroleum jelly carefully; it is difficult to remove from the tubing. Neither will create an airtight seal if the plant stem does not fit tightly in the tubing.

6. To prevent water from spilling out of the tubing, hold the tubing in a "U" shape and remove the tubing and plant sample from the water. Place the stopper in the test tube clamp on the base and support rod and tighten the clamp to secure the stopper. Be sure the plant remains upright.

7. Create a 2–3 cm air gap at the other end of the tubing—the end that will connect to the barometer. Either flick the tubing or use a pipet to remove a small amount of water, creating the air gap.

8. Insert the quick-release connector into the tubing far enough so it does not easily come out. Attach the connector to the barometer sensor and twist it to complete the connection between the sensor and the tubing.

   Check that your setup looks like the one illustrated on the previous page.

   - There must be an unbroken water column that extends from the water in the tubing to the water in the xylem of the plant sample in order for transpiration to occur. Any air bubbles in the tubing or at the surface of the cut stem will affect the data. Additionally, an air gap is important to have near the barometer sensor to be able to detect the small changes in pressure that result from the movement of water from the tubing into the plant xylem and through its leaves.

COLLECT DATA: NORMAL CONDITIONS

9. Leave the potometer undisturbed for at least one minute before beginning data collection. Collect data for 10 minutes. Answer the following questions while you wait.

10. If transpiration occurs in the plant sample, what will happen to the water in the tubing? What will happen to the pressure in the tubing? Explain your answer.

    As transpiration occurs, water will leave the plant through evaporation from the leaves. The water in the tubing will be pulled up into the plant to replace the water lost through transpiration. As the plant pulls water out of the tubing, the volume of the air gap at the end of the tubing connected to the barometer increases; the pressure in the air gap between the water column and the sensor will decrease. Because the tubing is air tight, as the volume of the air gap increases, the pressure exerted by the trapped air molecules in the gap decreases. Pressure and volume in a closed system have an inverse relationship.

11. What will happen if the seal between the plant stem and tubing is not air tight?

    If air gets between the cut end of the plant stem and the water in the tubing, the air pressure inside the tubing will equal the air pressure outside the tubing. Additionally, any air bubbles in the system will break the water column and the plant will no longer be able to pull water through its xylem. In either case, transpiration can no longer be measured by the barometer.

12. Consider the microscopic structure of a leaf.

    a. Through which structures does water evaporate from a plant? Be as specific as you can in your answer.

       Water moves out of a leaf through stomata, small openings in the epidermis of the leaf.

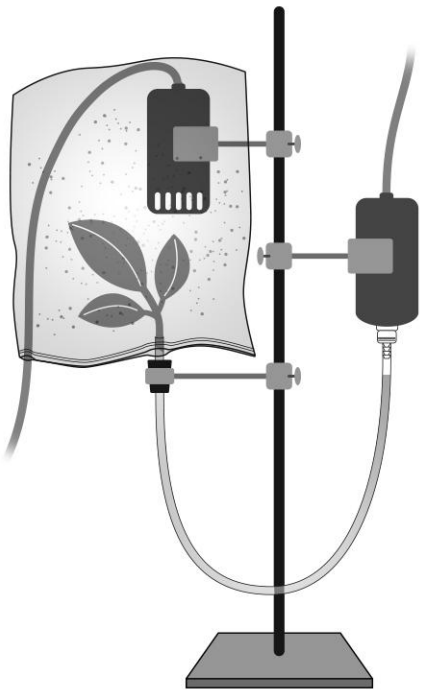
    b. Do you expect that these structures are the same regarding their location and density in the leaves of different plant species or do they vary? Explain your reasoning.

       Plants can adapt to environmental conditions that affect water loss by varying the density and location of their stomata. For example, plants adapted to drier conditions may have a lower density of stomata in order to prevent excess water loss. Plants such as cacti have reduced leaves (spines) with no stomata at all. Their stomata are located on the body of the plant. Plants that float on water may have stomata located only on the top of the leaves rather than underneath. (Most land plants have more stomata in the lower epidermis compared to the upper epidermis.)

13. After data collection stops, keep the plant sample, sensors, and other potometer components in place. Reset the pressure in the tubing by carefully twisting the quick-release connector to remove it from the sensor. Then reconnect it to the sensor so the tubing and sensor are once again connected.

COLLECT DATA: ALTERED CONDITION

14. Cover the plant sample and the weather sensor with a large, clear plastic bag. Mist the inside of the bag with water from a spray bottle and seal the plastic bag. Leave the system undisturbed for one minute and then begin data collection. Answer the following questions while the system records data for 10 minutes.



15. What environmental condition(s) that can be measured by the weather sensor do you expect to change in the plant's microclimate as a result of the plant being covered with the misted bag?

    By misting the air around the plant and trapping it inside the bag, the humidity in the enclosed space will increase.

16. The amount of water vapor in the air affects the water potential of the air. The rate of transpiration is directly related to the water potential difference between the leaves and the atmosphere.

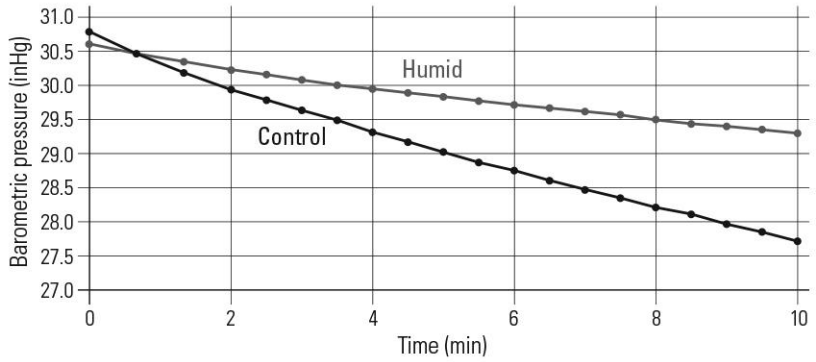
    a. Based on your knowledge of water potential, how should the water potential of the air in the "normal condition" test compare to the water potential of the air surrounding the plant covered with the bag?

       Adding water to the air around the leaves raises the water potential of the air. The air around the leaves in the first trial had a lower water potential than the microclimate of the covered plant.

b. How does water potential relate to transpiration? Predict whether or not you expect the rate of transpiration to be the same in both conditions? Explain your prediction.

Water moves along a water potential gradient, moving from regions of high water potential to regions of low water potential. There is a gradient of water potential from the soil around the roots to the air around a plant. Water molecules in the air spaces of the spongy tissue within a leaf move into the air, since the air has a lower water potential than the leaf. The drier the air around the leaf, the lower the water potential of the air and water will evaporate more quickly. Therefore, the rate of transpiration should be less in the second condition in which the leaves are surrounded by wetter air.

17. End data collection after 10 minutes. Print or sketch a graph of Barometric pressure versus Time to preserve a record of your data. Clearly label each run of data. Also, for each run, record the minimum, maximum, and average relative humidity (%) and temperature during each 10 minute period.



Transpiration under normal and humid conditions

Environment	Minimum Temperature (°C)	Maximum Temperature (°C)	Average Temperature (%)	Minimum Humidity (%)	Maximum Humidity (%)	Average Humidity (%)
Normal conditions (control)	21.6	21.7	21.6	36.1	37.2	36.7
Humid conditions	20.9	21.3	21.1	67.7	75.0	71.5

18. Create a data table to summarize the results for the two conditions and compare the rates of transpiration using the change in pressure/minute.

Potometer: Comparing the rate of transpiration under normal and humid conditions

Environment	Initial Pressure (inHg)	Final Pressure (inHg)	Change in Pressure (inHg)	Rate of Transpiration[1] (inHg/min)	Rate of Transpiration[2] (inHg/min)
Normal conditions	30.79	27.75	–3.04	–0.304	–0.294
Humid conditions	30.63	29.31	–1.32	–0.132	–0.124

[1]The rate was determined by dividing the overall change in pressure by 10 minutes.

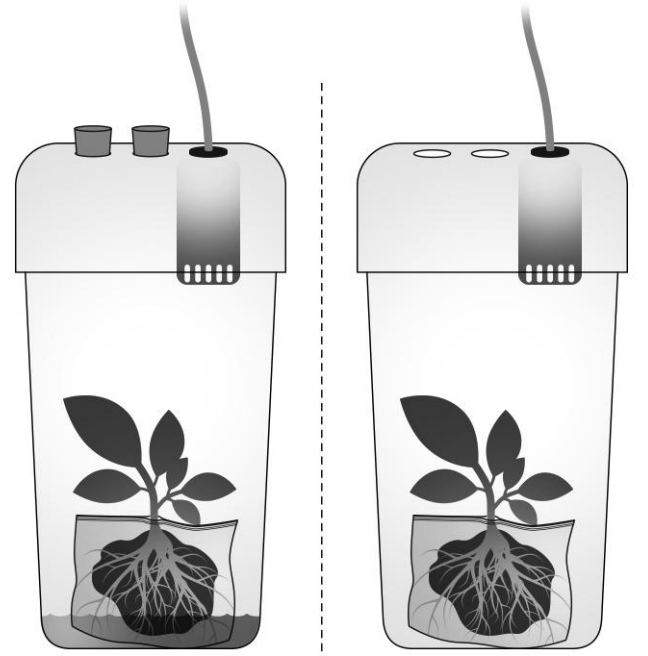
[2]The rate was determined by applying a linear fit to the data to find the slope of the line.

19. Was your prediction correct? Explain any differences between the control data (normal conditions) and the data collected for the covered, misted plant (altered condition). Use data from both sensors to support your explanation.

Answers will vary depending on the student's prediction. If the student predicted that the rate of transpiration under humid conditions would be less than the control conditions, the prediction is supported.

The rate of transpiration in the control (normal) conditions was greater because humid conditions slow the rate of evaporation from a plant. Under the control (normal) conditions, the air around the leaves was measured at an average relative humidity of 36.7% while the average relative humidity of the leaves covered with a misted bag was 71.5%. The water potential of the air in the humid conditions was higher than under the control conditions. Since there was less of a gradient between the water potential of the leaves and that of the air, water evaporated more slowly in the humid conditions. The temperature for the control condition averaged 21.6 °C and averaged 21.1 °C for the humid conditions. As this is a small difference in temperature, we can conclude that humidity is the factor that affected the outcome.

20. Refer to the table below, which shows data for a whole-plant transpiration experiment. The diagram illustrates the setup for the experiment.



A weather sensor monitored the microclimate in each chamber. One chamber contained only a plant. The plant's root ball was sealed in plastic so that evaporation could only occur through its leaves. The other chamber was set up in the same way—using a plant of the same size and species as the first chamber, but it also contained a small amount of water in the bottom of the chamber and the holes of the chamber were sealed. The mass of each plant was measured on the day of set up and again 72 hours later. External factors, such as the amount of daylight the plants received, were kept constant by placing the chambers side-by-side in the same location for the 72 hours.

Table 1: Whole-plant—Comparing the rate of transpiration under normal and humid conditions

Environment	Change in Mass (g)	Change in Mass (%)	Total Leaf Surface Area (cm^2)
Chamber 1	–0.69	–0.7	183
Chamber 2	–22.42	–21	175

a. Why does the mass of the plants decrease during the 72-hour time period?

The total mass of the plant and root ball decreases due to water lost through the leaves of the plants through transpiration.

b. Does the data from this whole-plant method support or contradict the results of the potometer investigation? Explain your answer.

The data from the whole-plant method supports the results of the potometer investigation. The plant in the humid EcoChamber container had greater total leaf surface area than the plant in normal conditions, yet the decrease in mass for the plant in high humidity was significantly less. The results indicate that very little water was lost through transpiration from the plant in the humid chamber. The rate of transpiration for normal conditions was 0.12% mass change/cm^2 and for humid conditions the rate was 0.0038% mass change/cm^2. In the potometer investigation, the humid conditions also caused a slower rate of transpiration than the control conditions.

21. To investigate additional factors that may affect transpiration, either the potometer method or whole-plant method can be used.

a. Identify one or more advantages to the potometer method of measuring transpiration. What might be some possible sources of error with this method?

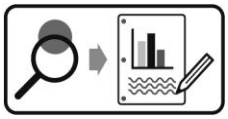
The potometer method gives information about the rate of transpiration rather than just a before and after "snapshot" of the plant mass. Also, you can more easily control external variables for the shorter time of data collection. Sources of error might include improper setup of the potometer system, such as air bubbles in the tubing or breakage of the stem when inserting it into the tubing.

b. What are some advantages to the whole-plant method for measuring transpiration? Are there possible sources of error with this method?

The whole-plant method is easy to set up but the method takes many hours and the mass comparison does not provide the rate of transpiration. Also, environmental conditions may not be as easily controlled over a number of days as they are in a short 10-minute potometer trial. On the other hand, slight variations in the local environment, such as a draft, may affect the potometer data but is not likely to affect whole-plant data.

Design and Conduct an Experiment

The initial investigation provides students with enough information to spark ideas for an experiment of their own design. In general, students can investigate additional environmental conditions, compare different types of plants, or apply a technique not practiced in the initial investigation, such as preparing microscope slides to observe stomata density.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Will windy conditions affect transpiration rate?*
- How do very dry conditions affect the rate of transpiration?
- Does temperature affect transpiration rate?*
- Do different plants species have different transpiration rates?*
- Do plants adapted to a dry environment have a different transpiration rate than plants adapted to more humid conditions?
- Does light intensity affect transpiration rate?
- Do plants with waxy leaves have lower transpiration rates than plants with non-waxy leaves?
- How does stomata density compare for different plant species?
- Is the rate of transpiration related to the number of stomata on each leaf?
- If a plant is surrounded by salt water, does it still transpire?*
- Are stomata located on both sides of a leaf (the upper and lower epidermis)?
- Does carbon dioxide concentration affect transpiration rates?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Will windy conditions affect transpiration rate?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected the rate of transpiration. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

      The independent variable in the experiment was the wind created by a fan blowing air on the leaves of the plant sample. My hypothesis is supported; wind increased the rate of transpiration. Under normal conditions the average transpiration rate was 0.304 mmHg/min while the average rate of transpiration under windy conditions was 0.485 mmHg/min.

   b. Based on the evidence you collected, explain why the results occurred.

      Water moves from the leaves to the air by evaporation. When water molecules evaporate, they increase the surface tension between water molecules at the surface of the xylem tube. This increase in tension creates a negative pressure that other water molecules move to fill. As water molecules adhere to each other, they pull up on the column of water in the xylem that extends to the roots.

      The lower the water potential of the surrounding air, the faster water will evaporate, increasing the negative pressure and pulling water up through the plant at a faster rate. By blowing air over the leaves, water molecules are moved away from the air directly above the leaf, lowering the humidity and lowering the water potential of the air. The lower water potential of the air caused by the "wind" increases the rate of evaporation of water from the plant.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   There is no evidence from the data that experimental error affected the results of this experiment, the data is reliable enough to support the hypothesis. The temperature was the same for both conditions and the same plant sample was used. However, only one trial was conducted. Collecting and averaging data from multiple runs would add confidence to the conclusion.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. Explain how prolonged drought contributes to a decrease in productivity of crop plants.

   Productivity is a measurement of the amount of energy a plant stores in carbon compounds that are not used for respiration. In drought conditions, there may not be enough water to keep guard cells turgid and stomata open. Many plants close their stomata when dehydrated.

   Prolonged periods of drought may therefore reduce the amount of carbon dioxide available to the plant for photosynthesis, reducing the amount of food the plant can store. Also, transpiration would be reduced. One of the effects of transpiration is the evaporative cooling of the plant. If there is not enough evaporation from the leaf, the temperature within the leaf may rise to levels that denature or inhibit enzymes responsible for photosynthesis.

2. Why is transpiration needed, even though the plant loses water in the process?

   To carry out photosynthesis, leaves need water. Water is provided to the leaves by transpiration: the evaporation of water from the leaves draws more water up the xylem—bringing water from the roots to the leaves. (Water is also needed by leaves to regulate the opening and closing of the stomata via guard cell shape.)

   Movement of water in the xylem is also an important part of transporting sugar molecules within plants, a mechanism known as "pressure flow" or "mass flow." Sugars are transported within phloem tubes, from the leaves to the other tissues of the plant and to the roots to be stored. The phloem sap has a very low water potential, due to the dissolved sugars. Since the xylem and phloem are adjacent to each other, water in the xylem can diffuse into the phloem (diffusing down the water potential gradient). The movement of water into the phloem increases turgor pressure and causes the sap to move downward.

   Another important consequence of transpiration is cooling. As water evaporates from a plant it carries away heat, helping to cool the plant and the air around it.

3. Name some of the structural or physiological adaptations that have evolved in plants that help limit water loss from their leaves. Explain how each adaptation is beneficial to the plant.

   Physiological adaptations:

   - Plants operate by circadian rhythms, an internal clock that helps regulate the opening and closing of stomata. Light triggers stomata to open by stimulating K^+ uptake in guard cells. Water diffuses into the guard cells, they become turgid, and the stomata are opened. This is beneficial so that plants don't lose unnecessary water at night when photosynthesis is not occurring.
   - Leaves monitor carbon dioxide levels. When CO_2 is depleted within the cells, stomata are opened to increase gas exchange, as long as enough water is available.
   - When plants are dehydrated, guard cells lose turgor pressure and collapse, closing the stomata to limit additional water loss. Also, when plants are water-stressed, abscissic acid (ABA) is produced from the roots and leaves and stimulates stomata to close.

   Structural adaptations:

   - To reduce surface area and decrease the rate of transpiration in dry environments, leaf size may be greatly reduced, as with conifers (needles) and cacti (spines). Some plants are leafless during hot seasons.
   - Location and density of stomata can vary in plant species. A low stomatal density would reduce water loss.
   - A thick cuticle is present in many plants adapted to arid and warm climates, helping to prevent water loss from leaves.
   - Stomata may be contained within pits (crypts) which help to protect them from wind, reducing the rate of transpiration.
   - Some plants utilize alternate photosynthetic pathways (C_4 and CAM) that help limit water loss during the day when temperatures are hottest.

4. Stomatal densities are shown in the table below for three plant species. The initial stomatal density was determined when plants were exposed to normal carbon dioxide (CO_2) levels. Plants were then exposed to CO_2 enriched environments and the final stomatal density corresponds to this enriched environment. The study included observations from 100 different plant species of various types (trees, shrubs, and herbs) and found the same trend occurred in 74% of plants observed.[12]

   Table 2: Effect of CO_2 level on stomatal density

Species	Stomatal Density, Normal CO_2 Level (stomata/mm^2)	Stomatal Density, Enriched CO_2 Level (stomata/mm^2)
Anthyllis vulneraria	154	137
Cynodon dactylon	321	249
Hypoestes sanguinolenta	110	61

   a. What effect did higher carbon dioxide levels have on stomatal density?

      Stomatal density decreased when plants were exposed to higher carbon dioxide levels.

[12] Woodward, F. I. and Kelly, C. K. (1995), The influence of CO_2 concentration on stomatal density. *New Phytologist*, 131: 311–327. doi: 10.1111/j.1469-8137.1995. tb03067.x

b. Propose an explanation for the change in stomatal density in response to higher carbon dioxide levels.

Stomatal density decreases when plants live in an environment with a high carbon dioxide concentration because the plant is able to acquire sufficient carbon dioxide from fewer openings in the leaves. The reduction in stomata limits water loss and helps conserve energy for the plant. The guard cells that regulate stomata opening depend on active transport of ions, so fewer guard cells means less energy expenditure on the part of the plant.

5. The table below summarizes data from a study of various species of *Eucalyptus* trees. During 24-hour time periods, a portable photosynthesis system was used to measure net carbon dioxide exchange for a sample of leaves from three or four trees of each species. Photosynthetic rate and stomatal conductance were calculated from data collected with the portable system. Stomatal conductance relates to the proportion of open stomata; the greater the number of open stomata, the greater the stomatal conductance.[13]

Table 3: Effect of time of day on photosynthetic rate and stomatal conductance in *Eucalyptus* trees

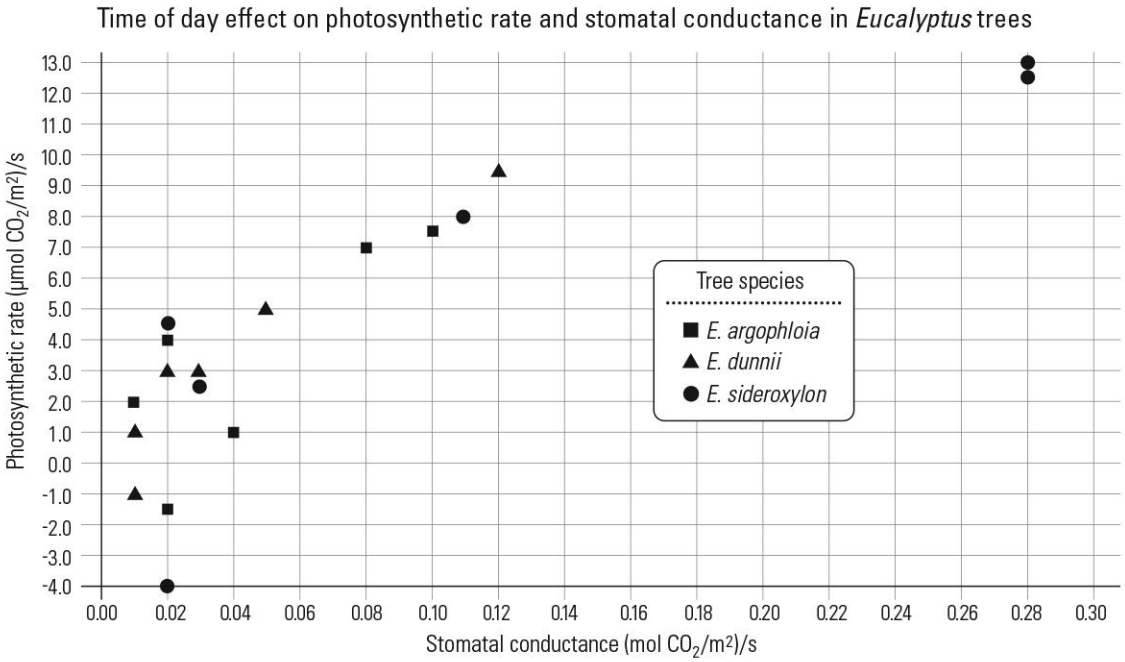
Species	Time of Day and Photosynthetic Rate (μmol CO_2/m^2)/s)		Stomatal Conductance (mol CO_2 /m^2)/s)
E. argophloia	06:00	–1.5	0.02
	09:00	*1*	*0.04*
	11:00	*7.5*	*0.10*
	13:00	*7*	*0.08*
	16:00	*4*	*0.02*
	19:00	*2*	*0.01*
E. dunnii	06:00	–1	0.01
	09:00	3	0.03
	11:00	9.5	0.12
	13:00	5	0.05
	16:00	3	0.02
	19:00	1	0.01
E. sideroxylon	06:00	–4	0.02
	09:00	2.5	0.03
	11:00	13	0.28
	13:00	12.5	0.28
	16:00	8	0.11
	19:00	4.5	0.02

a. Describe the trend in stomata opening and closing over the course of 24 hours. Describe the environmental factors that are likely to be the cause of this trend.

For all three species, stomata conductance was highest at 11 am. The increase in open stomata between 6 am and 11 am is likely due to an increase in light levels. Stomata open in response to light. As temperatures increase in the afternoon, the plant closes some stomata to limit water loss, so the decrease in stomatal conductance between 11 am and 4 pm is likely a response to increased temperatures. The decrease between 4 pm and 7 pm is a response to decreasing light levels as night approaches.

[13] Lewis, J.; Phillips, N.; Logan, B.; Hricko, C.; Tissue, D. Leaf photosynthesis, respiration and stomatal conductance in six *Eucalyptus* species native to mesic and xeric environments growing in a common garden. *Tree Physiol* (2011) 31 (9): 997-1006. doi: 10.1093/treephys/tpr087

b. Create an appropriately labeled scatter plot graph of Photosynthetic rate versus Stomatal conductance. Use a different symbol for each species.



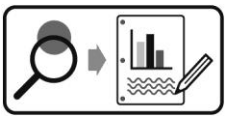
c. Describe the relationship between photosynthetic rate and the proportion of open stomata in leaves.

A higher stomatal conductance corresponds to a greater photosynthetic rate. When more stomata are open, carbon dioxide is plentiful in a leaf due to the ability of gas exchange with the atmosphere. This availability of carbon dioxide drives a high photosynthetic rate. When a low percentage of stomata are open, carbon dioxide acquisition is limited, therefore limiting photosynthesis.

NOTE: Students might inquire about data that seems not to fit the trend. For example, E. argophloia *has a stomatal conductance of 0.02 (mol CO_2/m²)/s at 6 am as well as 4 pm, but the photosynthetic rate is quite a bit greater at 4 pm. Plants can use carbon dioxide taken into its leaves earlier in the day for photosynthesis occurring hours later.*

Design and Conduct an Experiment Key

Humidity is only one of a number of environmental factors that can affect transpiration in plants. Transpiration rates also depend on the structural and physiological adaptations plants have to help them survive in the myriad of habitats on earth. Consider an aspect of transpiration or plant anatomy that you can explore further through an independent investigation.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of plants and transpiration, what environmental factors (abiotic or biotic) could affect this process?

   Environmental factors that can affect transpiration include: relative humidity, temperature, wind, levels of carbon dioxide, and availability of light and water. Plant adaptations may include: greater or lesser leaf surface area, density of stomata, and features of the leaf epidermis (upper and lower).

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   Will windy conditions affect the rate of transpiration?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   Factors such as wind may increase the evaporation of water from plants. Since plants require water, it is important that they regulate water loss so they don't wilt. Excess water loss can also lead to less gas exchange and therefore, less productivity. Farmers may consider this factor when determining a proper location to plant crops.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable in this experiment will be wind or the lack of wind. Windy conditions will be created using a fan to blow air over the leaves of the plant. The control plant will be tested at normal, indoor room conditions. The experimental plant will be tested under the same conditions, but will have a fan moving the air that is set at a distance of 0.5 meters from the plant.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable of the experiment will be the change in the pressure of the air in the tubing of a potometer. A barometer (low-pressure) sensor will be used to measure the decrease in pressure at 30 second intervals over a period of 10 minutes. The transpiration rate will be calculated from the slope of the line. A weather sensor will also be used to quantify the temperature and humidity in each trial.

6. Write a testable hypothesis (If…then…).

   If windy conditions lower the water potential of the air surrounding leaves, then there will be a greater rate of transpiration in the plant exposed to windy conditions than the plant exposed to normal (windless) conditions.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   Temperature will remain the same throughout the experiment (the room will be held at a constant temperature and plants will be placed in the same location in the room).

   The volume of air in the tubing at the sensor end should be between 2 and 3 cm. Too large a difference in the air volume between runs may affect the results. The potometer setup will be kept the same; only the amount of moving air will change.

   The pressure in the potometer air gap will be equalized with atmospheric pressure between each run. The same plant sample will be used for both conditions. Relative humidity and light level will be held constant as well.

8. How many trials will be run for each experimental group? Justify your choice.

   Two trials should be run for each group. This will allow us to see if data is consistent when conditions are held constant and two trials per condition can be achieved in a typical class period.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The rates of transpiration, calculated in (inHg/min)/cm^2 will be determined for each run and the rates from two trials will be averaged.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    As the volume of air in the potometer increases throughout each run, the atmospheric pressure outside the tubing will have a larger effect. Therefore, this volume of air will be kept as small as possible and similar between each run. Each run will begin with the air pressure in the tubing equal to the atmospheric pressure.

    Small amounts of air leaking into the potometer around the stem of the plant can affect the ability of the plant to pull up water. This end of the potometer will need to be kept air tight, but this may be hard to accomplish. Sometimes the stem of the plant sample breaks when pushed into the tubing.

    The plant sample may lose a leaf when the fan is turned on, which could affect the transpiration rate.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

    Acquire a plant sample that has numerous leaves and whose stem fits tightly in the tubing used for the potometer.

    Assemble a potometer with the plant sample and place a small fan 0.5 meters in front of the plant. Turn the fan on a low or medium speed.

    Collect data for 10 minutes, using a low-pressure barometer to monitor pressure inside the tubing and a weather sensor to monitor the microclimate.

    After 10 minutes, turn the fan off and stop data collection. Leave the plant sample and other potometer components in place.

    Reset the pressure in the tubing (disconnect and reconnect the tubing to the barometer).

    Repeat data collection with non-windy conditions—with the fan off.

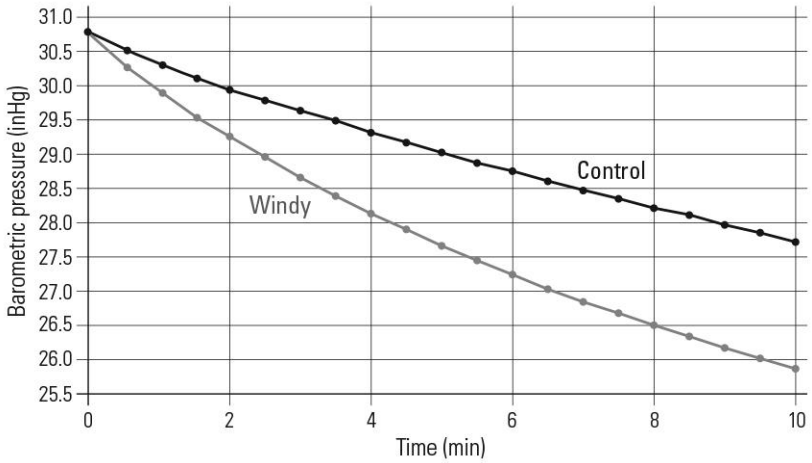
    Calculate the surface area of the leaves of the plant sample.

    Calculate the transpiration rate in (inHg/min)/cm^2 for each run.

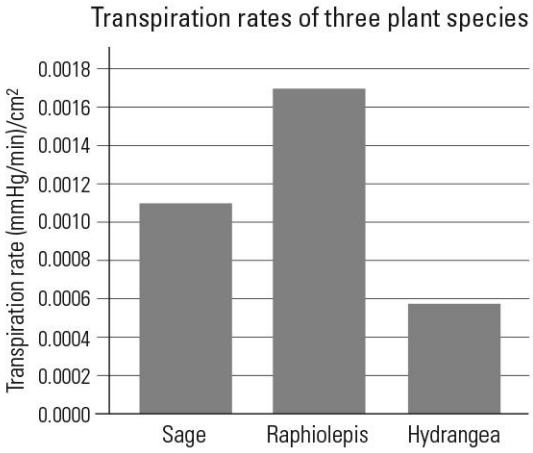
    Repeat the procedure using a plant sample from the same source as the first trial.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

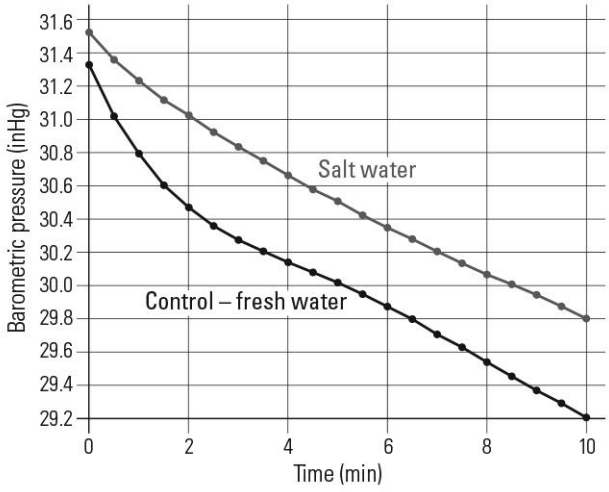
Sample Data



The results above were from an inquiry using hydrangea leaves in a potometer subjected to windy conditions, caused by a large fan placed 0.5 meters from the plant.



A potometer was used to determine the rates of transpiration for three plant species. The data was normalized by determining the leaf surface area, in square centimeters, for each plant sample.



The results shown above are from an inquiry that compared the rate of transpiration when a sample of sage was provided fresh water and then salt water (7%) in the tubing of a potometer.

Effect of temperature on change in mass of small plants due to transpiration

Environment	Average Temperature (°C)	Average Relative Humidity (%)	Change in Mass (g)	Change in Mass (%) $(m_i - m_f/m_i) \times 100$	Total Leaf Surface Area (cm^2)	Change in Mass (g/cm^2)
Room temperature (CFL lamp)	20.5	22	–18.43	–30.1	382.5	0.048
Warm temperature (heat lamp)	31.0	20	–45.2	–60.9	265.0	0.17

Using EcoChamber containers and the "whole-plant" transpiration method, two primrose plants were exposed to different temperature conditions for a 72-hour period. Small desk lamps were used to provide both plants with light, but the type of light bulb differed in each setup. A reptile-warming UVA bulb was used to heat one of the chambers. A compact fluorescent bulb was used to provide light but not heat to the control group.

12. MITOSIS

Lab Overview

After learning the technique for growing roots and preparing root tip squashes for microscope analysis, students observe the root tips for evidence of mitosis. They compare the number of cells in mitosis to the number of cells in interphase. They apply the chi-square "test of independence" to compare their results with provided data. Following the initial investigation, students can move to independent inquiry and test a particular treatment to see if it affects the rate of mitosis in roots. Chi-square analysis can be applied to evaluate the significance of the results.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	10 min
Initial Investigation (over the course of 3 days)	90 min

Student-Designed Experiment (Optional)	
Experiment Design	20 min
Experiment (over the course of 3–4 days)	90 min
Data Analysis	45 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	3.A.1, 3.A.2
Science Practices	2.1, 2.2, 3.1, 4.1–4.3, 5.1, 6.1
Learning Objectives	2.37, 3.3, 3.8, 3.9

Materials and Equipment

For Each Student Station

- Dissection scissors
- Forceps
- Razor blade or scalpel
- Glass test tube
- Glass microscope slides (3)
- Cover slips (2)
- Compound microscope with 400× magnification
- Disposable pipets (2), 1-mL
- Plastic cup, 16-oz
- Personal protective equipment:
  Disposable gloves and chemical apron
- Spot plate
- Carbol fuchsin solution, 1 mL[1]
- 1 M Warm hydrochloric acid (HCl), 1 mL[1, 2]
- Onion bulb[3]
- Paper towel
- Large toothpicks (4)
- Pencil with eraser
- Plastic wrap
- Disposable plastic gloves
- Permanent marker
- Distilled water

[1]These reagents are easily obtained from a chemical supply company. Many vendors have a kit available for purchase that includes onions, reagents for staining, and one or more compounds that can be used in the student-designed experiment.

[2]A warm water bath and a few test tubes are needed to prepare this for students. Refer to the Lab Preparation section.

[3]Green onions or small white onions work well. Garlic can be used as an alternative.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Additional equipment recommended for the student-designed experiment:

- Data collection system
- Conductivity sensor
- pH sensor
- Herbicide samples[1]
- Additional onion bulbs, or other plant samples (such as garlic)
- Plant food samples[1]

[1]These samples can be purchased from local hardware stores or nurseries. Examples of food samples include fertilizers or root growth stimulants (transplant solutions).

Prerequisites

Students should be familiar with the following concepts:

- The purpose of mitosis and its phases: prophase, metaphase, anaphase and telophase.

  While knowing the *names* of the phases is not requisite knowledge for AP Biology, students need to recognize the stages that comprise mitosis in order to distinguish dividing cells from cells in interphase.
- The cell cycle and its component phases: G_1, S, G_2, mitosis, and cytokinesis
- Viewing cells with a microscope and identifying cells in interphase and cells in mitosis

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Wear disposable gloves and a chemical apron while performing the staining and microscope slide preparation steps.
- Use caution when cutting with the razor blade or scalpel. Cut away from the body and away from other students, and do not use excessive force when cutting.
- Wear disposable plastic gloves when handling treated onion bulbs.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Onion bulbs

   Purchase small white onion bulbs or green onions from a grocery store. Alternatively, onion bulbs can be purchased from a chemical supply company.

   NOTE: Store-bought onions are sometimes treated with rooting inhibitors and may not work well for this activity. In PASCO trials, store-bought onions were used, however it is suggested that you test store-bought onions prior to student use.

2. Warm hydrochloric acid (HCl) for the root squash and staining procedures

   Set up a 50–60 °C water bath. Add 3 mL of 1 M hydrochloric acid to four test tubes and place the test tubes in the water bath. Place a 1-mL pipet in each test tube.

Teacher Tips

Tip 1 – Identifying cells in interphase and cells in mitosis

- If this is the first time in the current school year that students are using microscopes, you may want to plan a microscope review activity. Before starting the investigation, students need to be comfortable adjusting the focus and making small changes to slide placement to be able to view different regions of a sample.
- Students will be counting a large number of cells and determining if the cells are in interphase or in one of the phases of mitosis. To be efficient and obtain valid results, students need to be able to quickly distinguish between these. Prepared slides of onion root tips can be used *prior to* the investigation to review with students what the phases of mitosis look like in actual cells.

Tip 2 – Preserving root tips

The steps of the Initial Investigation assume that students will stain, squash, and observe root tips on the same day they are harvested from the onions. Alternatively, students (or the teacher) can harvest root tips after they become 1.5–2 cm long and preserve the tips for analysis at a later time. For most situations, this step will not be necessary.

NOTE: Root tip squashes yield the best results when fresh; preserving root tips for analysis later is not recommended.

To preserve root tips, follow the steps below.

a. Wear safety goggles, disposable gloves, and a chemical apron.

b. Cut roots from the onions and place them in 10–20 mL of Carnoy's fixative. (Use a glass or polypropylene container.)

c. Allow the roots to remain in the fixative for 4–24 hours.

d. Transfer the roots to 10–20 mL of ethyl alcohol. The roots can be stored indefinitely in the alcohol.

Tip 3 – Capturing images with SPARKvue software or the SPARK Science Learning System

Some digital microscopes, such as the ken-a-vision® Digital Monocular Comprehensive Microscope 2 (SE-7246), are compatible with SPARKvue software running on a computer or can be connected to the SPARK Science Learning System (SLS).

Root squash slides can be viewed on the computer screen or SLS screen and images can be captured and saved. Students can also use measurement and annotation tools that can enhance their experience with this investigation. The use of a digital microscope to preserve images expedites data collection and the images can be used to verify counts of cells.

Tip 4 – Chi-square analysis

Many biology teachers are familiar with the chi-square "goodness-of-fit" test, which tests how well observed data fits with expected data. However, this investigation requires the less familiar chi-square "test of independence," which tests whether two categories are independent of each other.

In many cases the goodness-of-fit test and the test of independence have similar outcomes for the same data set. For example, the chi-square value obtained from each method might indicate that the investigator should reject the null hypothesis. The value obtained from the test of independence will be more conservative in "treatment" situations—like the mitosis investigation—and is therefore a more valid statistical method to apply. (The value is less than what would be obtained for the goodness-of-fit test and makes it less likely that the null hypothesis is rejected.)

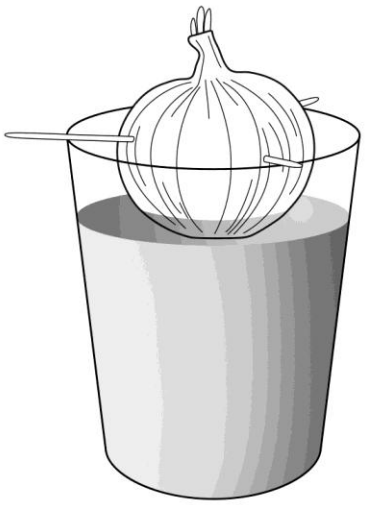
Rather than use the observed cells in the control group to calculate expected values for the treatment group, as would be done in a goodness-of-fit test, observed cells in both control and treatment groups are used to calculate expected values in a 2×2 contingency table for the test of independence.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. The understanding of mitosis and the skills acquired here prepare them to pursue additional investigations if time and materials allow for this.

From the student handout:

Part 1 – Growing root tips



1. Obtain a plastic cup and fill it two-thirds full with water. Label the cup with today's date and your initials.
2. Obtain a small onion. Remove any dry outer skin and any green leaves.
3. Use a razor blade to carefully cut off dry roots.

   NOTE: Make a shallow cut, such that the existing roots are cut back to the base of the bulb, but take care not to cut into the bottom of the bulb.
4. Stick long toothpicks into the sides of the onion to suspend it in the water of the cup. The area with the cut roots should be submerged in the water.
5. Loosely cover the cup with plastic wrap and place it in a dark location for 48 hours.

6. Following the growth period, you will squash and stain some of the root tips and observe the cells under the microscope. Answer the following questions in your lab notebook.

   a. Considering the purpose of mitosis, why are root tips a good source of tissue for observing cells undergoing this type of cell division?

      Mitosis is a type of cell division that produces new, genetically identical cells during growth of a tissue or to repair damaged tissue. Elongation of roots involves the addition of cells at the tip of the roots, so root tips should have a large number of cells undergoing mitosis.

   b. It will be critical that you can accurately determine if cells are in interphase or in mitosis. What cell features will you use to determine that a cell is in interphase?

      A cell in interphase will have a visible nucleus and nuclear membrane. The nucleus may contain visible nucleoli. Chromosomes will not be visible.

   c. What cell features will you use to make a determination that a cell is in mitosis?

      A cell in mitosis will have visible chromosomes. Many cells in mitosis will lack a nucleus and nuclear membrane and chromosomes will be condensed, dark structures. Many mitotic cells will have chromosomes aligned at the midline or two groups of chromosomes may be present if the cell is in anaphase. Spindle fibers may be visible.

7. After 48 hours, observe the onion roots and record your observations. If the roots are at least 2 cm in length, continue to Part 2. Otherwise, place the onions back into the dark location for another 24 hours.

   Answers will vary. Students should observe that the onion roots have grown at least a few centimeters in length.

Part 2 – Staining Root Tips for Observation

NOTE: Wear safety goggles, disposable gloves, and chemical aprons during this activity. Work in a well-ventilated area—ideally a chemical hood.

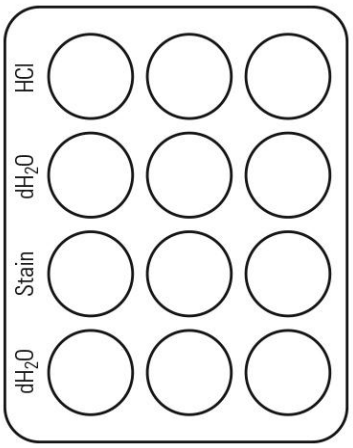
8. After the onion has been 2–3 days in the dark, remove it from the water and harvest the root tips for microscope analysis:

   a. Obtain a plastic spot plate.

   b. Use scissors to remove at least 4 or 5 roots from the onion. Trim the roots to approximately 1 cm in length. Discard the remainder of the root, being sure to keep the root tip.

   c. Use forceps to place the root tips into the first well of the spot plate.

   d. Use a disposable pipet to fill the spot containing root tips with 1 mL of warm (50–60 °C) 1 M hydrochloric acid (HCl). Soak the tips for 5 minutes.



9. While you wait, fill the remaining wells of the spot plate with the following solutions in the order shown in the diagram. Use a separate pipet for each solution.

10. After soaking the root tips for 5 minutes in the HCl, transfer them to the next well, containing distilled water (dH_2O). Rinse the tips in water for 1 minute.

11. Transfer the root tips to the stain (carbol fuchsin solution). Stain the tips for 2–3 minutes.

12. Transfer the root tips to distilled water and rinse them for 1 minute. Change the water during the rinse process until the rinse water is light pink.

    - Change the water by pipetting the colored water into a waste container and adding new water (with the pipet used for water).

    - Alternatively, if you have extra wells in the spot plate, you can fill them with water and transfer the roots from one spot to another.

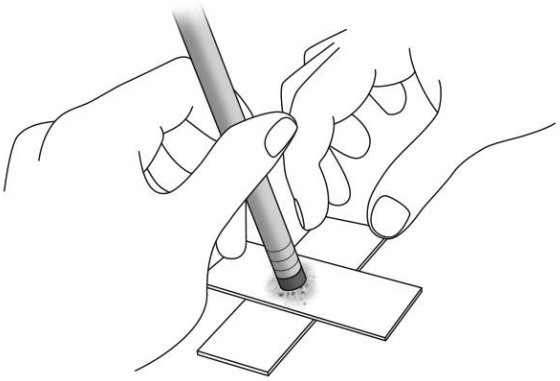
13. Place the stained roots on a paper towel. Use a scalpel or razor blade to trim the roots so that you keep only 3–5 mm of the root tip (the tapered end).

    NOTE: You can hold the root with forceps and cut across it with a razor blade. Be sure to discard the root cut away from the tip and keep only the tapered end.

14. Using a permanent marker, label one of the glass microscope slides “A” and the other “B”.

15. Place at least three stained root tips on the slide, close together but not overlapping. Place a cover slip over the tips.

16. Place the second glass slide over the coverslip, perpendicular to the slide with the roots. Using the eraser of a pencil, press down firmly on the top slide to squash the root tips. Move the eraser and push down on each root tip. Do not twist the eraser. Remove the top slide.



17. Repeat the process to prepare the second slide of squashed root tips.

18. Observe Slide A on high magnification (400×).

    NOTE: Observe areas where cells can be seen clearly, that is, areas where there is a single layer of cells. Do not focus on areas where there is more than one cell layer.

19. Some cells will appear elongated and rectangular, other cells will appear small and square in shape. In regions with small, square cells, look for cells in metaphase or anaphase. These phases will be the easiest to recognize and can help you locate a good field of view (FOV) to count.

20. When you find a FOV that has at least two cells showing evidence of mitosis, determine a systematic way of viewing and counting all of the cells in that FOV. Every well-stained, distinct cell in the field of view should be counted.

21. For each cell in the FOV, determine if the cell is in interphase or mitosis. Put a tally mark in the appropriate column of Table 1 (copied to your notebook). Continue to tally the cells until you have counted all cells in the FOV. Repeat this procedure for two additional fields of view.

    NOTE: You do not need to track the number of cells in specific phases, just note whether a cell is in interphase or in mitosis.

Table 1: Comparing the number of cells in interphase and in mitosis in root tissue

Slide	Cells in Interphase				Cells in Mitosis				Total Cells Counted
	FOV 1	FOV 2	FOV 3	Total	FOV 1	FOV 2	FOV 3	Total	
A	64	45	49	158	5	4	6	15	173
B	52	54	46	152	6	5	6	17	169

22. Observe and tally the number of interphase and mitotic cells in three fields of view for the second root tip slide (Slide B).

23. For each field of view observed for Slide A, determine the percentage of cells in interphase. Then determine the mean and standard deviation for the 3 FOVs. Do the same for Slide B.

$$\text{Mean: } \bar{x} = \frac{1}{N}\sum_{i=1}^{N} x_i \qquad \text{Standard deviation: } s = \sqrt{\frac{\sum(x - \bar{x})}{n - 1}}$$

Statistical analysis of the proportion of cells in interphase in root tips

Slide	Percentage of Cells in Interphase				Standard Deviation
	FOV 1	FOV 2	FOV 3	Mean	
A	92.7	91.8	89.0	91.2	1.93
B	89.6	91.5	88.5	89.9	1.52

NOTE: While a sample calculation is shown, students can use the SD function of a calculator, spreadsheet, or an online calculator to find the SD for their data. Students should understand the meaning and application of the SD equation, even if they do not calculate the value by hand.

Calculation for the sample data of Slide A:

$$\bar{x} = \frac{1}{3}(92.7 + 91.8 + 89.0)$$

$$= 91.2$$

$$s = \sqrt{\frac{(92.7 - 91.2)^2 + (91.8 - 91.2)^2 + (89.0 - 91.2)^2}{2}}$$

$$= 1.93$$

24. Consider all six fields of view as a single sample and report the mean percentage of cells in interphase to the class.

For the sample data provided above, the mean percentage of cells in interphase is 90.6%.

Data Analysis

1. Find the mean and standard deviation for the data provided by all groups.

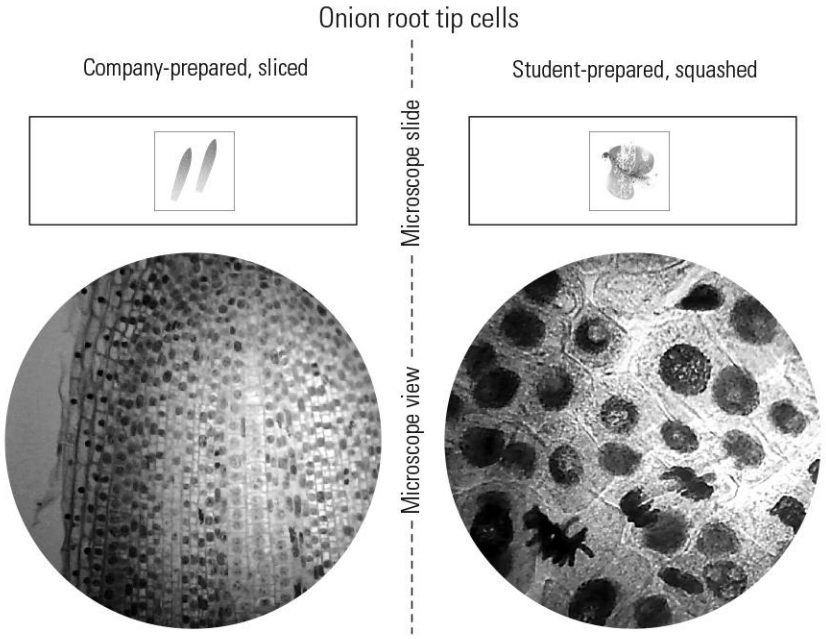
   Results will vary for the class data. Expected results for student groups is a mean near 90% for the proportion of cells in interphase. The standard deviation is expected to be in the range of ±1% – ±3%.

2. Given the standard deviation for your group data and class data, what can you conclude regarding the amount of variation in the samples?

   It is expected that there will be variation in the mean percentage of cells in interphase between different fields of view on a slide, or between slides of different root tips. Standard deviation indicates the spread in the data; the dispersion of the values on either side of the mean. For example, for Slide A, the percentage of cells in interphase was 91.2% ±1.93%. So any FOV would be expected to have between 89.3% and 93.1% of the cells in interphase. The standard deviation for Slide B was similar to A, indicating that FOVs on the same slide, as well as FOVs for different root tips (from the same onion), had means within a few percentages of each other.

Chi-square analysis

Science education supply companies offer prepared slides of onion root tips for purchase. You have probably viewed such slides before. Preparation of these slides differs from the technique you used in the Initial Investigation. Using special equipment, supply companies are able to create very thin slices (cross-sections) of the root rather than squashing the root tips. Their technique results in slides that make it easy to view the actively dividing region of the root tip and find cells in the stages of mitosis. Since the purpose of professionally prepared slides is to provide students with experience finding dividing cells, as opposed to cells in interphase, it is plausible that companies treat the roots with a compound that promotes mitosis—ensuring that a slide will have a large number of dividing cells for a student to observe.



A chi-square test can be used to determine if prepared cross-section slides are significantly different than squash slides (such as those in the Initial Investigation) in terms of the proportion of cells that are in interphase or mitosis. The table below provides data for such a comparison.

Table 2: Observed values *o*

Source	Number of Cells		
	Interphase	Mitosis	Total
Student-prepared root tip squash	310	32	342
Prepared slide from a company	315	35	350
Total	625	67	$N = 692$

The formula used to calculate a chi-square value is:

$$X^2 = \sum \frac{(o - e)^2}{e}$$

Typically, a chi-square test is a *goodness-of-fit* test. This test is applicable in situations where expected results can be calculated from theory, such as a predicted phenotypic ratio in the F_1 generation of fruit flies based on the principles of Mendelian inheritance. The null hypothesis for a goodness-of-fit test states there is no difference between the observed *o* and expected *e* (theoretical) values.

However, in treatment studies, a different type of chi-square test is applied. This *test of independence* operates under the null hypothesis that there is no association between two groups (or two variables)—the two groups are independent. **For the provided data, the null hypothesis is that the probability of a cell being in interphase or mitosis is independent of its source: a prepared slide from a company or a student-prepared squashed root tip.** The same chi-square formula is used for goodness-of-fit tests and tests of independence. However, in the test of independence expected frequencies are derived from observed frequencies, rather than from theory. The observations for the two groups for each category are recorded in a *contingency table*. Table 2 is an example of a contingency table (also known as a *2 × 2 contingency table).*

NOTE: The degrees of freedom (df) *for this type of chi-square test is one.*

In the chi-square test of independence, to calculate the expected values from observed values, apply the following Law of Probability:

If A and B are independent, then the probability *P* of A and B both occurring is:

$$P(\text{A and B}) = P(\text{A}) \times P(\text{B})$$

Considering the total number of cells observed, the probability of A and B both occurring in a sample size of *N* would be:

$$[\text{P(A)} \times \text{P(B)}]/\text{N}$$

3. Copy Table 3 into your lab notebook.

Table 3: Calculation of chi square

Source	Observed	Expected	$(o - e)$	$(o - e)^2$	$[(o - e)^2]/e$
Root tip squash Interphase	310	309	1	1	0.00
Root tip squash Mitosis	32	33	–1	1	0.04
Prepared slide Interphase	315	316	–1	1	0.00
Prepared slide Mitosis	35	34	1	1	0.04
				$X^2 = \Sigma[(o - e)^2]/e =$	0.08

From the observed values o and the total number of cells counted N, calculate each of the following:

a. What is the probability that a cell will be in interphase and sourced from the student-prepared squash? Record the value in the "Expected" column of Table 3.
[(cells in interphase × cells in root tip squash)/N], or (625 cells × 342 cells)/692 cells, or 309 cells

b. What is the probability that a cell will be in mitosis and sourced from the student-prepared squash? Record the value in the "Expected" column of Table 3.
[(cells in mitosis × cells in root tip squash)/N], or (67 cells × 342 cells)/692 cells, or 33 cells

4. Calculate and record the remaining expected values to complete the Expected column of Table 3.
Refer to Table 3.

5. Perform the calculations necessary to complete the remainder of Table 3. Find the sum of the values in the last column to determine the chi-square value.

$$X^2 = \sum \frac{(o - e)^2}{e}$$

$$X^2 = (0.00 + 0.04 + 0.00 + 0.04) = 0.08$$

6. With regard to the null hypothesis, what can you conclude from the calculated chi square?
Chi square is less than 3.84, the critical value for 1 df and $p = 0.05$. The investigator fails to reject the null hypothesis.

Table 4: Chi-square distribution

	Fail to reject				Reject	
Degrees of Freedom	Probability p Value					
	0.75	0.50	0.25	0.10	0.05	0.01
1	0.10	0.46	1.32	2.71	3.84	6.64
2	0.58	1.30	2.77	4.60	5.99	9.21
3	1.21	2.37	4.11	6.25	7.82	11.34
4	1.92	3.36	5.39	7.78	9.49	13.28

df = (number of groups – 1) × (number of categories – 1)

7. Do the chi-square results support the conjecture that supply companies treat roots with a compound to increase the rate of mitosis? Does the data provide conclusive evidence? Explain your answer.

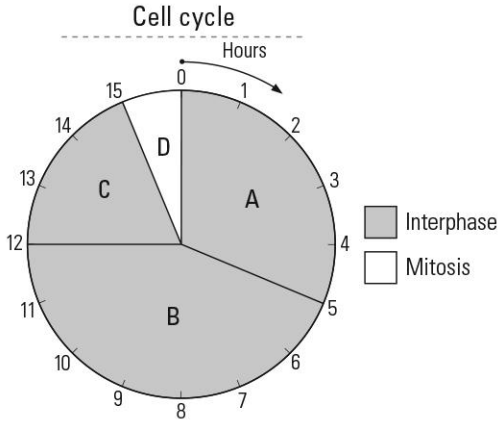
   Because the results of the chi-square test indicate that the null hypothesis (that the probability of a cell being in interphase or in mitosis is independent of its source) cannot be rejected, the results do not support the conjecture that supply companies treat roots. However, because the sample size was small, the data does not provide conclusive evidence—only one prepared slide was viewed. Further investigation is needed, using numerous prepared slides from more than one supply company.

8. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. The diagram summarizes the cell cycle of an organism.



   a. Is the relative length of interphase and mitosis in the diagram consistent with the proportion of these stages observed in the root tips you harvested? Explain your answer.

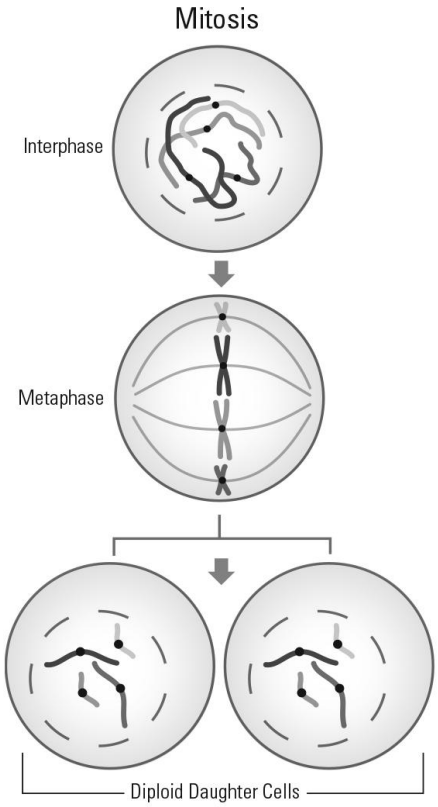
      The diagram indicates that mitosis extends one hour within the 16-hour cell cycle of the organism. This is 6.25% of the cell cycle, with interphase comprising 93.75% of cycle.

      In the root tips grown for this investigation, interphase was observed in 90.5% of the cells (based on the sample data: 310 cells in interphase of the 342 cells counted). This is similar to the diagram, where the root tip cells spend a majority of their time in interphase. However, the mitotic time-period may be longer in onions than the diagram indicates.

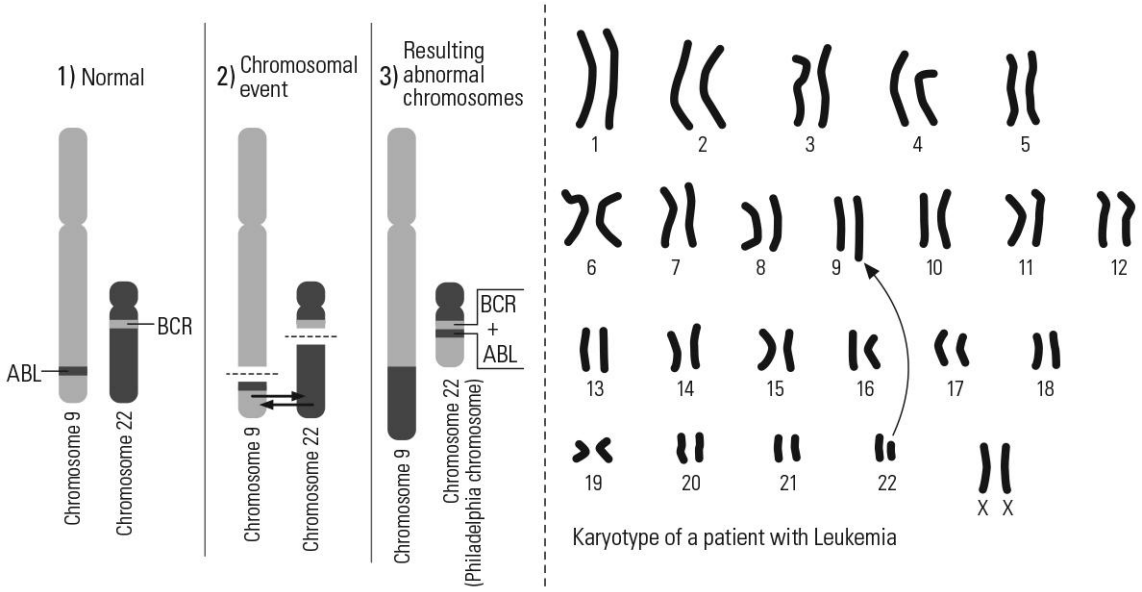
   b. Compare and contrast the amount and organization of DNA in a cell at the following stages of interphase: A and C.

      During $G_1$of interphase, which is indicated by "A" in the diagram, DNA is in the chromatin state and is unduplicated. Each chromosome is a single molecule of DNA (no sister chromatids). The S phase ("B") occurs between G_1 and G_2, in which replication of DNA creates identical sister chromatids. Therefore, in G_2 ("C") the amount of DNA in the cell is double that present in G_1. The sister chromatids are held together at the centromeres of the chromosomes and the DNA remains in the chromatin state and not visible under the microscope until the early phases of mitosis.

2. Mitosis, followed by cytokinesis, produces two genetically identical daughter cells. Draw a diagram that summarizes the transmittance of heritable information during the process of mitosis.



3. Cancer is characterized by a high mitotic rate that leads to formation of tumors. Research has shown that mutations or alterations to DNA can cause changes in the regulation of the cell cycle. An example of this is the *Philadelphia chromosome* associated with many forms of leukemia. The development of leukemia is related to a genetic change on chromosomes 9 and 22.



a. What is the name for the chromosomal event that occurs between chromosomes 9 and 22, creating the *Philadelphia chromosome*? Describe what happens during this event.

The event is called *translocation*. During this event, genetic material is exchanged between non-homologous chromosomes. This transfers genes to new locations and can affect the expression of the genes. For example, the Philadelphia chromosome is produced when the ABL gene from chromosome 9 is transferred to chromosome 22.

b. The cell cycle has a G_2 "checkpoint." The cell checks that DNA replication is complete and checks for DNA damage or mutations before moving into the mitotic phase. There are additional checkpoints in the cell cycle. Identify and describe one of these checkpoints and describe its importance.

One checkpoint occurs at metaphase. At this point, the cell checks that all mitotic spindles are properly attached to the kinetochores of the chromosomes. This checkpoint is essential for ensuring that proper separation of chromosomes will occur during anaphase.

4. A study was performed to determine the effects of 24-epibrassinolide (BL), a plant steroid hormone, on the mitotic index of onion root tips.[14] Investigators compared meristematic tissues of control onion bulbs to onion bulbs in experimental groups after 48 hours of root growth.

The mitotic index is the percentage of cells in mitosis relative to the total number of cells examined. The mitotic index was calculated by counting 400 cells from five root tips obtained from each group (2000 cells total from each group). Data from this study is summarized in Table 4.

Table 4: Effects of BL on mitosis in *Allium* roots

Concentration of BL	Number of Cells			Mitotic Index ± SD
	Interphase	Mitosis	Total	
Control (spring water)	1907	93	2000	4.65 ± 1.34
0.5 ppm BL	1918	82	2000	4.10 ± 1.34
0.05 ppm BL	1867	133	2000	6.65 ± 0.69

a. What can be concluded from this study? Use chi-square analysis to provide evidence for your conclusions.

First create a contingency table for each condition, then calculate the expected values and chi square to determine if the null hypothesis, that there is no association between BL and mitosis in roots, is accepted or rejected.

Contingency table for the first experimental group (0.5 ppm BL)

Source	Number of Cells		
	Interphase	Mitosis	Total
Control (spring water)	1907	93	2000
0.5 ppm BL	1918	82	2000
Total	3825	175	*N* = 4000

The number of expected interphase cells: (3825 × 2000)/4000 = 1913

The number of expected mitosis cells: (175 × 2000)/4000 = 88

[14] Howell, W.M.; Keller III, G.E.; Kirkpatrick, J.D.; Jenkins, R.L.; Hunsinger, R.N.; McLaughlin, E.W. Effects of the plant steroidal hormone, 24-epibrassinolide, on the mitotic index and growth of onion (*Allium cepa*) root tips. *Genetics and Molecular Research*, Online Journal. Dept. of Biology, Samford University, Birmingham, AL. 2007 Retrieved April, 2014 from http://www.funpecrp.com.br/gmr/year2007/vol1-6/gmr0259_full_text.htm

Calculation of chi square for the first experimental group (0.5 ppm BL)

Source	Observed	Expected	$[(o - e)^2]/e$
Control Interphase	1907	1913	0.02
Control Mitosis	93	88	0.28
0.5 ppm BL Interphase	1918	1913	0.01
0.5 ppm BL Mitosis	82	88	0.41
		$X^2 = \Sigma[(o - e)^2]/e =$	0.72

Contingency table for the second experimental group (0.05 ppm BL)

Source	Number of Cells		
	Interphase	Mitosis	Total
Control (spring water)	1907	93	2000
0.05 ppm BL	1867	133	2000
Total	3774	226	$N = 4000$

The number of expected interphase cells: (3774 × 2000)/4000 = 1887

The number of expected mitosis cells: (226 × 2000)/4000 = 113

Calculation of chi square for the second experimental group (0.05 ppm BL)

Source	Observed	Expected	$[(o - e)^2]/e$
Control Interphase	1907	1887	0.21
Control Mitosis	93	113	3.54
0.05 ppm BL Interphase	1867	1887	0.21
0.05 ppm BL Mitosis	133	113	3.54
		$X^2 = \Sigma[(o - e)^2]/e =$	7.50

At higher concentrations, BL does not appear to affect mitosis in allium roots. However, at lower concentrations, such as 0.05 ppm, BL significantly affected root growth. For $p = 0.05$ and 1 degree of freedom, the critical value in the chi-square distribution table is 3.84. Since the chi-square value for a BL concentration of 0.5 ppm was less than 3.84 (it was 0.72), the null hypothesis fails to be rejected. We cannot reject the hypothesis that the presence of BL and rate of root growth and mitosis are independent. At the lower concentration of 0.05 ppm, the chi-square value (7.50) was higher than 3.84. It is even higher than the critical value for $p = 0.01$, which is 6.64. Therefore, at a concentration of 0.05 ppm, BL does affect root growth and mitosis. The null hypothesis of independence is rejected.

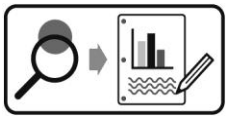
b. Another study showed that hormones like BL induce transcription of a cyclin gene. Explain the role of cyclins in the cell cycle.

Cyclins are proteins that bind to cyclin-dependent kinases (CDKs). Together these proteins form a complex that can activate other proteins (typically through phosphorylation) which in turn initiate aspects of cell division, such as the condensing of chromosomes.

One cyclin–CDK complex is *mitosis-promoting factor* (MPF), a complex that contains cyclin A or B bound to a CDK. MPF plays an important role in advancing a cell from the G_2 phase to mitosis. In general, cyclin concentration increases during the stages of interphase. The resulting increase in activity of different CDKs as cyclin concentration increases triggers the progression of changes that lead to and facilitate mitosis. Cyclin is degraded during mitosis, thus decreasing CDK activity and bringing an end to mitosis.

Design and Conduct an Experiment

The Initial Investigation provides students with the procedural skills needed to be successful conducting experiments of their own design and with familiarity with the statistics needed to analyze their results. If time allows, students can investigate factors that are likely to affect the rate of mitosis in organisms. For example, a number of studies involve testing compounds to see if they stimulate or inhibit mitosis.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet is provided below. The key describes a sample inquiry question and experimental protocol.

Suggested Inquiry Questions

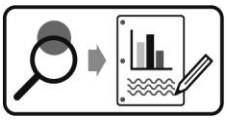
The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Does vitamin B-1 affect root growth and the rate of mitosis in onion bulbs?
- Does caffeine affect mitosis in onion root tips?*
- Does the plant hormone IAA affect mitosis in onion root tips?
- Is the rate of mitosis similar in roots of different plants, such as onion and garlic?
- Does pH affect mitosis in plant roots?
- Does light exposure to roots decrease the rate of mitosis in plant roots?
- Does the presence of another plant species affect mitosis in roots?

Design and Conduct an Experiment Key

A number of factors, internal and external, are likely to affect the rate of mitosis in organisms. Identify one of these factors and design an experiment to determine how that factor stimulates or inhibits mitosis.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of mitosis, what factors (abiotic or biotic) could affect this process?

   Mitosis can potentially be affected by any factor that stimulates or inhibits regulation of the cell cycle. These factors could be hormones or other internal signals, or compounds or conditions in the environment that may interact with internal signals or induce changes in intracellular molecules. Such factors could include: plant hormones, minerals in the soil, light level, pH, and compounds produced by other plants competing for space, light, or other resources.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   Does vitamin B-1 affect root growth and the rate of mitosis in onion bulbs?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   Vitamin B-1 is present in many solutions sold in gardening stores. It is advertised to encourage root growth and help reduce transplant shock. It would be informative to have scientific evidence to support or refute these claims.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable of the experiment will be the solution plant roots are placed in. Solutions will be prepared with differing concentrations of vitamin B-1 and onion bulbs will be placed in the solutions to grow roots. A store-bought root stimulant containing vitamin B-1 will be used as the stock solution. The first dilution will be prepared according to the recommendation on the label (1 tbsp, approximately 15 mL, in 1 quart of water). Three additional solutions will be prepared: 0.5 tbsp in 1 quart of water, 0.25 tbsp in 1 quart of water, and 0.5 tsp in 1 quart of water. A control using only water will also be prepared.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variables of the experiment will be root growth and mitotic index. Photographs will be taken on a daily basis to document root growth. Following 48–72 hours of growth, roots will be harvested, stained, and viewed with a microscope to determine the number of cells in mitosis.

6. Write a testable hypothesis (If…then…).

   If vitamin B-1 stimulates root growth, then roots harvested from treated bulbs will have a higher proportion of cells in mitosis compared to control bulbs.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   Temperature and light level will be held constant by placing all onion bulbs in the same dark cabinet during the growth period. There will be one bulb in each cup. All bulbs will come from the same large bag purchased at the grocery store, theoretically being of the same age. Each cup will have the same volume of liquid in it.

8. How many trials will be run for each experimental group? Justify your choice.

   Two replicates of each group will be set up. For example, two cups with spring water, two cups with the first dilution of the stock vitamin B-1 solution, and so on, with one onion bulb in each cup. Counting cells will be time consuming, so setting up numerous replicates is not feasible. Two will provide confidence that if an effect is observed it is due to the treatment and not something unique to a particular bulb.

9. It is beneficial to estimate at the beginning of the experiment the sample size needed to make statistically valid conclusions. The formula below can be used for this purpose. In the formula, n represents an adequate sample size, that is, the number of cells that need to be counted to compare the control and experimental groups. Solve for n in the equation. How many cells should you count in each of your groups?

   NOTE: The formula makes assumptions of other statistical values, such as margin of error and critical value.

$$0.03 = 2 \times \sqrt{\frac{0.9 \times 0.1}{n}}$$

   n = 400; At least 400 cells should be counted in each group.

10. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

    A qualitative comparison of root growth will be documented. Additionally, root tips will be stained and squashed and the number of cells in mitosis and interphase will be counted. A chi-square test of independence will be used to evaluate the results.

11. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    It might be difficult to make uniform cuts to remove the old roots from the onion bulbs before placing them in the solutions. Sometimes the root tip is inadvertently disposed of during the squash procedure and cells from the upper section of root may be viewed instead, which would have far fewer cells in mitosis. The dilution directions on the label of the root stimulant bottle may not provide concentrations good for submerging roots in for prolonged periods since the more common use of the stimulant is to saturate the soil around a plant.

12. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)
    a. Prepare 2 cups, filled two-thirds full, for each of the following:

       Spring water, 1 tbsp/quart vitamin B-1 solution, 0.5 tbsp/quart vitamin B-1 solution, 0.25 tbsp/quart vitamin-B1 solution, and 0.5 tsp/quart vitamin B-1 solution.

    b. As was done in the initial investigation, remove old roots from 10 small onion bulbs. Use toothpicks to suspend one onion bulb in each cup.
    c. Place the cups in a box and place the box in a dark cabinet in a room that is expected to remain at a stable temperature between 65–75 °C.
    d. Photograph the roots in each cup after 24 hours and again after 48 hours.
    e. Remove the onions from the dark cabinet when the roots are at least 2 cm long.
    f. Repeat the staining and squash procedures of the Initial Investigation to prepare one slide with two root tips from each onion. Label each slide to prevent mix-up.
    g. View each slide with a microscope and tally the number of cells observed in interphase and the number observed in mitosis. Count at least 200 cells on each slide so at least 400 cells total are counted for each group.

13. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

Chi-square distribution

	Fail to reject				Reject	
Degrees of Freedom	**Probability *p* Value**					
	0.75	0.50	0.25	0.10	0.05	0.01
1	0.10	0.46	1.32	2.71	3.84	6.64
2	0.58	1.30	2.77	4.60	5.99	9.21
3	1.21	2.37	4.11	6.25	7.82	11.34
4	1.92	3.36	5.39	7.78	9.49	13.28

The data below are from an inquiry investigating the effect of caffeine on mitosis in *Allium* root tips. Onion roots were grown in sand that was soaked with water containing dissolved caffeine on Day 1. Roots were harvested after 48 hours in the dark, then fixed and stored in 70% ethanol prior to microscopic examination.

Effect of caffeine[15] on mitosis, 1 mM

Calculation of chi square

Source	Observed	Expected	$(o - e)$	$(o - e)^2$	$[(o - e)^2]/e$
Control interphase	460	486	–26	693	1.42
Control mitosis	75	49	26	693	14.23
Treated interphase	549	523	26	693	1.33
Treated mitosis	26	52	–26	693	13.24
				$X^2 = \Sigma[(o - e)^2]/e =$	30.22

Since 30.22 is greater than 3.84, the null hypothesis is rejected.

Effect of caffeine on mitosis, 5 mM

Calculation of chi square

Source	Observed	Expected	$(o - e)$	$(o - e)^2$	$[(o - e)^2]/e$
Control interphase	460	484	–24	594	1.23
Control mitosis	75	51	24	594	11.74
Treated interphase	449	425	24	594	1.40
Treated mitosis	20	44	–-24	594	13.39
				$X^2 = \Sigma[(o - e)^2]/e =$	27.76

Since 27.76 is greater than 3.84, the null hypothesis is rejected.

[15] Elizabeth Cowles. Eastern Connecticut State University. Research data on the effect of caffeine on mitosis. 2014.

Effect of caffeine on mitosis, 10 mM

Calculation of chi square

Source	Observed	Expected	$(o - e)$	$(o - e)^2$	$[(o - e)^2]/e$
Control interphase	460	482	–22	481	1.00
Control mitosis	75	53	22	481	9.06
Treated interphase	330	308	22	481	1.56
Treated mitosis	12	34	–22	481	14.16
				$X^2 = \Sigma[(o - e)^2]/e =$	25.79

Since 25.79 is greater than 3.84, the null hypothesis is rejected.

13. MEIOSIS

Lab Overview

Students use physical models of chromosomes to explore the topics of meiosis and genetic variation. First, students use "paper chromosomes" to model the independent assortment and inheritance of fruit fly chromosomes. Fruit flies are a good candidate for a modeling activity since they have only 8 chromosomes, compared to 46 chromosomes in humans. Analyzing the variation in the phenotypes of five virtual offspring flies provides a springboard for students to connect the events of meiosis with its purpose of creating haploid cells and with the genetic variation that results from sexual reproduction.

Students then use "pop-bead chromosomes" to model the events of meiosis I and meiosis II and finally they determine the crossover rate between alleles that determine spore color in the fungus *Sordaria fimicola.* Spore color is a single gene trait with black being the wild-type form of the gene, symbolized by "+." Tan color is the result of a mutation and its allele is symbolized by "tn." Students determine the crossover rate by observing the arrangement of black and tan colored spores produced by a hybrid fungus (+ strain crossed with the tn strain). Using the crossover rate, students can determine the location of the gene for spore color relative to the chromosome centromere.

Pacing and Length of the Lab

***Drosophila* Investigation**		**Modeling Meiosis**		***Sordaria* Investigation**	
Teacher Preparation	5 min	Teacher Preparation	10 min	Teacher Preparation	10 min
Investigation	25 min	Investigation	30 min	Investigation and Analysis	55 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	3.A.1–3.A.3, 3.C.2
Science Practices	1.1, 1.4, 2.2, 4.3, 5.1, 5.3, 6.1, 6.2, 7.1, 7.2
Learning Objectives	3.1, 3.3, 3.9–3.12, 3.14, 3.15

Materials and Equipment

For Each Student Station

- *Drosophila* Chromosome Sheet[1]
- Karyotype of Offspring Fly Sheet[1]
- Scissors
- Tape
- Pop bead chromosomes (4), 2 colors, 2 sizes[2]
- String (2 pieces), approximately 1 m and 0.5 m
- Pop beads, 2 colors, enough to make sister chromatids[2]
- Cards with images or photographs of *Sordaria* asci[3]

OR

- *Sordaria* crossing over kit[4]

[1]These sheets are included at the end of this document and should be photocopied for student use.

[2]About 150 pop beads (75 of each color) are needed for chromosomes and chromatids. Refer to the Lab Preparation section to create them.

[3]Cards with images or photographs can be purchased from supply companies such as Flinn Scientific or Ward's Science.

[4]Crossing-over kits can be purchased from many different science supply companies. Refer to the documentation included with the kit for additional preparation directions if students prepare their own slides to observe asci.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

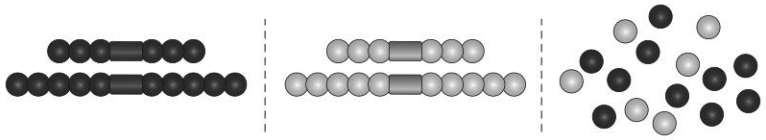
- Diploid cells (2n) are cells that contain two sets of chromosomes, one maternal set and one paternal set. In animals, all cells are diploid except gametes. Haploid (n) cells are cells that contain half the number of chromosomes as a diploid cell from the same species. Meiosis produces haploid cells.
- Sexually reproducing species produce non-identical offspring. Sexual reproduction is an important part of generating genetic variation in a population.
- Genes are located on chromosomes and different forms of a gene (alleles) are typically symbolized with uppercase and lowercase letters, indicating the dominant and recessive forms. The *genotype* is the combination of genes an organism inherits; the *phenotype* is the expression of these genes (the appearance of the organism).
- Chromosomes exist in the "X" shape only during some phases of cell division. X-shaped chromosomes are composed of identical sister chromatids formed when DNA replication occurs prior to cell division beginning.
- Meiosis consists of two rounds of cell division and produces four genetically unique daughter cells.
- *Crossing over* is the exchange of genetic material between non-sister chromatids during meiosis.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Photocopy the *Drosophila* Chromosome Sheet and Karyotype of Offspring Fly sheet for students. You may choose to have students work independently or in pairs.

2. Create sets of pop-bead chromosomes. Each student or student group needs four chromosomes representing two homologous pairs. One chromosome pair should be longer and the other shorter. The two chromosomes of a pair should be the same length but differ in color (use red and yellow pop beads, for example). Initially, the chromosomes provided to students should be in the unduplicated state. Provide students extra pop beads to create sister chromatids during the activity.



Teacher Tips

Tip 1 – Using the chromosome and karyotype sheets

The chromosome sheet provides eight chromosomes each for the (virtual) male and female flies that will be mated. The homologous chromosomes are identified by: length, alleles for a particular trait (such as wing appearance), and number. Students cut out the chromosomes along the solid lines, keeping each homologous pair together, and then fold them along the dashed lines, using tape to keep the pair folded in half. For many of the homologous pairs, the allele on the paternal chromosome differs from the allele on the maternal chromosome. To symbolize the randomness of a particular gamete receiving a particular allele, students toss the paper chromosome model in the air and record which allele of the pair is visible.

The karyotype sheet provides a place for students to place the "chromosomes" that have been tossed to determine which chromosomes (alleles) are present in the gametes. Like an actual karyotype, chromosomes are arranged in pairs based on length and other features. With this visual representation, students can easily see what traits the offspring has inherited.

Tip 2 – Understanding the Sordaria *experiment*

Experiments that investigate crossover frequency in *Sordaria* involve growing true-breeding black and tan strains in the same Petri dish. Where filaments of each strain overlap, haploid nuclei from each strain can fuse to produce a diploid zygote. Unlike the animal life cycle, in the fungus life cycle the diploid stage is brief. The fungal zygote undergoes meiosis to form haploid cells. Some of these cells undergo mitosis and are responsible for the growth of the fungus. Other haploid cells also undergo mitosis but become spores that are contained within tube-like structures called *asci*. The asci are located in fruiting bodies (called *perithecia*) and the fungus reproduces by releasing spores from the fruiting body into the environment to produce new *Sordaria*.

In the crossover experiment, the fruiting bodies can be collected from the surface of the agar and crushed to observe the spores contained within. Students then observe the color arrangement of spores within the asci. If 4 black spores are next to 4 tan spores (4:4 arrangement) there was no crossing over during meiosis. Any other arrangement indicates crossover occurred.

The proportion of asci that show crossover relates to the distance between the gene for spore color and the centromere of the chromosome. If the gene is close to the centromere, it is unlikely to be exchanged during a crossover event. That is, a part of the chromatid near the centromere is unlikely to overlap the chromatid of a homologous chromosome. Genes far away from the centromere are much more likely to be swapped between chromosomes during meiosis. So a high frequency of crossover corresponds to a greater map distance.

A map unit (mu) is equal to a recombination frequency of 1%. For example, if 14% of the fruit flies have recombinant phenotypes for a trait, then the gene that controls that trait is 14 map units from the centromere. Each recombinant fly is the result of one recombinant gamete, one crossover event. For the *Sordaria* investigation, the recombination frequency must be divided by two to determine the map units. This is because we are not observing the *offspring* of *Sordaria*; we are observing the structure that contains the gametes (an ascus). Each ascus that shows crossover is counted as "1" even though the recombinant ascus is actually only 50% recombinant; two of the spores have parental chromatids that had no exchange of genetic material and two of the spores contain recombinant chromatids. (The diagram at the end of the *Sordaria* investigation illustrates this.)

NOTE: If you purchase a kit for this activity, refer to the kit directions for additional information and safety precautions.

Drosophila Investigation

NOTE: Because of the length of the investigations, time may not allow for a student-designed experiment. However, several extension options are provided at the end of the lab.

This investigation is designed to familiarize students with the relationship between meiosis, sexual reproduction, and genetic variation in a population. Students toss paper chromosomes into the air to determine the random assortment of chromosomes passed along to an offspring organism by its parents.

From the student handout:

1. Obtain one copy each of the *Drosophila* Chromosome Sheet and Karyotype of Offspring Fly sheet.

2. In your lab notebook, answer the following questions before cutting and folding the chromosomes.

   NOTE: Refer to the Drosophila *Chromosome Sheet to help answer the questions. The letters on the chromosomes represent alleles, although, in the case of chromosome pair #1, the letter represents the sex chromosome: X or Y.*

   a. Each fly has four homologous pairs of chromosomes. What are homologous chromosomes?

      Homologous chromosomes are a pair of chromosomes of the same length that carry genes for the same characteristics.

   b. What is the diploid number for *Drosophila*? What is the haploid number?

      The diploid number (2n) for Drosophila is 8. The haploid number is 4.

   c. What cells in a fruit fly would be haploid? How are these cells produced?

      The reproductive cells, the gametes (sperm and egg cells), are haploid. These cells are produced by a type of cell division called *meiosis*.

      NOTE: The letters on the chromosomes represent alleles, although, in the case of chromosome pair #1, the letter represents the sex chromosome: X or Y.

   d. With regard to sex, eyes, wings, and body color, describe the phenotypes of the two flies.

      One fly is female and has normal eyes, vestigial wings, and normal body color. The other fly is male and has normal eyes, normal wings, and ebony body color.

   e. If the male and female mate and produce offspring, can the offspring have normal wings? Can the offspring have vestigial wings? How do you know?

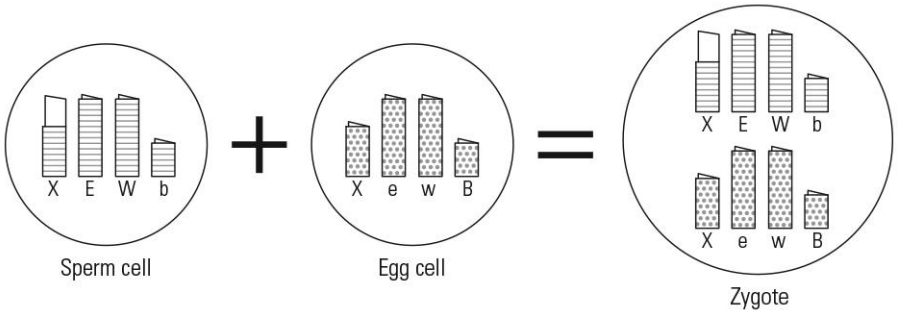
      The offspring of these parents can have either normal wings or vestigial wings. The male and female genotypes for the wing characteristic are Ww and ww, respectively. The offspring has a 50% chance of inheriting the dominant "W" allele, in which case it will have normal wings. If the offspring inherits a "w" from each parent, it will have vestigial wings (also a 50% probability).

   f. To model the inheritance of chromosomes by an offspring organism, you will cut out each homologous pair from the female and male flies. The two chromosomes of a homologous pair will remain together when cut, but then will be folded in half. Why do you think the pair is being folded in half?

      The pair is folded in half because a parent passes on only one chromosome from each homologous pair during reproduction. By providing four chromosomes from each parent, the two fruit fly parents provide the offspring with the correct diploid number of chromosomes (8).

3. Follow the instructions on the *Drosophila* Chromosome Sheet to cut and fold the pairs of chromosomes. Keep the female fly's chromosomes separate from the male fly's chromosomes.

4. Simulate the segregation and independent assortment of chromosomes by tossing the female fly's chromosomes into the air. The chromosomes that land face up on the table represent those present in an egg cell in this female. In your notebook, copy the diagram below and draw the appropriate chromosomes in the "egg cell."



5. Toss the male fly's chromosomes into the air. Draw the appropriate chromosomes in the "sperm cell" on the diagram.

6. Draw the chromosomes that would be in the zygote, following fertilization.

7. Pair up the female and male flies' chromosomes on the Karyotype of Offspring Fly sheet. Put them onto the sheet with the side facing up the way the chromosome landed.

   a. Create a table in your notebook to organize the characteristics (genotype and phenotype) of five offspring.

   Results of Fertilization for Five Offspring

Offspring	Genotype	Phenotype
1	XX, Ee, Ww, Bb	Female, normal eyes, normal wings, normal body
2	XX, EE, Ww, Bb	Female, normal eyes, normal wings, normal body
3	XX, Ee, Ww, bb	Female, normal eyes, normal wings, ebony body
4	XY, Ee, ww, Bb	Male, normal eyes, vestigial wings, normal body
5	XY, ee, ww, bb	Male, eyeless, vestigial wings, ebony body

   b. Record the genotype and phenotype in the table for the first offspring fly, based on the chromosomes placed on the Karyotype Sheet.

   c. Toss the male and female sets of chromosomes into the air again and pair them on the Karyotype page. Determine the genotype and phenotype of the second offspring fly. Repeat the procedures until five offspring are produced.

8. The offspring flies were all produced from the same two parents. Are the offspring identical? If so, explain why. If not, explain the variation.

   In a PASCO trial, two of the five flies were identical, but there was variation in the traits of the other offspring. Some have ebony body color instead of normal body color. Two have vestigial wings instead of normal wings. Since each parent only passes on half of their chromosomes and it is random as to which chromosome in a pair ends up within a gamete, there are a number of different chromosome sets that can be inherited from the same two parents.

9. Due to the *independent assortment* of chromosomes during meiosis, there are 2^n types of gametes that can be produced by an organism. That is, there are 2^n different assortments of chromosomes possible, where n is the haploid number of the organism. How many genetically unique egg cells can be produced by a female fruit fly? How does this compare to the number of different gametes a human female can produce?

   A female fruit fly can produce 16 different egg cells ($2^4 = 16$). A human female can produce over eight million different egg cells ($2^{23} = 8{,}388{,}608$)!

10. The 2^n calculation does not take into account an event called *crossing over* that increases the genetic variation among gametes and therefore the variation in a population of a sexually-reproducing species. Describe how crossing over occurs during the formation of gametes and explain why it increases genetic variation.

    When homologous chromosomes form tetrads during prophase I of meiosis, small sections of chromosomes can be exchanged—a chromatid from one homologous chromosome overlaps a chromatid of the adjacent homologous chromosome and genetic material is exchanged. This produces a recombinant chromosome with unique genetic information, a chromosome that was not present in either the mother or the father. If crossing over occurs at more than one locus, or on other homologous chromosome pairs, the offspring inherits a number of recombinant chromosomes that make it even less like its parents or siblings.

Modeling Meiosis Investigation

Even a cell containing only 8 chromosomes, such as that of *Drosophila*, can be cumbersome to use for a model of meiosis. In this activity, you will use only four pop-bead chromosomes to simulate the events of meiosis.

1. Obtain four chromosomes. Each chromosome should be a single string of pop beads and there should be two homologous pairs. You should also have some loose pop beads for making additional chromosomes.

2. Before you begin the simulation, answer the following questions in your notebook.

   a. Why are two colors used for the pop-bead chromosomes to simulate meiosis?

   Although they are the same length and carry genes for the same trait, two colors are used to indicate that the DNA sequences of the homologous chromosomes are not identical: one chromosome is paternally derived and the other is maternally derived.

   b. The chromosomes you are starting with are not "X-shaped," that is, there are no sister chromatids attached at a centromere. What happens to DNA to create X-shaped chromosomes? Summarize this event and identify when it occurs.

   The chromosomes are only X-shaped after DNA replication, which occurs prior to prophase I of meiosis. During DNA replication, the two strands of DNA are separated by a helicase enzyme that moves along the DNA, breaking the hydrogen bonds between the bases. (The bonds that link the nucleotides into a strand are covalent and are not broken.)

   Each parent strand serves as a template for the construction of a new complementary daughter strand by DNA polymerase. Because the strands are antiparallel, one daughter strand is constructed in a continuous fashion (the "leading" strand) and the other is constructed in fragments that are bonded together by ligase (the "lagging" strand).

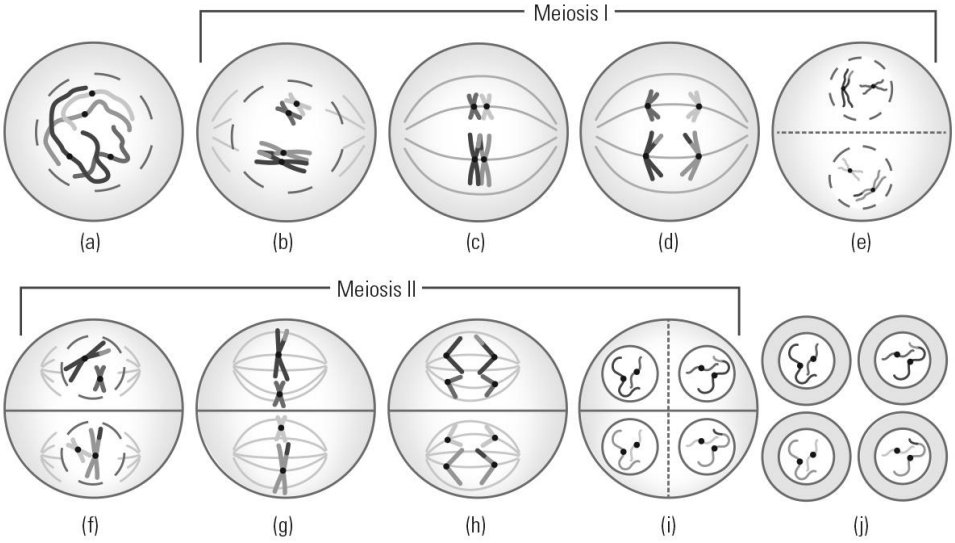
   Replication results in two double-stranded molecules, each identical to the parent molecule and composed of one parent strand and a complementary daughter strand. Replication occurs quickly due to multiple origin-of-replication sites that are opened on each DNA molecule, forming replication "bubbles" that expand as the process continues in both directions.

3. Build sister chromatids for each of the four chromosomes. Attach each sister chromatid to its replicate to create duplicated chromosomes.

4. Use string to make a large circle at your table to represent a cell. Use a smaller piece of string to make a nucleus. Place the chromosomes in the nucleus.

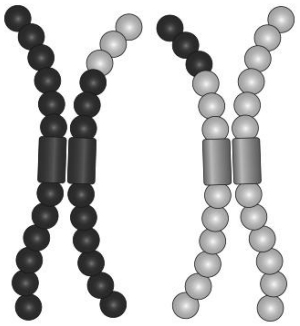
5. Work with your group to simulate and sketch the phases of meiosis I and meiosis II. Discuss the following questions with your group during the simulation activity. Answer the questions in your notebook and include sketches to help illustrate your explanations where appropriate.

Students should use the pop-bead chromosomes to show the progression from one diploid cell to four haploid cells.



a. How can you show crossing over with the pop-bead chromosomes? Would the cross over frequency be the same for all genes? Why or why not?

Crossing over can be shown by removing pop beads from one chromatid (3 yellow pop beads in this case) and switching them with the same number of pop beads from a chromatid of the homologous chromosome (in this case, red). The length of each chromosome remains the same; the chromosomes would just be recombinant, mostly yellow with a few red beads, or mostly red with a few yellow beads.



Cross over frequency depends on the distance between the gene and the centromere of the chromosome. If a gene is located close to the centromere, it is less likely to be exchanged. Exchange of genetic material occurs at *chiasmata*, where non-sister chromatids of homologous chromosomes physically overlap. The overlap tends to occur along areas of the chromatids that are farthest from the centromeres.

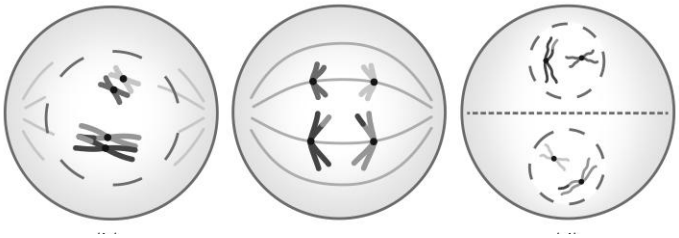
b. In this simulation of meiosis, a person is moving and arranging the chromosomes. What is the physical mechanism of chromosome movement and separation in an actual cell?

Spindle fibers, composed of kinetochore microtubules, connect to the centromeres (also known as *kinetochores*) of chromosomes and can move the chromosomes to the midline of a cell (the metaphase plate) or can pull homologous chromosomes to opposite sides of a cell. The microtubules can also separate sister chromatids from each other.

The microtubules use ATP and motor proteins to move chromosomes along the microtubule toward a pole. Also, the microtubules shorten in length, helping draw the chromosome toward the pole from which the microtubule originates.

c. At what point does the change from diploid to haploid occur? Why is it important that meiosis creates haploid cells?

The change from diploid to haploid occurs during meiosis I. During anaphase I, homologous chromosomes are pulled to opposite sides of the cell. In telophase I, the nuclei of each daughter cell contains half the number of chromosomes compared to the diploid progenitor cell at the beginning of meiosis.

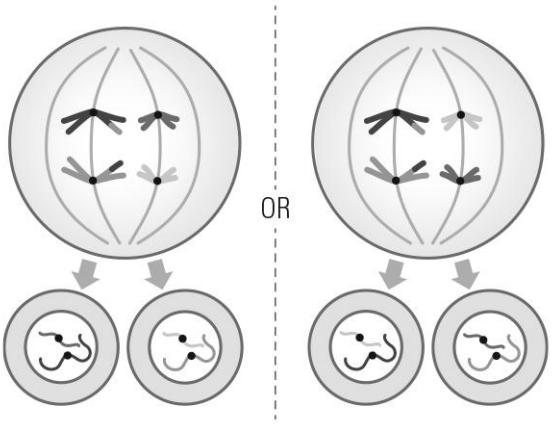


It is important for meiosis to produce haploid cells because gametes need to be haploid to maintain the diploid number in an offspring formed from fertilization.

d. What does *independent assortment* mean with regard to meiosis? How can you use the pop-bead chromosomes to explain this concept?

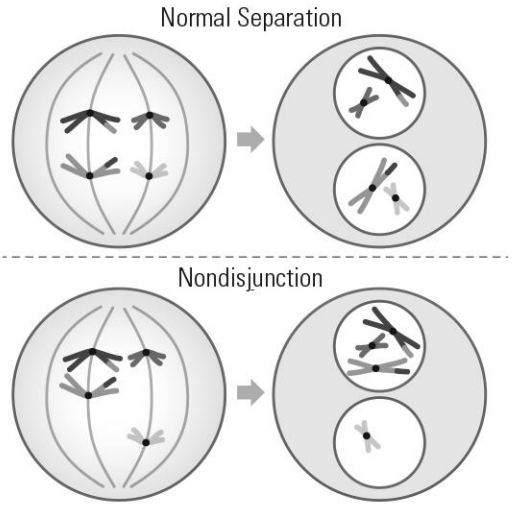
"Independent assortment" means that a daughter cell (gamete) can end up with one of many combinations of maternal and paternal chromosomes at the end of meiosis. The daughter cell will always have half the number of chromosomes, but not the same assortment of chromosomes.

Each homologous pair consists of a paternal chromosome and a maternal chromosome, but how these align at the metaphase plate is random. The alignment of one pair is random with respect to the other homologous pairs. For a simple cell with four chromosomes, there are two possible alignments and gametes will have one of four possible chromosome sets (the number of possibilities = $2^n = 2^2 = 4$). The greater the number of homologous chromosomes *n*, the greater the number of paternal and maternal alignments that are possible at metaphase I.



e. What is the effect on gametes if homologous chromosomes fail to separate? How does nondisjunction affect the outcome of fertilization?

Nondisjunction, or the failure of homologous chromosomes to separate, results in gametes that have an extra chromosome or a missing chromosome. If a gamete with an extra chromosome fuses with a normal gamete during fertilization, the resulting offspring will have the diploid number of chromosomes plus 1. For example, in humans, the offspring would have 47 chromosomes instead of 46. The offspring may not be viable, or it may have a genetic disorder such as Down syndrome or Klinefelter's syndrome, depending on which chromosome is affected.



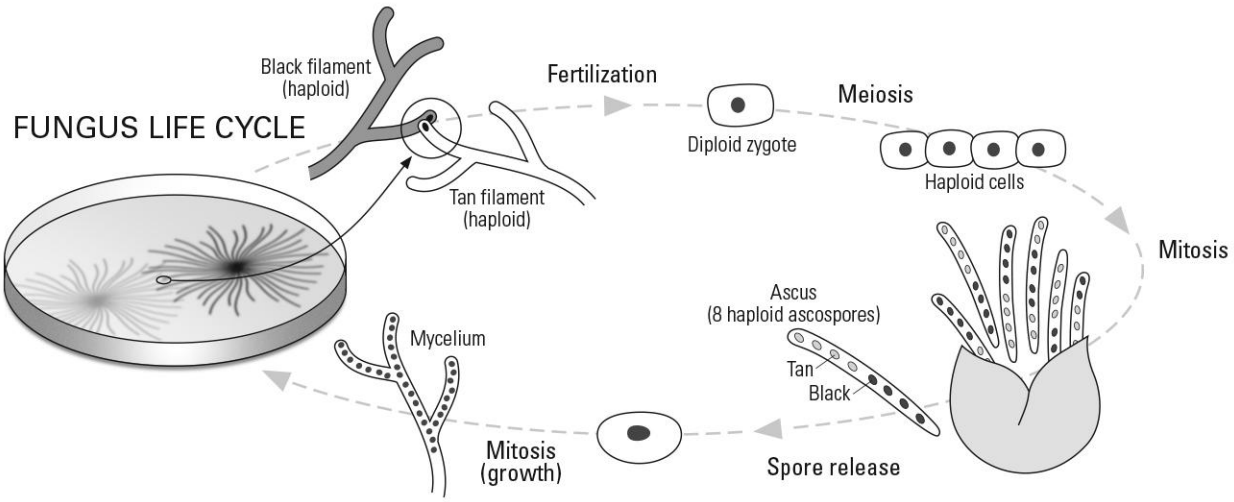
f. How is the process and outcome of mitosis different from meiosis? What is the purpose of each type of cell division in the life cycle of animals?

The daughter cells produced in mitosis are identical to each other and to the progenitor cell. In mitosis, homologous chromosomes do not pair up, so there is no crossing over, nor is there independent assortment (separation) of chromosomes, so there is no genetic diversity in the cells produced. Rather, the separation that occurs in mitosis is of sister chromatids, so that the daughter cells end up with identical sets of chromosomes.

Meiosis produces genetically unique haploid gametes that provide for genetic variation in a sexually reproducing species, such as an animal species. The role of mitosis is to produce new diploid cells for growth and development of the organism and to maintain tissues throughout the organism's life.

Sordaria Investigation

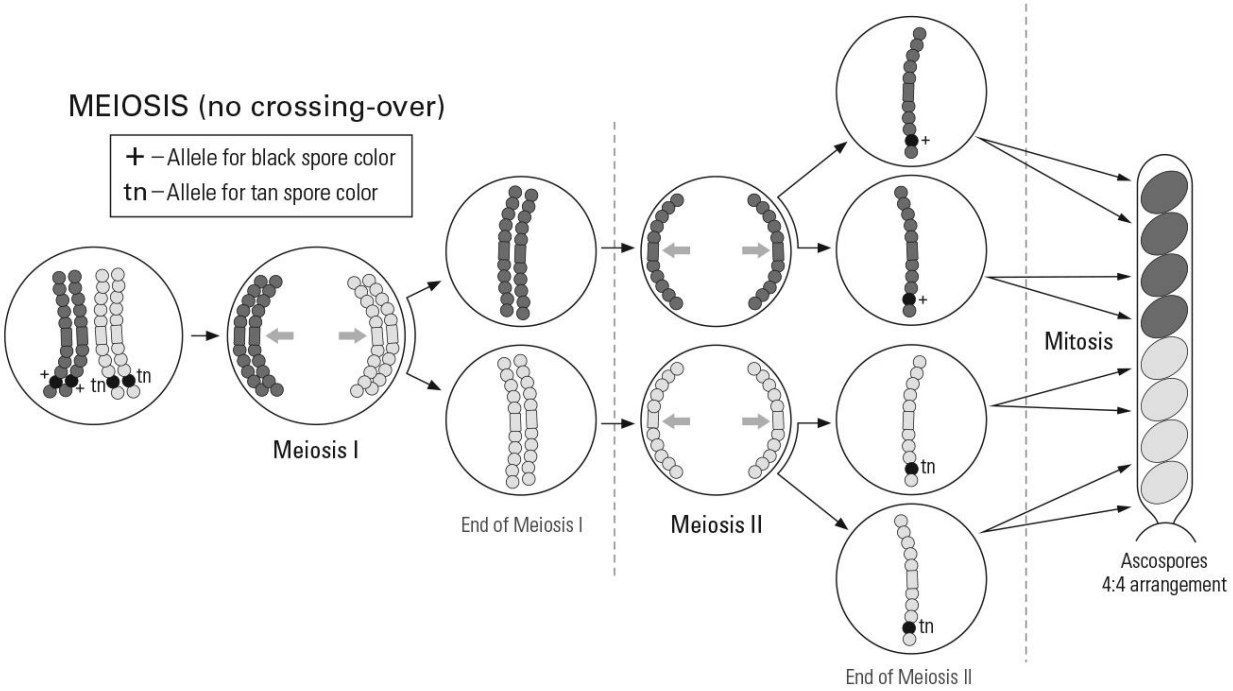
Mitosis does not always produce diploid cells from a diploid cell; sometimes haploid cells undergo mitosis to form more haploid cells. Such is the case in the life cycle of Sac Fungi (Ascomycota). The species investigated in this activity is the fungus *Sordaria fimicola*.



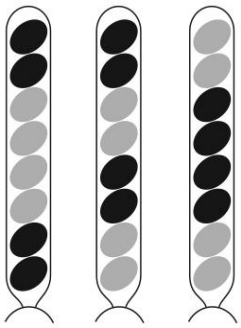
A mature fungus grows from an *ascospore*, a cell with a haploid nucleus. The cells of the fungus are all haploid, which is different from the cells of animals like fruit flies or humans, which are diploid. As the fungus grows, it produces many filaments (hyphae) which form the mycelium, the "fuzzy" structures you observe when you see fungus growing on bread.

If two strains of *Sordaria* are grown together, their filaments can overlap and fertilization can occur. Two haploid nuclei, one from each strain, fuse to form a diploid zygote. But rather than growing by mitosis, like an animal would, the fungal zygote undergoes meiosis, returning to the haploid state. The haploid cells undergo mitosis, forming more ascospores. The fungus keeps the ascospores in a structure called the ascus. The fungus produces fruiting bodies (the *perithecia*) which contain numerous asci. The ascospores in these asci are released into the environment and new fungi establish themselves and grow.

The *Sordaria* life cycle allows for a relatively straightforward investigation of gene mapping: determining the distance between a gene and the centromere of the chromosome it resides on. Spore color is a single-gene trait. By analyzing the colors and arrangement of spores within asci produced on a plate containing black and tan fungal strains, one can determine if crossing over occurred when the zygote underwent meiosis. If there is *no* crossing over during meiosis, the spores will be in a 4:4 arrangement within the ascus: four tan spores next to four black spores. (Refer to the diagram on the following page.) Other color arrangements are observed when crossing over has occurred. The frequency of crossover corresponds to a gene's location on the chromosome.



1. Obtain cards that show asci collected from a black × tan *Sordaria* cross plate. (Alternatively, follow your teacher's directions for preparing slides of asci to view with a microscope.)



2. Locate some asci that have the 4:4 spore color arrangement. Then find instances that differ from this arrangement. Sketch at least three arrangements that differ from the 4:4 arrangement.

3. How many cells are produced by meiosis? Why are there eight cells in each ascus?

   Meiosis produces four cells. There are eight cells in each ascus because each daughter cell produced in meiosis undergoes mitosis, doubling the cell number from four to eight.

4. Copy the table below into your notebook. Count at least 50 asci on one or more cards (or microscope slides) and indicate in the table if each ascus shows no crossover or if there is evidence of crossover. You can tally as you go and then calculate the total.

Table 1: Determining rate of crossover in *Sordaria*[1]

No Crossover (4:4 arrangement)	**Asci That Show Crossover**	**Total Asci Counted**	**Percentage of Asci Showing Crossover**
𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 ||	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 |	68	36/68 = 53%
Total: 32	Total: 36		

[1]This data was obtained using cards from Ward's Science.

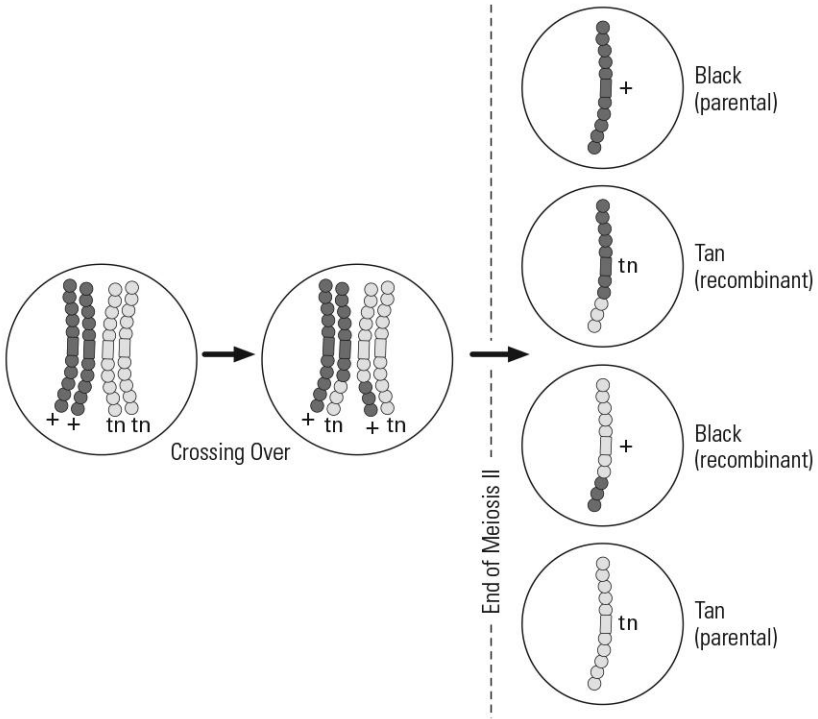
NOTE: If an ascus contains all black or all tan spores, do not count this ascus (it results from self-fertilization). Count only the asci that contain both black and tan spores.

Data Analysis

1. Determine the total number of asci you observed and calculate the percentage of asci that showed crossover.

   Refer to Table 1.

2. When reporting gene-to-centromere distance, *map unit* (mu) is the unit associated with this distance. One map unit is equal to 1% recombination frequency. In most instances, the percentage of offspring with non-parental phenotypes is equal to the number of map units. In the *Sordaria* investigation, however, you observed asci not offspring. Each ascus you counted as recombinant—meaning the ascus showed crossover—actually contained both parental spores and recombinant spores. Only half of the spores in these asci were actually recombinant.



   Therefore, the gene-to-centromere distance for *Sordaria* is calculated as:

   $$\text{Distance (mu)} = \% \text{ of crossover} / 2$$

   Calculate the gene-to-centromere distance from your data and compare your results to others in your class, and to the published map distance.

   Results will vary. In the PASCO trial: Distance = 53% / 2 = 26.5 map units

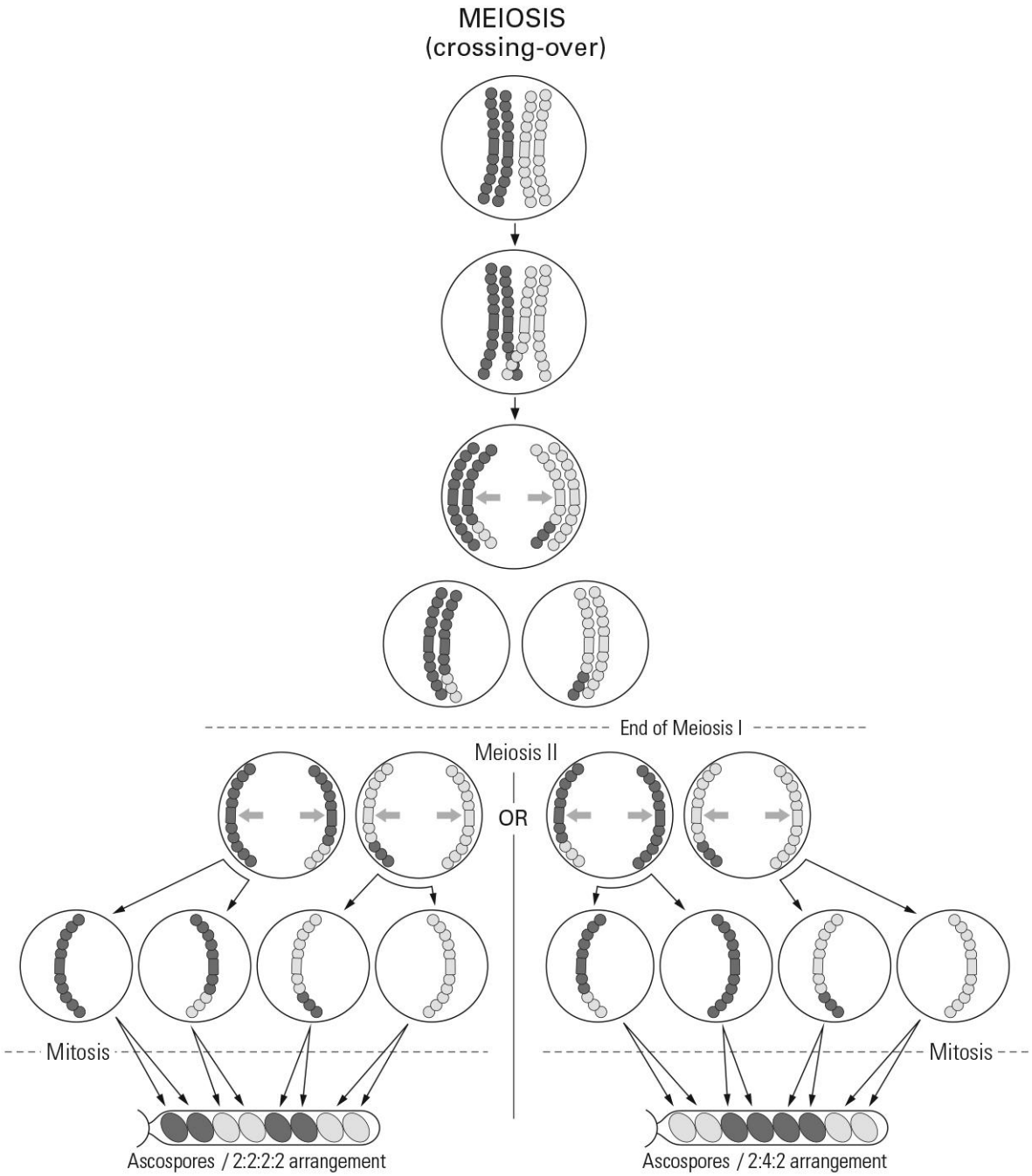
   The published distance is 26 map units.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. Suppose pop beads of one color are used to represent a chromosome from the black *Sordaria* strain (carrying the "+" allele) and pop beads of a contrasting color are used to represent a chromosome from the tan *Sordaria* strain (carrying the "tn" allele). Use the pop bead chromosomes to model crossing over during meiosis and follow the chromosomes through meiosis II and the mitosis that follows. Draw pictures to show how a 2:2:2:2 and a 2:4:2 arrangement can be produced in asci.



2. A *map unit* (mu) is the same distance as a *centiMorgan* (cM), a unit named after the geneticist Thomas Hunt Morgan. Morgan's work was crucial to understanding that some genes are inherited as a unit, due to being on the same chromosome. In other words, they are "linked" genes. Morgan produced a number of mutant fruit flies that he used in breeding experiments to deduce gene locations. He first crossed true-breeding flies that differed in phenotype to obtain an F_1 generation.

   In some of Morgan's experiments, he performed a test cross between a wild type double heterozygous female fly (which was a fly from the F_1 generation) and a mutant fly with a double homozygous recessive genotype. The table below displays the results of an experiment similar to Morgan's.

   NOTE: The wild type traits ("+") are dominant to the mutant forms. The allele symbols used are those that Morgan used: "pr" for eyes and "vg" for wings.

   Table 2: Results of a cross between pr^+ pr vg^+ vg × pr pr vg vg

F2 Generation Phenotypes	Expected Number of Flies		Number of Flies Observed
	If Genes Sort Independently	If Genes Are Inherited as a Unit	
Wild type (red eyes, normal wings)	268	536	482
Purple eyes, vestigial wings	268	536	475
Red eyes, vestigial wings	268	0	56
Purple eyes, normal wings	268	0	59
Total number of flies[1]	1072	1072	1072

[1]Included in the Teacher Resources version only.

   a. First suppose that the two genes, those for eye color and wing shape, are on different chromosomes. What would be the expected phenotypic ratio in the F_2 generation? Based on the number of flies observed, how many flies would be expected for each phenotype listed in the table? (Copy the table into your notebook and complete the second column based on your prediction.)

      A 1:1:1:1 phenotypic ratio would be expected in the F_2 generation. The double heterozygote can produce four possible gametes: pr+ vg+, pr+ vg, pr vg+, pr vg. The double homozygote will only produce pr vg gametes. Therefore, all four phenotypes are equally likely to occur in the offspring of this cross. Since 1072 flies were observed, there should be 268 flies (0.25 x 1072) of each phenotype.

   b. Now suppose that the two genes are located on the same chromosome and are inherited as a unit. Complete the third column of the table, based on your prediction of the number of flies that should be observed exhibiting each phenotype if the genes are linked.

      See the table above. If the genes are linked, the offspring will show only parental phenotypes. The double heterozygote will produce two types of gametes, pr+ vg+ and pr vg. Therefore, 50% of the offspring, or 536 flies out of 1072 offspring, are expected to be wild type and 50% are expected to have the mutant phenotype.

   c. Does the data support independent assortment of these genes, or linkage between the genes? Explain your answer.

      The data indicates linkage between the genes. Even though the actual number observed for each phenotype does not match the expected number for linkage, it more closely matches the linkage condition compared to the independent assortment condition. When almost all of the offspring are of the parental phenotypes, genes for those traits are very likely to exist on the same chromosome, that is, the genes are linked.

d. Assuming the genes are linked, how can the recombinant phenotypes observed be explained? What is the distance between the body color and eye color genes?

The recombinant phenotypes are the result of the genes crossing over during meiosis. Without crossing over, the double heterozygote parent passes on a chromosome with pr+ vg+ or pr vg. Crossing over produces recombinant chromosomes that have pr+ vg or pr vg+.

Of 1072 total offspring, 115 flies, or 10.7%, were recombinant. This means the genes are approximately 11 map units apart.

NOTE: In this case, the percentage of recombinants is not divided by two, as was done with *Sordaria*. The *Sordaria* calculation is unique because it involves the observation of the actual products of meiosis rather than the observation of offspring. Teacher Tip 2 provides further explanation.

3. Explain the difference between *linked* genes and *sex-linked* genes. How did Thomas Hunt Morgan show that the white-eye mutation in *Drosophila* was located on the X chromosome?

Linked genes are genes that exist on the same chromosome. Being on the same chromosome, linked genes are inherited as a unit (unless separated by crossing over). Sex-linked genes are genes that are located on a sex chromosome (rather than an autosome). If the gene is on the X chromosome, for example, it is said to be "X-linked."

Morgan carried out a number of breeding experiments using wild type flies and white-eye mutant flies. (The wild-type eye color is red.) Morgan first noticed the white-eye phenotype in a male fly. Crossing a wild type female with a white-eye male resulted in red-eyed offspring (100%). When two F_1 flies were mated, the white-eye phenotype appeared again, but only in male flies.

Further experiments showed that females could have white eyes if they were the offspring of a white-eyed male and a heterozygous red-eyed female. The trait appears in males more often than in females because males have only one X chromosome. If they inherited an X chromosome with the mutation, they had white eyes. Whereas, females have two X chromosomes and can only have white eyes if they inherit two copies of the mutation.

4. Why does crossing over contribute more to genetic diversity in a sexually reproducing species than mutation does?

Mutation is an infrequent occurrence whereas crossing over takes place on many chromosomes every time meiosis occurs. The diversity of gametes in the gene pool of a population is due mostly to crossing over and independent assortment of chromosomes. A smaller proportion of gametes in the gene pool are different as a result of mutation, which therefore contributes less to genetic diversity.

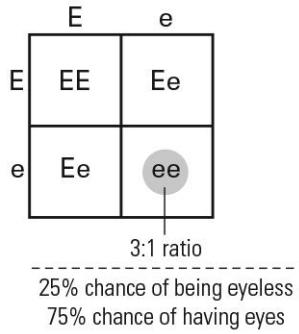
5. Mendel's studies of inheritance in pea plants occurred during the years 1856–1866, though the significance of his work was not realized until much later. During the years 1883–1901, enhanced microscopy allowed scientists to observe and describe the details of meiosis.

a. Explain how the behavior of chromosomes during meiosis supports the conclusions Mendel arrived at from his studies of inheritance.

Mendel concluded that during the formation of gametes, each parents' pair of *factors* (alleles) for a trait were segregated so that each gamete contained only one factor. (This is called the Law of Segregation.) This conclusion is supported by the observation that homologous chromosomes separate during meiosis. The separation takes the alleles on those chromosomes into different daughter cells so that gametes contain only one of each type of chromosome and only one allele of a pair for each trait.

From his dihybrid crosses, Mendel also concluded that the pairs of factors that code for different traits segregate independently of each other. For example, the segregation of flower color alleles had no influence over the segregation of plant height alleles. (This is called the Law of Independent Assortment.) This law is supported by observations that chromosomes line up at the metaphase plate in an orientation that differs in each meiotic event. The maternal and paternal chromosomes are randomly oriented and the way one pair lines up does not influence the orientation of the other pairs.

b. If genes are located on different chromosomes and follow a Mendelian pattern of inheritance, then the rules of probability can be applied to predict or explain the outcome of a genetics experiment. Consider the male and female fruit flies in the initial *Drosophila* investigation. Calculate the probability that an offspring of these flies will be female, eyeless, and have vestigial wings and normal body color. Explain how the rules of probability are applied in your calculation.



A Punnett square for the cross Ee × Ee can be used to determine the probability of an offspring fly being eyeless. The Punnett square shows that the probability of the eyeless trait is 25%.

Instead of using a Punnett square, the following rules of probability can be considered: the rule of multiplication (for independent events) and the rule of addition (for events that can occur in two or more independent ways). *In this example, only the law of multiplication applies.*

The probability of an "e" in the female gamete (egg) is 50% and the probability of an "e" in the male gamete (sperm) is 50%. At fertilization, there is a random fusion of gametes, so the probability of an "e" gamete fusing with another "e" gamete is the product of the two independent probabilities, which is 0.5 x 0.5, or 0.25 (25%).

The probability of each chromosome pair outcome is independent of the other, so you would calculate the product of the four independent probabilities to determine the probability of the specified phenotype in one of the offspring. The probability of each trait in the following cross is given below:

XXEewwBb × XYEeWwbb

Female = 0.5 × 1.0 = 0.50

Eyeless = 0.5 × 0.5 = 0.25

Vestigial wings = 1.0 × 0.5 = 0.50

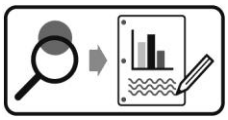
Normal body color = 0.5 × 1.0 = 0.50

The probability of these 4 traits occurring together in an offspring fly is 0.50 × 0.25 × 0.50 × 0.50, which is 0.031, or 3.1%.

Design and Conduct an Experiment

The Initial Investigation provides students a comprehensive study of meiosis and introduces some procedural skills for observing the results of crossover in *Sordaria*. If time allows, students can culture *Sordaria* under different conditions to investigate whether environmental factors, such as pH or nutrient composition of the agar, affect the frequency of crossing over during meiosis.

Alternatively, students can extend this investigation by researching further the connection between chromosome abnormalities and cancer (HeLa cells, for example), or by carrying out experiments with fruit flies to learn more about gene mapping.



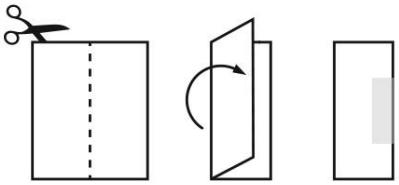
Discuss with students their experiment design or their plan for additional research.

Suggested Inquiry Questions

Inquiry questions are left up to the students.

Drosophila Chromosome Sheet

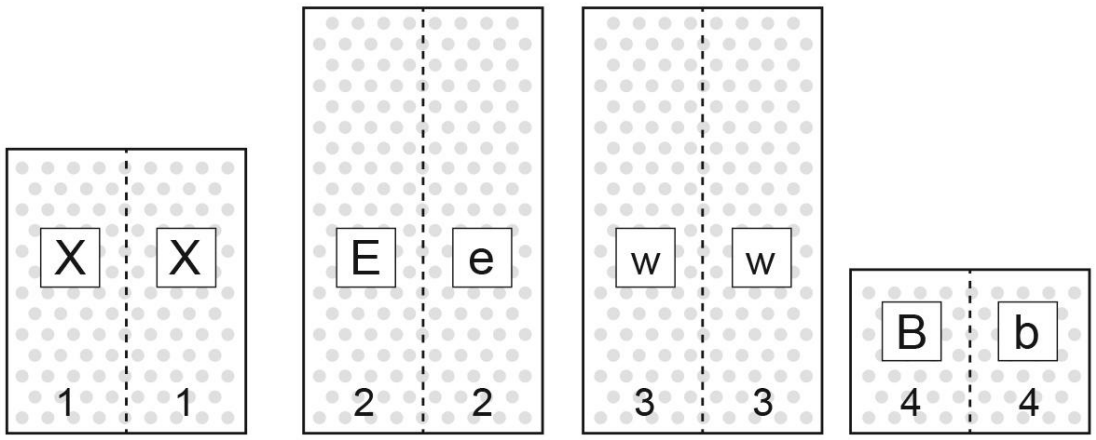
Instructions



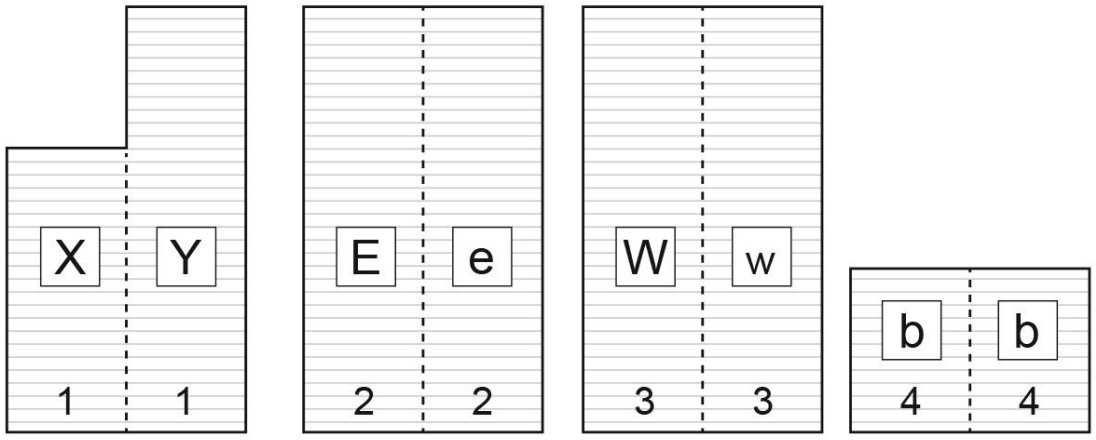
Legend

XX/XY = male / female
E/e = normal eyes / eyeless
W/w = normal wings / vestigal wings
B/b = normal body color / ebony body color

Female Fly

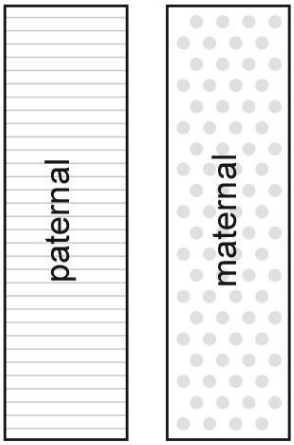


Male Fly



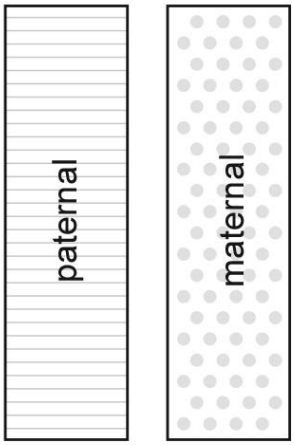
Karyotype of Offspring Fly

Paternal: inherited from the male (“father”)
Maternal: inherited from the female (“mother”)



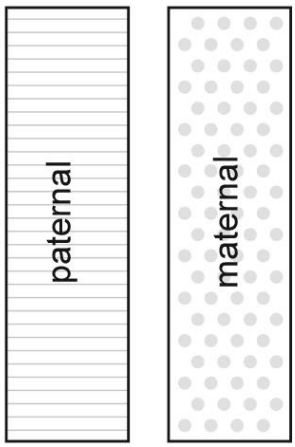
1 1

Sex _____



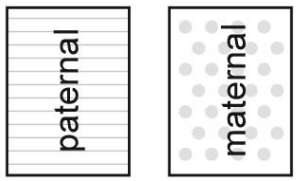
2 2

Eyes _____



3 3

Wings _____



4 4

Body Color _____

14. TRANSFORMATION

Lab Overview

The discovery of bacteria transformation played an important role in understanding that DNA stores and transmits heritable information. Transformation remains important to biological research today. This lab is meant to help students solidify their understanding of the central dogma of molecular biology: the gene–protein connection.

The plasmid DNA used for the transformation contains both a gene providing resistance to the antibiotic ampicillin (*ampR*) and one that will cause bacteria to fluoresce (*gfp*). Students relate the change in the bacteria's phenotype to the genes in the plasmid. They learn that not all genes are expressed and that the fluorescence due to the *gfp* gene depends on a factor called IPTG. Students apply the concept of an inducible promoter to explain the results of the investigation (differential gene expression). Students also learn to calculate the efficiency of the transformation.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	90 min
Initial Investigation—Day 1	55 min
Initial Investigation—Day 2	40 min

Student-Designed Experiment (Optional)	
Experiment Design	30 min
Experiment	1–3 days
Data Analysis	30 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	3.A.1, 3.B.2, 3.C.1, 3.C.2
Science Practices	1.4, 2.2, 4.3, 5.1, 5.3, 6.2
Learning Objectives	3.3–3.5, 3.19, 3.21, 3.23, 3.27

Materials and Equipment

For Each Student Station[1]

- LB (Luria Broth) Petri plate
- LB/Amp Petri plate (2)
- LB/Amp/IPTG Petri plate
- Inoculating loops (2), sterile
- Transfer pipets (4), 1-mL, sterile
- Micropipet with a sterile tip
- Microcentrifuge tubes (2)
- Small cup or beaker, 100-mL, for ice
- Tube with 0.5 M Calcium chloride ($CaCl_2$), 1 mL[2]
- Tube with Recovery Broth, 1.5 mL
- Tube with pFluoroGreen™ (pGFP) plasmid, 12 µL[2]
- Toothpick, sterile
- Ice
- Permanent marker, fine
- Masking tape

[1]Refer to the Lab Preparation section for directions regarding the preparation and organization of the materials listed.

[2]Keep these materials on ice.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

For Class Use

- *E. coli* host cells (on 5 large Petri plates)
- Warm water baths (2), 37 °C and 42 °C
- Incubator (37 °C)
- Long wave UV light source
- Disinfectant

Additional equipment recommended for the student-designed experiment:

- Additional *E. coli* and other bacteria species[1]
- Other antibiotics: kanamycin, penicillin, or others[2]
- Other plasmids: pUC18, pBLU®, pKAN, or others[2]
- Sterile forceps
- Additional Petri plates and LB agar
- Filter paper
- Hole punch

[1]Only purchase classroom-approved bacteria strains from science supply companies. Do not use any bacteria that may pose a risk to students.
[2]Many supply companies offer these items for purchase. Antibiotics can be ordered as liquid solutions or as discs in canisters.

For Teacher Preparation

- EDVO-Kit: 223/AP08[1]
- Water bath, 60 °C
- Pipet pump and 10 mL glass pipet (optional)
- Sterile water, 50 µL
- Micropipet with sterile tips (to aliquot plasmid)
- Microwave or hot plate and beaker with water
- Heat-resistant glove
- Incubator
- Permanent marker, fine

[1] Materials are often purchased as a kit. This investigation is based on the materials in EDVO-Kit: 223/AP08, available at www.edvotek.com or from www.pasco.com (BP–6948). Similar kits can be purchased that use arabinose instead of IPTG to regulate gene expression. This document can be edited to reflect that difference if you choose to use a different kit.

Prerequisites

Students should be familiar with the following concepts:

- Plasmids are small, circular pieces of DNA commonly found in bacteria. The plasmids exist in addition to the bacterial chromosome and many copies may be present in a cell.
- Transformation is the uptake of naked DNA from a bacterium's environment. A bacterium can express the genes present in a plasmid and acquire new phenotypic traits.
- While transformation can occur naturally, in biotechnology applications cells are made *competent* using chemical and temperature treatments.
- The genotype of an organism results in a phenotypic trait when genes are transcribed and translation occurs to produce proteins. *Promoters* play a role in gene transcription (expression).
- Antibiotics are chemicals that interfere with bacteria reproduction. Plasmids often include genes for antibiotic resistance; these genes enable bacteria to survive on a medium containing a specific antibiotic, confirming transformation occurred.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Students with an allergy to antibiotics such as penicillin, ampicillin, kanamycin, or tetracycline should not participate in this experiment.
- Follow proper sterile technique while carrying out all procedures.
- Always wash hands thoroughly with soap and water after working with bacteria and use a 10% bleach solution to disinfect lab surfaces before and after the experiment.

  NOTE: wear gloves and goggles when working with bleach.
- Disinfect used materials (Petri plates, loops, pipets, and tubes) by placing them in an autoclavable, disposable bag and autoclaving at 121 °C for 20 minutes, or by soaking the materials in a 10% bleach solution overnight.
- Never look directly at a UV light. If available, wear UV safety goggles when using a long-wave UV light source.

Lab Preparation

Use the components of the kit to prepare the materials and equipment for each student group or for the class prior to the lab .

Transformation of E. coli *with Green Fluorescent Protein Kit*[1]

- Petri plates (5), large
- Petri plates (40), small
- Transfer pipets (40), 1-mL, plastic
- Pipet (4), 10-mL, (for teacher prep only)[3, 4]
- Toothpicks (26)[4]
- Inoculating loops (26)[4]
- Microcentrifuge tubes (65)
- ReadyPour Luria Broth ("LB") agar[4]
- Luria Broth Medium for Recovery ("Recovery Broth")[4]
- BactoBeads™ *E. coli* GFP Host, 1 vial[5]
- Supercoiled pFluoroGreen™ (pGFP) plasmid DNA[2]
- Ampicillin[2]
- IPTG[2]
- 0.5 M Calcium chloride ($CaCl_2$)
- Growth Additive[2]

[1]The kit contains quantities of all materials sufficient for 10 groups.

[2]Keep frozen until used.

[3]A pipet pump is not included in the kit but is needed to use the 10-mL pipets.

[4]Sterile

[5]Bactobeads may be substituted with FluoroCells™. If this is the case, refer to http://www.edvotek.com/Quick-Guides for modified preparation procedures.

The day before the transformation experiment

NOTE: The E. coli *source plates need to be prepared and inoculated 16–20 hours before students perform the transformation. Older source plates will compromise the success of transformation.*

1. Prepare the ReadyPour™ Luria Broth (LB) agar for the Petri plates:

   a. Melt the LB agar: Loosen (but don't remove) the cap of the bottle containing the prepared agar. Squeeze and vigorously shake the plastic bottle to break up the solid agar into chunks. Heat the bottle using a hot plate or a microwave as follows:

      - Microwave: Heat on high for 1 minute. Use a heat-resistant glove to swirl the bottle. Heat on high for additional 30-second intervals until the agar is completely melted. Wearing a heat-resistant glove, swirl between each interval. Do not shake the agar, as this creates bubbles.

      - Hot plate: Place the bottle in a pan or beaker partially filled with water. Heat the water to boiling. Wearing a heat-resistant glove, swirl the bottle occasionally. Do not shake the agar as this creates bubbles. Remove the bottle from the water when the agar is completely melted.

      CAUTION: Failure to loosen the cap prior to heating may cause the bottle to break or explode. Swirl gently and watch carefully as the agar comes to a boil. Do not allow the agar to boil over.

   b. Allow the melted agar to cool to 60 °C. Swirl the agar occasionally during cooling to evenly distribute the heat and prevent solidifying.

   NOTE: You may want to place the bottle in a 60 °C water bath to prevent premature solidifying, although this will cause the agar to take longer to cool. Do not allow the agar to cool below 45 °C.

2. Prepare the Petri plates:

   While the agar cools, use a permanent marker to label the Petri plates. Make all labels small so as not to obscure the view of bacteria colonies that will form.

   a. Label the five large Petri plates "*E. coli*". These will be used as the source plates from which students acquire the bacteria colonies for transformation.

   b. Label 10 small Petri plates "LB".

   c. Label 20 small Petri plates "LB/Amp".

   d. Label the remaining 10 small Petri plates "LB/Amp/IPTG".

3. Pour the *E. coli* source plates:

   Lay the five large sterile Petri plates on the laboratory table. Use one hand to open the plate "clamshell" style and carefully pour, or use a pipet pump, to add approximately 10 mL of cooled agar into the plate. You may need to rock the plate back and forth to fully cover the bottom of the plate.

   Repeat the process with the remaining four plates, then leave the plates, with their lids on, undisturbed until the agar solidifies.

   CAUTION: Do not mouth pipet any reagents.

4. Prepare the transformation plates:

   a. Add the entire amount of the Growth Additive to the remaining cooled (60 °C) agar. Re-cap the bottle and swirl it to mix the additive evenly with the agar.

   b. Carefully pour, or pipet, approximately 5 mL of agar into the 10 plates labeled "LB."

   c. Add the entire amount of ampicillin to the remaining agar. Re-cap the bottle and swirl it to mix well.

   d. Pour, or pipet, approximately 5 mL of agar to the 20 plates labeled "LB/Amp."

   e. Add the entire amount of the IPTG liquid to the remaining agar and swirl it to mix. Pour, or pipet, approximately 5 mL of the agar into the "LB/Amp/IPTG" plates.

   f. Leave the plates, with the lids on, undisturbed until the agar solidifies. Twenty minutes is recommended. Plates should then be stored inverted and sealed in a plastic bag to prevent dehydration. For optimal results, store plates at room temperature overnight.

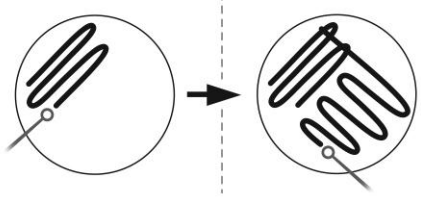
   NOTE: Plates can be stored for up to two days at room temperature, or longer if placed in the refrigerator. If placed in a refrigerator, warm plates to 37 °C in an incubator thirty minutes before use.

5. Prepare the five source plates:

   For best results, streak the source plates 16–20 hours before the transformation experiment.

   a. Use a sterile inoculating loop to remove a single BactoBead™ from the vial and place the bead near the edge of one of the large Petri plates. Immediately place the lid on the Petri plate and re-cap the BactoBead vial.

   b. Dissolve the bead by adding 10 µL of sterile water to it. Streak the loop back and forth through the dissolved BactoBead to make a primary streak at the top of the plate. Take care to not gouge the medium with the loop.



   c. Streak the loop through the primary streak to a clean part of the agar several times to create a secondary streak.

   d. Rotate the plate and make a third streak. Then rotate the plate once more to make a fourth streak. Cover the plate and repeat the procedure for the other four plates

   e. Invert the plates and incubate them at 37 °C for 16–20 hours.

   f. Disinfect the *E. coli* source vial and the loops used for streaking the source plates before disposal.

The day of the transformation experiment

The water baths and reagents for student groups should be prepared one hour or more in advance of students performing the experiment.

1. Prepare the water baths:

   Equilibrate two water baths, one at 37 °C and one at 42 °C.

2. Aliquot reagents:

   Determine whether student groups will have their own microcentrifuge tubes of reagents or whether student groups will share tubes of reagents. If groups will be given their own reagents, dispense the specified volumes into microcentrifuge tubes and label each tube.

   NOTE: The Materials and Equipment list for each student station includes these reagents. Inform students if the groups are to share the tubes of reagents.

   a. 10 tubes (assuming ten groups) with 1 mL each of 0.5 M $CaCl_2$. Place the tubes on ice.

   b. 10 tubes with 1.5 mL each of Recovery Broth.

   c. 10 tubes with 12 µL each of pGFP (plasmid). Before dispensing, take the tube of plasmid from the freezer and place it on ice to thaw. Tap the tube to ensure the entire sample is at the bottom and use a micropipet with a sterile tip to dispense into the tubes. After dispensing, tap each tube and place it on the ice.

   NOTE: The plasmid and $CaCl_2$ tubes should always remain on ice.

Teacher Tips

Tip 1 – Preparing the students for a successful transformation experiment

In a typical class period (45–55 minutes) students will need to work quickly through the procedures. Use the class period prior to the investigation to discuss the steps they will follow and the purpose of each step. Students can design their own flow chart, or a class flow chart can be constructed on the board during class discussion.

If students are inexperienced with culturing bacteria, take time to teach sterile techniques and the proper method of collecting bacteria cells and streaking an agar plate. For more experienced students, it is still suggested that you review proper sterile technique with them.

Tip 2 – Use small disposable cups and crushed ice for microcentrifuge tubes

The calcium chloride and plasmid microcentrifuge tubes need to remain on ice during the experiment. Small disposable cups filled halfway with crushed ice work well for this purpose.

Tip 3– Incubation Period

Do not allow the students' plates to incubate longer than 16–18 hours at 37 °C. Depending on the time of day the class completes the procedures, you may take the plates out of the incubator the next morning (prior to 18 hours) or you may set the temperature of the incubator to be cooler than 37 °C.

Tip 4 – Save Materials

If you are having students carry out student-designed experiments, then after completing the lab preparation steps, save any extra materials or solutions. Students may also make use of the bacteria resulting from the Initial Investigation.

Tip 5 – Understanding the purpose of each Petri plate

Students are asked, in the Data Analysis and Synthesis Questions sections, to think about and relate each plate's purpose to their observations of the presence or absence of bacteria on the plate. The table below is a quick teacher-reference regarding the plates used in the investigation.

Preparation of Petri plates

Label and Contents	Number of Plates	Purpose
LB Luria Broth agar	10	These plates are the positive control for the investigation. They are used to test whether the *E. coli* cells survive the chemical and temperature treatments of the transformation procedure. Since there is no antibiotic in the medium, bacteria growth is expected. A "lawn" of bacteria is expected for this plate, given that there is no selection against non-transformed cells.
LB/Amp Luria Broth agar that contains ampicillin	20	Each student group will use two of these plates. One of the plates will contain bacteria from the tube with no plasmid (–DNA) and the other plate will contain bacteria from the tube with the plasmid (+DNA tube). The plate labeled "–DNA" is a *negative* control. No bacteria growth is expected on this plate since ampicillin prevents reproduction of *E. coli by inhibiting cell wall formation and causing lysis of the cells*. The second LB/Amp plate, labeled "+DNA," will show bacteria growth (isolated colonies) as long as the transformation procedure is successful: the gene that confers resistance to the antibiotic will only be acquired by bacteria in the +DNA tubes. This agar is missing the factor that promotes protein synthesis of GFP. Transformed bacteria will grow but will not fluoresce.
LB/Amp/IPTG Luria Broth agar that contains ampicillin and IPTG	10	The bacteria on this plate will be from the +DNA tube, so some of these bacteria will have acquired the plasmid (with the Amp*R* gene) and the plate should have isolated colonies, similar to the LB/Amp/+DNA plate. Unlike the transformed bacteria on the LB/Amp plates, the bacteria on the plates with IPTG will fluoresce green. The *gfp* gene is regulated by an inducible promoter. The promoter is only "on" when IPTG is present; that is, green fluorescent protein is only produced in the presence of IPTG.

Tip 6 – Explaining the role of IPTG

Allow students to independently discover that the presence of IPTG affects expression of the *gfp* gene. In other words, the discussion about gene regulation and expression is meant to occur *after* the students have completed the transformation experiment. Plan to use some class time to lead the discussion; an example discussion is outlined below.

1. All transformed bacteria incorporated the plasmid with *gfp*; they all have the gene that codes for production of green fluorescent protein. However, some bacteria actively express the gene and fluoresce while other bacteria do not. Ask students to identify the two plates that contain transformed bacteria and ask them why only one plate contains fluorescent bacteria.

   The plates with the transformed bacteria from the +DNA tube are the LB/Amp and LB/Amp/IPTG plates. Since the bacteria only fluoresce on the plate that contains the IPTG, the IPTG must have something to do with the fluorescence.

2. Ask students to recall the two steps to protein production: transcription and translation. Have them summarize the process and purpose of each. Ask them to consider how transcription can be regulated so that a gene can be turned "on" or "off."

   During transcription, DNA is used to produce a single-stranded mRNA molecule that has the same nucleotide sequence (except it uses uracil instead of thymine). That mRNA sequence is then translated into a sequence of amino acids, three bases at a time. This occurs on ribosomes in the cytoplasm. Each set of three bases, a "codon," is the code for a specific amino acid. The amino acids are joined together to form a polypeptide which then folds and coils into a complex 3-dimensional shape, and may combine with other polypeptides, to form a functional protein.

   Proteins are one or more long polypeptides (100+ amino acids) that fold and coil into complex 3-dimensional shapes. Some become enzymes, some act as hormones, and others form the structural parts of tissue, like muscle.

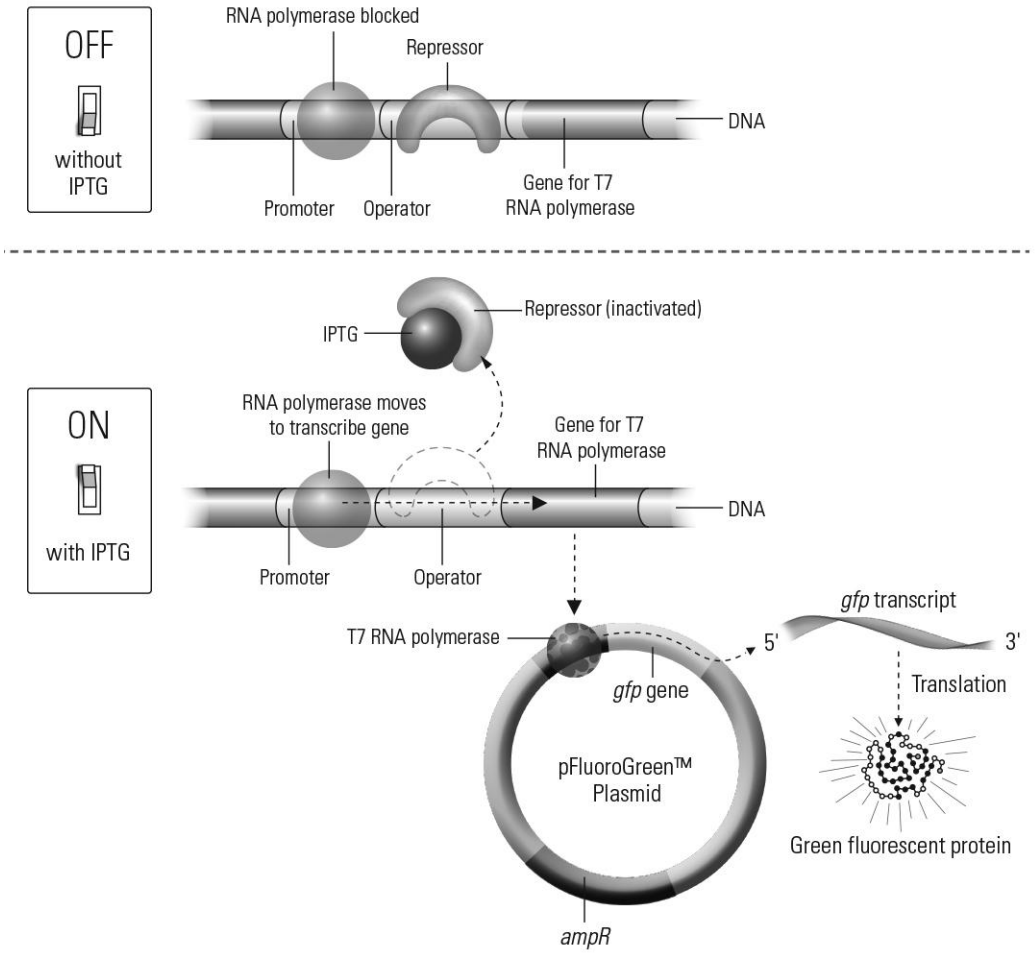
   A gene is "on" if it is actively transcribed, resulting in the mRNA that will be translated to make a protein such as GFP. Transcription of the gene begins when an RNA polymerase molecule binds to a promoter region. If this promoter region is blocked, denying RNA polymerase access, the gene is turned "off."

3. The *E. coli* bacteria used in this lab have been genetically engineered to produce a special RNA polymerase: T7. The gene for T7 is controlled by the *lac* promoter. This promoter is *inducible*. Show students a diagram of the *lac* operon and ask students to describe what an inducible promoter is.

   An *inducible promoter* is a section of the DNA that regulates gene expression. By default, the inducible promoter keeps gene expression "off." The promoter typically has a repressor molecule bound to it that prevents transcription of the gene. (The repressor blocks RNA polymerase.) A molecule in the environment, such as lactose, can "turn on" gene expression by binding to and inactivating these repressors. This allows RNA polymerase to bind to the promoter, initiating transcription of the gene.

4. IPTG (isopropyl-beta-D-thiogalactopyranoside) is a molecule that can bind to the *lac* repressor. Ask students to sketch a diagram to show how they think the presence of IPTG affects production of T7 RNA polymerase.

   Student answers will vary. Students should include the basic components of the operon system in their sketch: promoter region, gene, RNA polymerase, and repressor. Under normal conditions, the repressor is bound to the DNA and prevents transcription of the adjacent gene. To show how IPTG affects production of T7 RNA polymerase, students should show the repressor not attached to the DNA; rather IPTG should be shown bound to the repressor. This leaves the operon "open" for the native RNA polymerase to access the gene and transcribe it. Transcription results in production of T7 RNA polymerase. (The following diagram also illustrates the relationship between T7 RNA polymerase and green fluorescent protein.)



5. IPTG allows T7 RNA polymerase to be made within the cell. Ask students to consider the diagram in the Background section of the handout showing the pGFP plasmid. Have students explain the relationship between T7 RNA polymerase and GFP production.

   Refer to the diagram on the previous page. The promoter sequence for the *gfp* gene requires T7 RNA polymerase to initiate transcription. (*E. coli's* natural RNA polymerase cannot recognize the promoter.) So when T7 RNA polymerase is made within cells, the *gfp* gene is expressed. If there is no T7 polymerase in the cell, then the *gfp* gene remains "silent" and the green fluorescent protein is not made.

6. Bring students back to the original question about why some transformed bacteria fluoresce and why some do not.

   The bacteria colonies on the plates with IPTG were the only bacteria producing T7 RNA polymerase. The other transformed bacteria contained the plasmid but the *gfp* gene remained silent because the *lac* repressor remained bound to the *lac* promoter (prior to the coding sequence for T7) so the T7 RNA polymerase was not produced.

   The IPTG promotes production of the GFP protein by allowing production of the T7 RNA polymerase that transcribes the *gfp* gene, so only the colonies growing on the LB/Amp/IPTG plates fluoresce. Colonies on plates lacking IPTG lack the polymerase needed to transcribe *gfp*, so no GFP is produced by these colonies.

7. Ask students to explain the difference in gene expression between the *gfp* gene and the *ampR* gene.

   The *gfp* gene was not always expressed; it was expressed only when IPTG was present in the medium. Yet the transformed bacteria expressed the *ampR* (ampicillin-resistance) gene independent of IPTG. The *ampR* gene must not be regulated by a T7 RNA polymerase promoter.

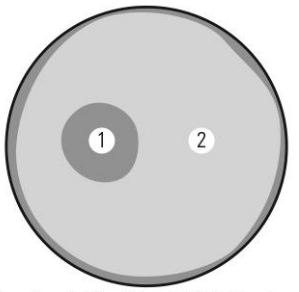
Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. The understanding of the transformation process and the skills acquired here prepare them to pursue additional investigations involving bacteria, if time and materials allow for this.

From the student handout:

Before the transformation experiment

1. *E. coli* was grown on Luria Broth (LB) agar containing two paper discs. One disc was soaked in ampicillin and the other was soaked in distilled water before being placed on the surface of the agar. The dark area on the Petri plate indicates bacteria growth. What does the diagram suggest about the effect of ampicillin on *E. coli*? Explain your answer.



1 = Ampicillin / 2 = Distilled water

Ampicillin kills the *E. coli* bacteria cells or keeps the cells from being able to reproduce. On the majority of the plate, away from the disc, are many colonies ("lawn" growth). Those bacteria survived and reproduced, creating the colonies, in the absence of the antibiotic. The ampicillin prevented bacteria growth, resulting in a clear area of agar around the disc.

Disc 2, containing water, indicates that it is the chemical in the antibiotic that inhibits growth, not the paper or moisture.

2. Some bacteria are resistant to antibiotics such as ampicillin. Bacteria that are sensitive to an antibiotic can become resistant if they acquire a resistance gene. The ability to survive in the presence of the antibiotic results from the gene coding for a beneficial protein, for example, for an enzyme that degrades the antibiotic. In nature, how might bacteria acquire new genes? Identify two or more possibilities and give a description of each.

Here are four ways a bacteria might acquire new genes:

1) Mutation: a change can occur within an existing gene when DNA is being replicated.

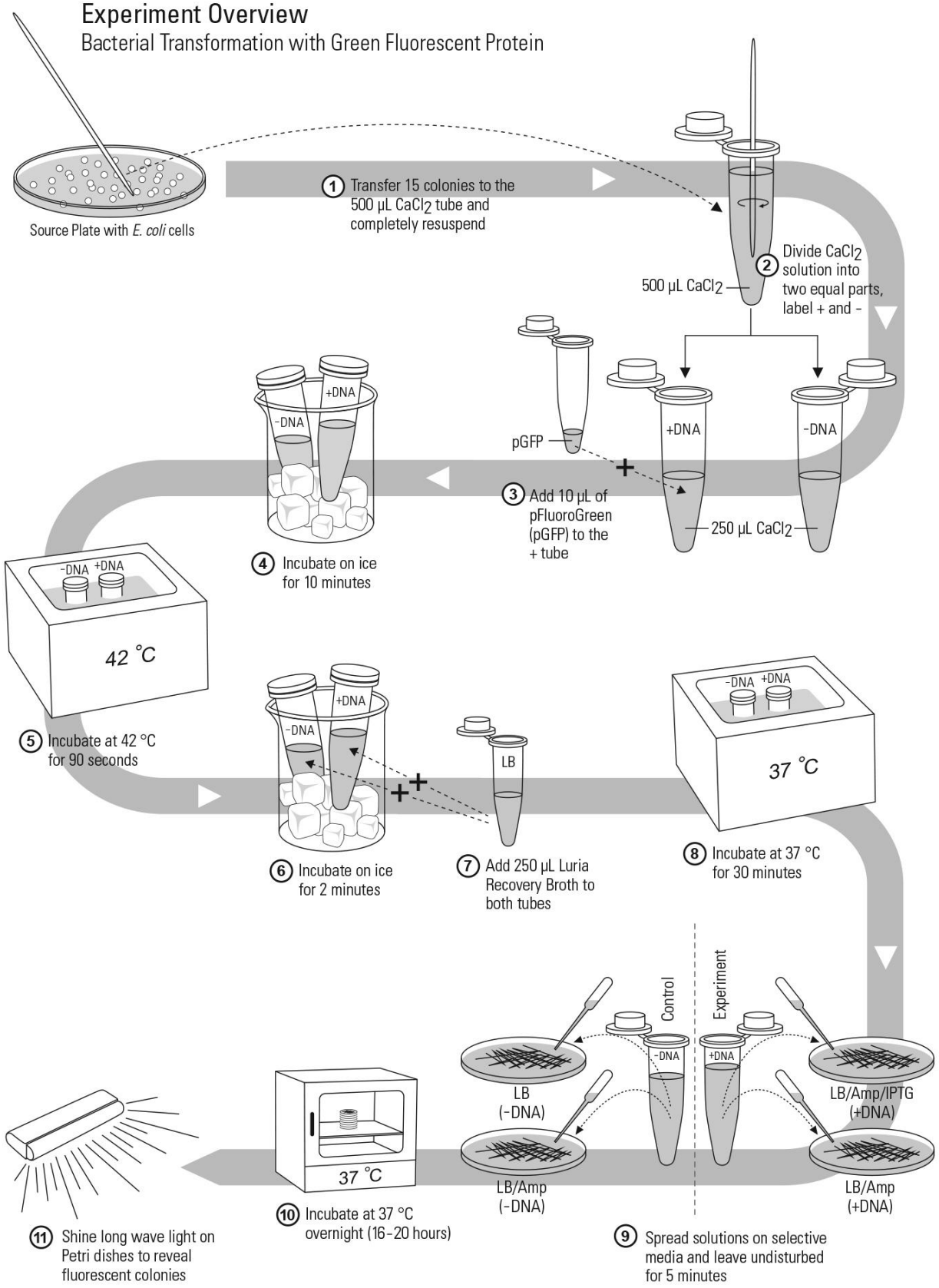
2) Transformation: bacteria take in DNA from the environment.

3) Conjugation: a bacterium transfers a copy of a plasmid to another bacterium.

4) Transduction: DNA from a bacterium is transferred to another bacterium via a virus.

3. For successful transformation, you will need to work efficiently. You will not have time to figure out the steps along the way. To prepare for a successful experiment, read the procedures of the investigation carefully and create a flow chart to guide you through the steps of the transformation procedure.

Refer to the following flow chart. This diagram is not part of the student version. Students are encouraged to create their own diagrams as they read the directions carefully. You may wish to provide this diagram to students for them to check their work and understanding.


Experiment Overview
Bacterial Transformation with Green Fluorescent Protein
Source Plate with E. coli cells
1 Transfer 15 colonies to the 500 µL CaCl2 tube and completely resuspend
500 µL CaCl2
2 Divide CaCl2 solution into two equal parts, label + and -
pGFP
+DNA
-DNA
3 Add 10 µL of pFluoroGreen (pGFP) to the + tube
250 µL CaCl2
4 Incubate on ice for 10 minutes
42 °C
5 Incubate at 42 °C for 90 seconds
6 Incubate on ice for 2 minutes
LB
7 Add 250 µL Luria Recovery Broth to both tubes
37 °C
8 Incubate at 37 °C for 30 minutes
Control
Experiment
LB (-DNA)
LB/Amp/IPTG (+DNA)
LB/Amp (-DNA)
LB/Amp (+DNA)
9 Spread solutions on selective media and leave undisturbed for 5 minutes
10 Incubate at 37 °C overnight (16-20 hours)
11 Shine long wave light on Petri dishes to reveal fluorescent colonies

4. By introducing a plasmid into the cells you are altering the genotype of the bacteria. How will this affect the phenotype of the bacteria? In other words, how will you know if the transformation is successful?

   The plasmid being used contains two genes of interest: *gfp* and *ampR*. The *ampR* gene is an antibiotic resistance gene. The *gfp* gene codes for green fluorescent protein. If the bacteria are transformed, they will have two new traits in their phenotype. The transformation can be shown to be successful if the bacteria grows on a plate that contains ampicillin or if it fluoresces green.

5. Identify the purpose of each of the following:

   a. The labels "+" and "–" on the microcentrifuge tubes.

      The "+" indicates the tube with bacteria that receives the plasmid solution. The "–" represents the control group that does not receive the plasmid.

   b. Suspending cells in $CaCl_2$.

      The ions of the calcium chloride solution increase the permeability of the bacteria, creating small pores that make it possible for the plasmid to enter the cell.

   c. Incubation of cells in 42 °C water, followed by incubation on ice.

      The movement of the cells from ice to warm water, the "heat shock", causes the plasmids to move into the cells. Returning the cells to ice helps the plasmids remain within the cytoplasm.

   d. Culturing cells on an agar plate containing ampicillin ("LB/Amp").

      Since normal *E. coli* cells are sensitive to ampicillin, they should only grow on medium containing ampicillin if the bacteria have incorporated the plasmid with the resistance gene in it. Culturing the bacteria on the LB/Amp plates will show if any have been successfully transformed.

Transformation experiment

6. Put on your safety goggles.

7. Add ice to half-fill the small beaker or cup and put the plasmid and calcium chloride tubes into it.

8. Use a sterile 1-mL pipet to transfer 0.5 mL (500 µL) of cold $CaCl_2$ solution to one of the microcentrifuge tubes. Place that tube into the ice.

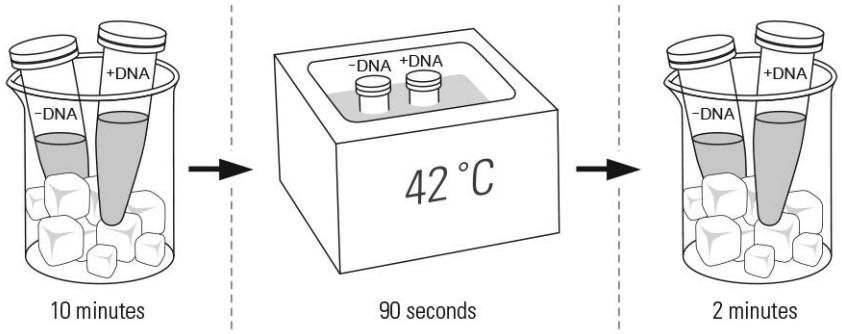
9. With a sterile toothpick, transfer the cells of 15 colonies from an *E. coli* source plate to the tube you just filled with the $CaCl_2$. Swirl the toothpick vigorously in the solution to dislodge the cells and flick the tube to fully suspend them. The suspension should look homogenous, without clumps.

10. Using the same pipet, transfer 0.25 mL (250 µL) of the cell suspension into a second microcentrifuge tube.

11. Use a permanent marker to mark one of the microcentrifuge tubes with the cell suspension with a "+" and the second one with a "–".

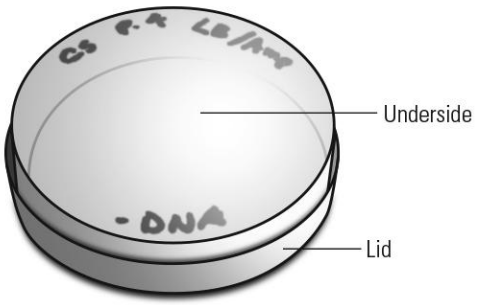
12. Place the "–" tube back in the ice. Use a micropipet with a sterile tip to add 10 µL of plasmid (pGFP) solution to the "+" tube and place it in ice. Leave both tubes in the ice for 10 minutes.

13. After the 10 minutes on ice, move the tubes to a 42 °C water bath for 90 seconds. Then return the tubes to ice for 2 minutes.



14. Use a fresh sterile pipet to add 250 µL of Recovery Broth to each of the tubes and mix them. Place the tubes in a 37 °C water bath for a 30-minute recovery period.

15. While you wait, label the four Petri plates with a permanent marker. Make all labels small and near the edge of the underside of the bottom of the plate (not on the removable lid) so as not to obscure the view of bacteria colonies that will form.



    a. Write your group's initials, class period, and the date on each plate.

    b. On the LB plate, write "–DNA".

    c. On one LB/Amp plate write "–DNA". Write "+DNA" on the second LB/Amp plate.

    d. On the LB/Amp/IPTG plate write "+DNA."

16. When the recovery period is over, bring the tubes back to your lab station. Use a sterile pipet to transfer 0.25 mL (250 µL) of cell suspension from the "–" tube to each of the two plates labeled "–DNA." Use a fresh sterile inoculating loop to spread the cell suspension over the entire plate (for both plates).

17. Use a fresh pipet to add 0.25 mL of cell suspension from the "+" tube to the plates labeled "+DNA" and use a fresh sterile inoculating loop to spread the cell suspension over the plates.

18. Leave the plates undisturbed for at least five minutes to allow the liquid to be absorbed into the agar in each plate.

19. On which plate(s) do you expect to observe growth of the transformed bacteria cells? Explain the reasoning for your prediction.

    The transformed bacteria will come from the "+" tube. If transformation was successful, bacteria growth should occur on both of the +DNA plates (LB/Amp and LB/Amp/IPTG). Even though these plates have ampicillin, the transformed bacteria will be resistant to the antibiotic.

20. After the liquid has been absorbed, stack the set of plates and tape them together. Write your group's initials and class period on the tape. Invert the plates and place them in a 37 °C incubator for 16–20 hours.

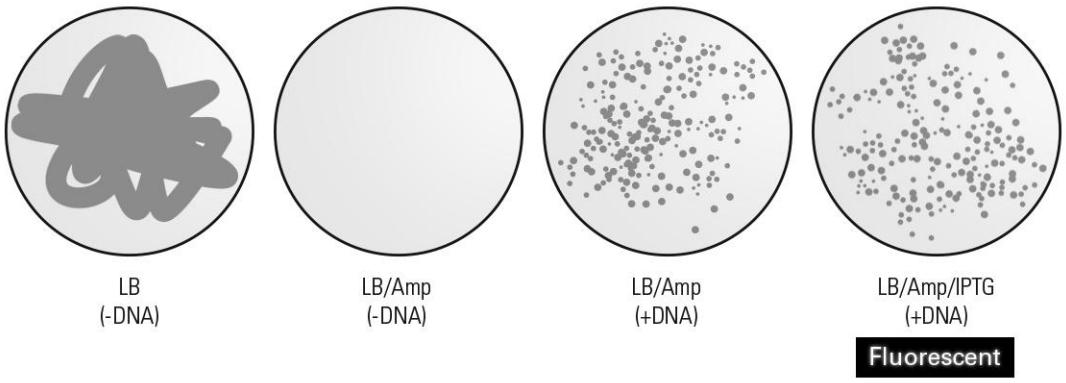
21. Thoroughly disinfect your work space, place the used materials in the location designated by your teacher for disinfection and disposal, and wash your hands with soap.

Results of the transformation experiment

NOTE: Do NOT remove the lid of the Petri plates. Colonies are easily observed by keeping the plates inverted and viewing them through the bottom of the plate.

22. After the incubation period, obtain your stack of Petri plates. Record detailed observations and sketch the appearance of each of the plates.

    The diagrams below illustrate the relative amount of growth on each plate (the colonies will actually appear white or light green in color). It is expected that there will be "lawn" growth on the LB plate: the plate will be covered in numerous small colonies wherever the solution was spread on the agar. The LB/Amp (–DNA) plate is expected to be clear, having no bacteria growth. The LB/Amp (+DNA) plates will both have many colonies, however there is less growth than on the LB plate, since not all cells are actually transformed.



23. Darken the room and use a long wave UV light to determine if any transformed colonies fluoresce, due to production of GFP. Record your observations.

    Colonies on the LB/Amp/IPTG (+DNA) plate fluoresce brightly. None fluoresce on the LB/Amp plate.

24. Did you get any surprising or unexpected results? Explain your answer.

    Answers will vary. Some students may not have any transformed bacteria. Students may have accidentally contaminated their plates with bacteria from the environment, in which case colonies will have different appearances, such as different colors.

    All groups should notice the difference between transformed bacteria on the plate containing only ampicillin and the transformed bacteria on the plate containing IPTG and ampicillin. Students are likely to be surprised that the bacteria on the LB/Amp plate do not fluoresce like those on the plate containing IPTG.

Data Analysis

1. Which plate has the greatest bacteria growth? Explain why this is the case.

   The LB (–DNA) plate has the greatest bacteria growth. The medium has nothing in it that inhibits growth; there is no ampicillin. Most of the cells should have survived the chemical and temperature treatments and reproduced during the 24 hours, so there should be numerous colonies on the plate ("lawn" growth).

2. Which plate has no bacteria growth? Explain why this is the case.

   The LB/Amp, –DNA plate had no bacteria growth. Without the plasmid, *E. coli* cannot survive in the presence of ampicillin, so the ampicillin killed the bacteria cells transferred to that plate and prevented formation of colonies.

3. Control groups are an important part of good experimental design.

   a. Which plate or plates represent a control group for the transformation experiment? Explain your answer.

   LB: This plate is a positive control. Bacteria growth is expected on this plate—it has no antibiotic, so bacteria growth should not be inhibited. The –DNA bacteria on this plate went through the transformation treatment (chemical and temperature changes) and this plate will show whether the treatment killed or harmed the cells.

   LB/Amp (–DNA): This plate is a negative control. Bacteria are not expected to grow on this plate as it contains antibiotic and cells without the plasmid. This will show that *E. coli* is not normally resistant to ampicillin.

   b. Why are control groups essential?

   Control groups are essential to make valid claims about the results of an experiment. The comparison of bacteria exposed to the plasmid to bacteria not exposed to the plasmid is important in showing that the plasmid is responsible for the growth in the presence of ampicillin. It verifies that testing for ampicillin resistance is a proper method of screening for transformed bacteria.

   The positive control confirms that the transformation procedure does not kill the bacteria, so if no growth occurs on the other plates, something other than the chemical and temperature treatments is responsible.

Transformation efficiency

Transformation efficiency is an indicator of the success of the experiment and is obtained by determining the number of cells transformed per 1 µg of plasmid DNA.

4. To calculate the transformation efficiency:

   a. Begin by counting the number of colonies on the LB/Amp/IPTG plate. It may be helpful to mark counted colonies with a dry erase marker on the outside of the plate. Record the total number of colonies in your lab notebook.

   For the sample data, there are 40 colonies on the Petri plate.

   b. Use the colony count to calculate the transformation efficiency using the formula below and the accompanying information:

   Total DNA used: 0.050 µg

   Recovery volume: 0.50 mL

   Volume plated: 0.25 mL

$$\text{Transformation Efficiency} = \frac{\text{Number of transformants/plate}}{\text{µg DNA/plate}}$$

$$\text{where the number of µg DNA/plate} = \text{Total DNA (µg)} \times \frac{\text{Volume plated}}{\text{Recovery volume}}$$

   The mass of the DNA applied to the plate:

$$\text{µg DNA/plate} = 0.050 \text{ µg DNA} \times \frac{0.25 \text{ mL}}{0.50 \text{ mL}} = 0.025 \text{ µg}$$

   For the sample data:

$$\text{Transformation Efficiency} = \frac{40 \text{ transformants}}{0.025 \text{ µg DNA}}$$

$$= 1.6 \times 10^3 \text{ transformants/µg}$$

5. In research laboratories, transformation efficiency ranges from 1×10^5 to 1×10^8 cells per microgram of plasmid DNA. How does the transformation efficiency of this investigation compare to that of a research laboratory?

   Results will vary. For the sample data, the efficiency was lower than that of a research laboratory.

6. How do the transformation efficiencies acquired by different student groups compare? What might account for differences in efficiency?

   Results will vary. Some students may follow procedures better than other students. Students may not accurately pipet the proper volume of plasmid or cell suspension onto the plates.

7. Count the colonies on the plate labeled LB/Amp (+DNA) and calculate the transformation efficiency. Record the data in your notebook. Is the efficiency similar between the two experimental group plates (LB/Amp and LB/Amp/IPTG)?

   Results will vary, but the efficiency should be very similar for the two plates. Both plates received bacteria from the +DNA tube and were incubated at the same temperature. Any difference would be due to the accuracy of pipetting or the difference in the medium (IPTG present or absent). The IPTG is not expected to affect the growth rate, however. It is also possible that the cells are not suspended uniformly within the +DNA tube so there may be variation in the number of cells acquired in each 250 µL sample taken from the tube.

8. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

The *E. coli* used in this experiment have been genetically engineered to be able to produce a special RNA polymerase, called *T7 RNA Polymerase*. The gene for this polymerase is under the control of the inducible promoter of the *lac* operon. IPTG is a substance that binds to the *lac* repressor, thereby inducing transcription of the T7 RNA polymerase gene. The pGFP plasmid used in the investigation was engineered to have a T7-specific promoter just upstream of the *gfp* gene. You may find it helpful to refer to the image in the Background section.

1. Think about how gene expression is regulated by an inducible promoter.

   a. What is the role of a repressor protein in gene regulation?

      A repressor protein binds to a promoter "upstream" of the gene being regulated. The repressor prevents transcription of the gene.

   b. What effect does IPTG in the growth medium have on the expression of the T7 RNA polymerase gene within the *E. coli* bacteria cells?

      IPTG induces the expression of the T7 gene. By binding to the *lac* repressor, IPTG allows the T7 gene to be actively transcribed.

2. IPTG affects the expression of the *gfp* gene, and therefore affects whether *E. coli* colonies fluoresce. Why is the expression of the *gfp* gene dependent upon IPTG?

   Without IPTG in the agar, the transformed *E. coli* cells do not produce T7 RNA polymerase molecules; when IPTG is absent, the *lac* repressor blocks transcription of the T7 gene, since this gene is regulated by the *lac* promoter. Without T7 RNA polymerase, transcription of *gfp* on the plasmid cannot occur, so transformants on the LB/Amp agar do not fluoresce even though they have acquired the plasmid.

   Without transcription of *gfp*, there is no production of green fluorescent protein. IPTG molecules block the *lac* repressor and induce production of T7 RNA polymerase. The T7 RNA polymerase molecules then bind to the promoter controlling the *gfp* gene on the plasmid and transcribe the gene, resulting in the production of GFP and the fluorescence of the cells.

3. Explain how the transformation experiment demonstrates:

   a. the relationship between genotype and phenotype.

      Genotype refers to the genes that comprise an organism's genome. Transformation changes the genotype of the affected bacteria cells because the bacteria acquire new genes when they take in the plasmid. Phenotype refers to the physical appearance of an organism, or its traits, that result from gene expression. Genotype controls phenotype. The change in genotype in transformed *E. coli* results in a change in phenotype: the cells acquire the traits of antibiotic resistance and green fluorescence.

   b. the relationship between phenotype and the environment.

      Organisms do not always express all of the genes that comprise their genotype, so the phenotype is not a reflection of the entire genotype of an organism. The experiment showed that a gene can be present in an organism, but only expressed under certain conditions. For example, the *gfp* gene was only expressed when IPTG was present in the medium.

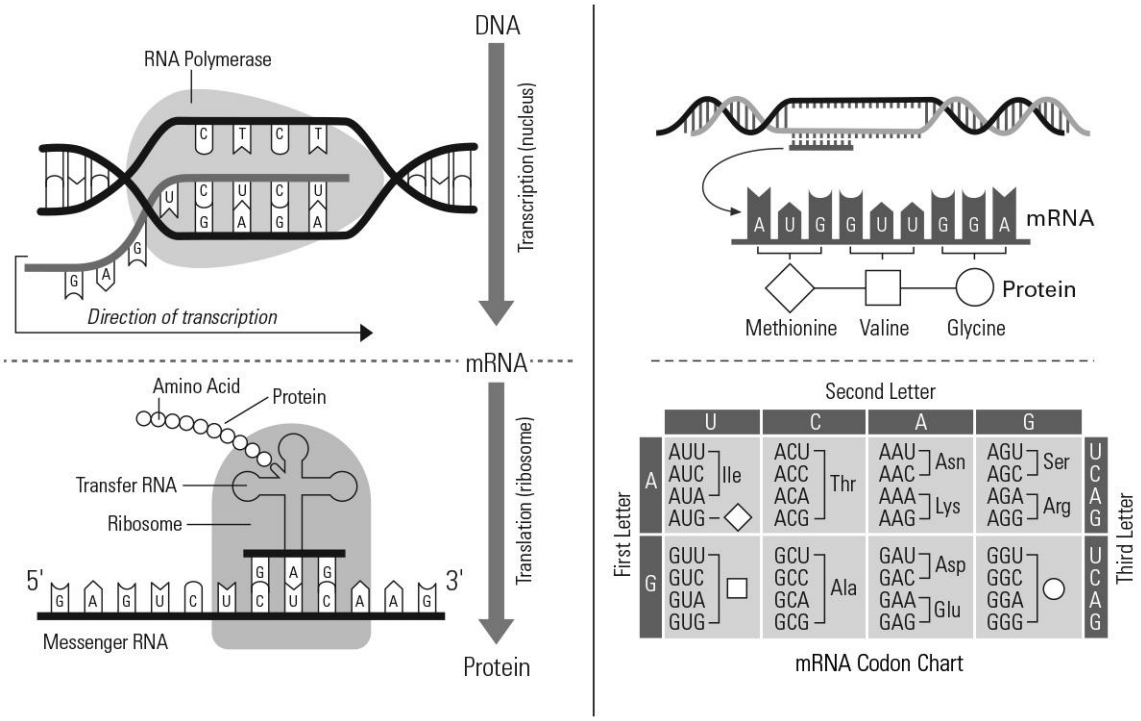
      The environment can contain factors that affect gene expression, and therefore phenotype. If an environmental factor, such as IPTG, determines whether a gene is “on” or “off” then the organism's phenotype will be different under different environmental conditions.

4. The *ampR* gene codes for a digestive enzyme that degrades ampicillin, thereby allowing *E. coli* cells to grow and reproduce in the presence of the antibiotic. Describe in detail the molecular processes involved in gene expression. That is, how does the DNA sequence of a gene result in the synthesis of a particular protein within cells?

   Gene expression begins when, within a chromosome or plasmid, RNA polymerase binds to a promoter sequence. This initiates the separation of a section of the double-stranded DNA. RNA polymerase then moves along the DNA, transcribing the sequence and building messenger RNA (mRNA). One strand of the DNA is used as a template to make this strand of RNA that contains bases complementary to those in the DNA strand. For example, a DNA template sequence of ACTTAG results in an mRNA with the sequence UGAAUC. A termination sequence signals the end of transcription.

   The RNA polymerase and the mRNA strand separate from DNA and the open section of DNA rewinds into the double helix. The small subunit of a ribosome binds to the 5' end of the mRNA molecule and when an initiator tRNA binds to the start codon on the mRNA, translation is initiated.

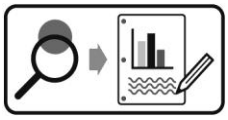
   A large ribosomal subunit attaches and provides active sites for peptide bond formation between amino acids delivered to the ribosome by activated tRNA molecules. Amino acids are bonded into a long polypeptide. The order of amino acids is specified by the codon sequence of the mRNA. For example, if the mRNA sequence is AUGGUU, the amino acid sequence is Met—Val. (The diagrams below summarize these processes. Note, however, that the mRNA Codon Chart is abbreviated and does not show all codons.)



When a stop codon is reached, the polypeptide separates from the ribosome. The polypeptide becomes a functional protein after it folds into the proper 3-dimensional conformation. Some proteins consist of more than one polypeptide.

Design and Conduct an Experiment

The Initial Investigation provides students with the procedural skills needed to be successful conducting experiments of their own design. If time allows, students can apply their skills in growing bacteria to explore other aspects of transformation or antibiotic resistance.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet is provided below. The key describes a sample inquiry question and experimental protocol.

Suggested Inquiry Questions

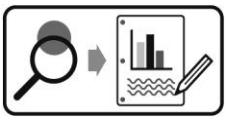
The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Sample data is not provided for the suggested inquiry questions in this lab.

- Are transformed *E. coli* also resistant to penicillin or other antibiotics, in addition to ampicillin?
- Will other salts, such as sodium chloride, make bacteria cells competent?
- Is transformation efficiency similar when a different plasmid is used?
- Is transformation efficiency similar for other types of bacteria?
- Are *E. coli* naturally resistant to any antibiotics, without being transformed?
- What process can be used to isolate the green fluorescent protein from cells?

Design and Conduct an Experiment Key

Explore other aspects of transformation or antibiotic resistance.



Develop and conduct your experiment using the following guide.

1. Create a driving question: develop a testable question for your experiment.

   Are transformed *E. coli* also resistant to penicillin or other antibiotics, in addition to ampicillin?

2. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable will be the type of antibiotic added to the Petri plates. Different antibiotic discs will be placed on the surface of the agar. Each plate will have an ampicillin disc, a penicillin disc, and a kanamycin disc.

3. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable will be bacteria growth—the number and appearance of colonies around the antibiotic discs. Following a 24-hour incubation, the plates will be observed to see if the antibiotic inhibited bacteria growth. If inhibition has occurred, the zone of inhibition will be measured in millimeters.

4. Write a testable hypothesis (If…then…).

   If the *ampR* resistance gene confers resistance to other antibiotics, there will be no zones of inhibition around the antibiotic discs.

5. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   Bacteria will be obtained from the same LB/Amp/IPTG Petri plate (except for the negative control). The same number of colonies (2) will be placed in the liquid agar used to pour each experimental plate. The antibiotic discs will be taken from the same canister and should therefore be of the same age and concentration. The same stock of Luria Broth will be used for the medium of each plate and the plates will be incubated at the same temperature (37 °C).

6. How many trials will be run for each experimental group? Justify your choice.

   Three replicate plates will be prepared, each containing the same three antibiotic discs. Additionally, two control plates will be prepared, one with three paper discs soaked in water. The second will have the same antibiotic discs as the experimental groups, but will be inoculated with bacteria from the LB/Amp (–DNA) plate (non-transformants). Having replicates of the experimental plates will ensure that results are valid and three can be set up in a relatively short period of time.

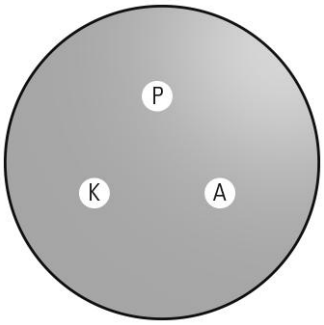
7. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   Bacteria growth will be compared between the plates. If there are zones of inhibition around the discs, these areas will be measured and an average will be calculated if the zone of inhibition occurs on each of the three replicate plates.

8. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

   There is a risk of accidental contamination of the plate with bacteria present in the classroom environment when procedures are performed. Also, the bacteria will be transferred from the LB/Amp/IPTG plate into liquid agar. If the agar is too hot when the transfer is made, the heat will kill the bacteria and there might be little to no growth on the Petri plates as a result. Third, if the antibiotic discs are placed too close together, it may be difficult to determine if inhibition of growth is due to one or both of the antibiotics.

9. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)
   a. Prepare standard Luria Broth agar and allow the agar to cool to 60 °C. Swirl to prevent the agar from solidifying and keep the agar in a hot water bath until it is used.
   b. Place five Petri plates on the table and label them: “Experiment 1”, “Experiment 2”, “Experiment 3”, “Control 1” and “Control 2.”
   c. Transfer transformed bacteria to the Experiment plates and Control 1.
      1. Fill a small, sterile test tube halfway with LB agar.
      2. Use a loop to take 2 colonies from the LB/Amp/IPTG plate and swirl the loop vigorously in the agar to dislodge the cells from the loop and mix them evenly into the agar.
      3. Quickly pour the agar (about 5 mL) into the “Experiment 1” plate and cover.
      4. Leave the plate undisturbed until the agar solidifies.
   d. Repeat the process to prepare the two replicate Experiment plates and Control 1.
   e. Transfer non-transformants to “Control 2”, following the same process as before but using colonies from the LB/Amp (–DNA) Petri plate. Use a new loop for this transfer.
   f. Add the antibiotic discs to the Experiment plates and Control 2:
      1. Use sterile forceps to remove the antibiotic discs from the canisters and place the discs on the surface of the agar. They should be evenly spaced, as shown.
      2. Gently press down on each disc to be sure it adheres to the agar.
   g. Moisten filter paper discs with distilled water and place three discs on the surface of the Control 1 plate.
   h. Place the plates inverted in an incubator. Observe bacteria growth after 24 hours.



10. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

15. UNDERSTANDING INHERITED MITOCHONDRIAL DISORDERS

Lab Overview

Students use pedigree analysis and DNA analysis to confirm or refute the initial diagnosis of MELAS for two patients. MELAS and certain other genetic mitochondrial disorders are unusual in their inheritance: the mutation is passed along maternally only. This maternal inheritance, combined with the complexity in symptoms associated with the disorder, provides a case study that requires students to think critically and evaluate multiple lines of evidence.

The two investigations, "Pedigree Investigation" and "DNA Investigation," both relate to the same two patients. Either investigation can be performed first or students can complete the investigations simultaneously. Based on the length of your class periods, your students' prior knowledge, and your own pedagogical preference, you can determine how best to organize the investigations. In addition to the time required for the investigations, allow time for a discussion of the background information regarding the mitochondrial genome and MELAS. (Photocopy the supplement at the end of this document for students.)

Pacing and Length of the Lab

Pedigree Investigation	
Teacher Preparation Time	10 min
Pedigree Analysis and Construction	30 min

DNA Investigation	
Teacher Preparation Time	45 min
DNA Electrophoresis	50 min
Gel Analysis	30 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.A.1, 2.A.2, 3.A.4, 3.C.1, 4.A.1, 4.A.2, 4.A.4, 4.B.1
Science Practices	1.1–1.4, 3.1–3.3, 5.1–5.3, 6.1–6.5, 7.1, 7.2
Learning Objectives	2.1, 2.5, 3.1, 3.4, 3.6, 3.15, 3.16, 3.17, 4.1, 4.3, 4.5. 4.6, 4.8

Materials and Equipment

For Each Student Station

- Horizontal gel electrophoresis apparatus
- DC power supply
- Automatic micropipet, 5 to 50 µL, with 5 tips
- Tray with 0.8% agarose gel
- QuickStrip™ DNA samples
- InstaStain® Blue card
- Plastic tray for gel staining
- Plastic wrap
- Graduated cylinder, 100-mL
- Waste receptacles (for used tips)
- Disposable gloves
- Distilled water or buffer, 75–100 mL, for staining
- **OPTIONAL** (for preserving a record of the result)
- Camera (USB or other)
- Permanent marker
- Transparency film (for tracing the results)

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

One per Class

- DNA visualization system (white light)[1]
- Spatula (for handling the gel)

[1]A visualization system is not required, but will allow students to optimize the view of the gel.

For Teacher Preparation

- Mitochondrial Genetics Kit[1] (BP-6946)
- Erlenmeyer flasks, 500-mL (2)
- Large beaker or container, 3-L (to dilute buffer)
- Balance
- Microwave or hot plate
- Heat-resistant gloves
- Gel casting trays, 1 per group
- Scissors
- Plastic wrap or aluminum foil
- Distilled water, 3 L

[1]To prepare lab materials using the materials in the kit, refer to the Lab Preparation section.

Prerequisites

Students should be familiar with the following concepts:

- Structure and function of DNA, RNA, and proteins, and the central dogma of molecular genetics
- Structure and function of mitochondria
- Cellular respiration, oxidative phosphorylation, and the importance of electron transport chains within mitochondria
- Typical patterns of inheritance and pedigree analysis

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times
- Make sure that all liquid reagents are safely stored and that areas are dry before plugging in and turning on electrophoresis equipment.
- Wear gloves when working with stain.

Lab Preparation

Use the components of the kit to prepare the materials and equipment for each student group or for the class prior to the lab:

Mitochondrial Genetics Kit

- Concentrated electrophoresis buffer, (50×) 60 mL
- Graduated transfer pipet
- Transfer pipets (10)
- InstaStain Blue Card (6)
- QuickStrip DNA samples (6 strips, 40 µL per well)
- UltraSpec-Agarose™ powder, 3 g

1. Dilute the concentrated electrophoresis buffer included in the kit:

   Add 60 mL of concentrated (5×) buffer to 2,940 mL of distilled water and mix. This makes 3 L of 1× buffer.

2. Prepare 0.8% agarose and cast the gels:

   a. Pour 250 mL of the dilute buffer (1×) into each of the two 500-mL Erlenmeyer flasks. Add 1.5 g of UltraSpec-Agarose from the kit into the buffer in each flask and swirl.

   b. Heat the mixture to dissolve the agarose powder.

      - Microwave method: Cover the flasks with plastic wrap and heat it for 1 minute on high. Swirl the mixture. Continue to heat it on high for 30 second intervals until all of the agarose is completely dissolved. The mixture should appear perfectly clear.

      - Hot plate method: Cover the flasks with aluminum foil and heat the mixture to boiling. Swirl occasionally. Boil until all of the agarose is completely dissolved. The mixture should appear perfectly clear.

      NOTE: Use heat-resistant gloves when preparing melted agarose for gels.

   c. Allow the agarose mixture to cool to 60 °C. Swirl the flask to promote even dissipation of heat.

   d. While the agarose is cooling, prepare the six gel casting trays (if more are needed, refer to Teacher Tip 3): Seal both ends of the casting trays using a rubber dam, lab tape, or masking tape. Place a gel comb in the first set of notches at the end of the tray. Make sure the comb fits firmly in the notches.

   e. When the agarose has cooled to 60 °C, cast the gel: Place the casting trays on a level surface and pour enough agarose mixture into each tray so the surface of the liquid is approximately halfway up the teeth of the gel comb. Do not allow agarose to overflow the tray (over the top of the dam or the tape). Leave the trays undisturbed until the agarose has completely solidified (approximately 20 minutes).

   f. After the gels have solidified, carefully remove the comb and the dam or tape from the ends of the casting tray. Store the gels (in the trays) in the refrigerator if they will not be used immediately.

3. Separate the block of QuickStrip™ samples into individual strips. Use scissors to cut carefully between the rows and do not puncture the protective overlay.

4. Set up an electrophoresis station for each student group with the items listed in the Materials and Equipment list.

5. Pour 300–400 mL of dilute (1×) buffer into each electrophoresis chamber.

Teacher Tips

Tip 1 – Leading a pedigree analysis

The pedigree investigation serves two purposes. First, students should become comfortable recognizing a "pattern of inheritance" (PoI) exhibited by the pedigree (autosomal recessive, for example). Second, and equally important, is the development of the thought process of making claims, using evidence to support those claims, and generating reasons to justify why the evidence matters.

1. Make sure your students get comfortable with this "linear" approach to scientific reasoning. One strategy is to divide students into groups of two or three and have each group make a claim. Then have different groups state and defend their claims and guide the class to a consensus regarding the claim.

   To do this, ask one group for its evidence supporting the claim. Poll other student groups to ensure that all lines of evidence are used. Then ask another group to give reasons why the evidence supports the claim. Work through this process until all lines of evidence supporting the claim are discussed. This pedagogical method ensures that all students understand how pedigrees are "read," and the questioning and consensus-building models real scientific communication.

2. The sample pedigrees (Pedigrees 1–3) are examples of clinical pedigrees and may look different than ones traditionally shown in textbooks. For instance, these pedigrees show unaffected carriers as half-shaded. This approach adds information that allows students to be more confident in their claims, and it makes the analysis more straightforward.

   You will also notice the diamond shapes with a number inside. This symbol represents offspring of unknown gender. This apparent lack of information also simplifies the analysis. If gender is unknown, then gender doesn't matter, and therefore the trait is not X-linked. Finally, you will see males and females with offspring but without a mate. This symbol doesn't defy logic; it merely indicates that the genetic status of the mate doesn't matter.

Tip 2 – Helping students understand MELAS

MELAS, like all mitochondrial genetic diseases, is complex both in genetic and clinical presentation. Given that inherited mitochondrial disorders are a significant yet underappreciated source of morbidity and mortality, it is important for advanced biology students to learn how mutations in mitochondrial DNA can lead to dysfunction at the organelle, cell, tissue, organ, organ system, and organismal level.

1. ***Make copies of the Background Information supplement page (at the end of this document) for students.*** They should read the information carefully and discuss it with their group, but they will still need teacher assistance to fully understand the disorder and how it manifests in patients. Emphasize the following facts about MELAS:

   - Mitochondrial DNA (mtDNA) is maternally inherited. Therefore, mitochondrial disorders exhibit a non-Mendelian pattern of inheritance.

   - MELAS is commonly caused by a point mutation (adenine replaced by guanine) in a gene coding for a tRNA that brings the amino acid leucine to growing polypeptides at ribosomes during translation. This mutation affects the synthesis of proteins of the electron transport chain (ETC): the tRNA that is supposed to bring leucine is missing. If one or more proteins of the ETC are nonfunctional, the production of ATP by oxidative phosphorylation will be severely limited.

- Patients with MELAS have a variable mixture of normal and mutated mtDNA, a condition known as *heteroplasmy*. (Having 100% mutant mtDNA would be a fatal condition.)

- The severity of symptoms can vary among individuals with MELAS, due to differences in the percentage of mitochondria containing mutated mtDNA and in the tissue distribution of the mutated mtDNA. If tissues with high metabolic demand, such as nerve tissue, have a lot of mitochondria with mutated mtDNA, the cells of the tissue are likely to die, resulting in a severe manifestation of the disease. (DiMauro & Schon, 2003)

2. For more information on mitochondrial genetics, please see: Reardon, R.A. and Daniel Sharer. 2012. Teaching Mitochondrial Genetics and Disease: A GENA Project Curriculum Intervention. American Biology Teacher. Volume 74, Number 4.

Tip 3 – Using the kit materials for more than six groups

While the kit is designed for six student groups, the amount of DNA provided in the QuickStrip samples and the amount of agarose provided are more than what is needed. The materials can be "stretched" in the following ways to accommodate a larger class size, or two small classes.

1. The melted agarose mixture can be used to pour more than six gels if the gels are poured thinner. Be sure the comb is still submerged in the mixture to ensure that wells form properly. Also, agarose can be purchased separately from a supply company to provide enough gels for the number of groups in your class or classes.

2. The DNA in the QuickStrip samples can provide the volume needed for two groups. While the lab procedures call for 30 µl per well when loading the gel, half of this volume will still provide good results. Instruct the students to set their micropipet to a smaller volume (15–20 µl) and to share the QuickStrip samples with another lab group.

3. If you prepare more than six gels, you will need to purchase additional InstaStain® Blue cards. These can be purchased in bulk (www.edvotek.com). Also, check for extra DNA stain in your chemical storage area, left over from previous DNA electrophoresis experiments or kits.

NOTE: Edvotek also has InstaStain cards containing a few micrograms of ethidium bromide. If you already own equipment for visualizing DNA using a UV light system, you may wish to purchase these InstaStain Ethidum Bromide cards as an alternative. They provide excellent results.

Tip 4 – Using micropipets and loading gels

Loading the gel correctly requires students to be skilled in the use of a micropipet. The day prior to the DNA investigation, review micropipet use with students.

1. Without "real" samples, review the steps of micropipet use with the students. They should practice putting a tip on the pipet, setting the volume of the pipet, drawing liquid into the pipet, and transferring and expelling liquid into a well. Science supply companies sell model gels for practice or you can make your own practice gels with agar and Petri plates.

2. Model for students the process of loading a DNA sample into the wells of a gel submerged under buffer solution. To keep the micropipet steady, both elbows can be placed on the table and both hands can be used to hold and position the pipet tip halfway into the well. Warn students that the well can be punctured if they put the tip too far down in the well. Explain that the DNA sample should be expelled from the tip by *slowly* pressing on the plunger.

   It is also important for students to remember that they have to keep their thumb on the plunger, with the plunger depressed, as they remove the tip from the well (to prevent drawing the sample back into the tip). Show students how to eject a tip and remind them that a new tip should be used for each sample.

NOTE: If your students have not loaded a gel or run electrophoresis before, you will need to provide additional instruction.

Tip 5 – Voltage and time recommendations

For most electrophoresis models and power supplies, best results are obtained by running the gel at 75–100 volts for 40–50 minutes. Refer to documentation from the manufacturer of your electrophoresis equipment for additional guidelines.

NOTE: Running at a higher voltage for quicker results can result in poor separation of the DNA and blurry results without distinct bands.

Tip 6 – Preserving a record of the gel

Students will sketch a diagram of the gel results following electrophoresis. For additional analysis, students can preserve their results in one of the following ways:

- Trace the results by placing a piece of transparency film over the gel. Use a permanent marker to draw the position of each band on the gel onto the transparency. Be sure to include the outline of the gel and the locations of the sample wells, in addition to the DNA bands.
- Photograph the gel.

  a. Place a ruler next to the gel, with the edge of the ruler (0 cm) placed at the sample wells.

  b. Use a USB camera to capture and save an image in SPARKvue or on the SPARK Science Learning System.

Tip 7 – Formative assessment: Student predictions regarding gel electrophoresis

Before students run the control and patient DNA samples, make sure they write and sketch their predictions about the patterns they expect to see for each of the four samples. The student version of this lab purposely withholds the expected size of each band. Students, however, are expected to think about the outcomes of the restriction digest and the resulting banding pattern in the agarose gels.

They should write down the predicted banding pattern (for example, one band, two bands) for all four DNA samples in Table 1. They should also draw the resulting DNA fragment pattern they expect to see in the gel. It is not important for students to determine the exact size of each band during the prediction phase, but they should be able to predict that the two bands resulting from the digestion of normal/wild-type control DNA are both smaller than the mutant DNA fragment that is not cut during the restriction digest (the point mutation erases the restriction site for Apa1).

Tip 8 – Helping students reconcile the two investigations, pedigree analysis and gel results

Regardless of how you sequence the activities in this investigation, reconciling the information from the electrophoresis and the student-generated pedigrees is essential.

After evaluating evidence from the electrophoresis results and pedigree construction, students should be able to make the following connections at the conclusion of the lab:

- Patient 1 does not have the point mutation that indicates MELAS at this locus. This lane of the DNA gel shows two DNA bands. One is 3000 bp and the other is roughly 1,300 bp long (the same sizes as the normal control DNA sample). Patient 1's pedigree does not indicate a maternal pattern of inheritance. In fact, her pedigree indicates no pattern of inheritance, suggesting the mutation causing her disease is spontaneous.
- Patient 2 is heteroplasmic for MELAS at this locus. The lane of his DNA gel shows three DNA bands. One band is roughly 4,300 bp long (the same as the mutant control), and the other two bands are 3,000 bp and 1,300 bp (these later two bands are the same size as the normal control DNA, and together they add up to the size of the original amplified DNA). Further, his pedigree indicates a maternal pattern of inheritance for several different symptoms of mitochondrial disease.

The investigations can be sequenced in either of the following ways:

SEQUENCE A

a. Have students read the patient case histories and family histories.

b. Have students build pedigrees for each patient based on the family history, shading any phenotype that sounds like mitochondrial disease.

c. Have students carry out electrophoresis on the patients' DNA.

d. Determine if either patient has MELAS caused by an adenine-to-guanine point mutation.

SEQUENCE B

a. Have students read the patient case histories and family histories.

b. Have students carry out electrophoresis on the patients' DNA.

c. Determine if either patient has MELAS caused by an adenine-to-guanine point mutation.

d. Have students build pedigrees for each patient based on the family history, shading any phenotype that sounds like mitochondrial disease.

Pedigree Investigation

NOTE: Because of the complexity of these investigations, no student-designed experiment is included.

This investigation challenges students to analyze example pedigrees for mode of inheritance and to support their claims with evidence. After this practice, students should be able to construct pedigrees for the two patients based on each patient's case history and determine if the patients' pedigrees display the same mode of inheritance as one of the example pedigrees.

The following table, also in the Background section of the student handout, summarizes each patient's case history.

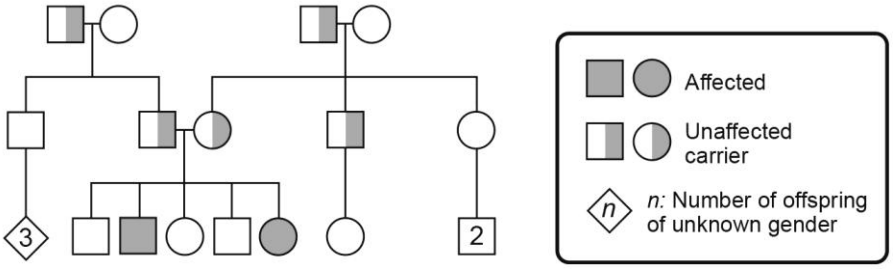
Patient 1	Patient 2
Description	**Description**
Seven year old female with a history of normal development until age two. At that point she developed episodic vomiting, acidosis, epilepsy, general weakness, ataxia (stiff, unsteady gait), and dystonia (involuntary muscle contractions).	Fifty-two year old male with sudden onset headaches and seizures. Patient has a history of diabetes and deafness. MRI detected bi-temporal lesions.
Family History	**Family History**
A patient history was taken going back to the patient's grandparents on her mother's side. No similar symptoms have occurred in the patient's siblings (patient has twin older brothers.) No symptoms appear in the patient's parents nor in the mother's parents (maternal grandparents). The patient's grandfather (mother's father) has adult-onset diabetes (Type II diabetes).	A patient history was taken going back to the patient's grandparents on his mother's side. The patient's brother was found to have asymptomatic mild lactic acidosis. The patient's mother had diabetes, exercise intolerance, and ptosis (drooping eyelids). The patient's maternal uncle died of a stroke at age 37 and had multiple health issues (poorly defined). The patient's maternal grandmother had diabetes and possibly other symptoms and the patient's father has rheumatoid arthritis, but no history of diabetes or neurological problems.

From the student handout:

Pedigree analysis is a powerful tool used by geneticists, physicians, and genetic counselors to understand the *pattern of inheritance* (PoI) of certain traits. Before constructing pedigrees for the two patients, review the following three pedigrees.

1. Work with a partner and analyze Pedigree 1. Look for patterns emerging from the pedigree (for example, how the trait passed from one generation to the next).

Pedigree 1



2. Create a chart in your lab notebook, like the one below, that includes space for the PoI claim and for the evidence you are asked to provide. Sketch a portion, or all, of Pedigree 1 into your notebook as well.

Analysis of Pedigree 1

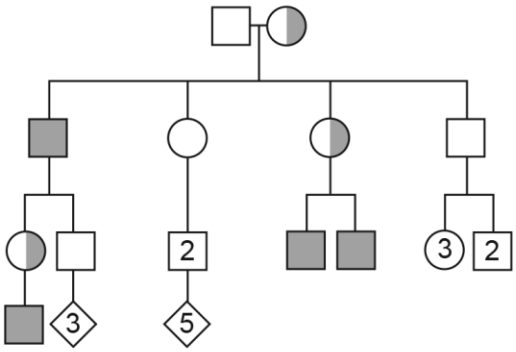
PoI Claim	Evidence
Autosomal Recessive	Carriers are unaffected; offspring are only affected when two carriers mate. Males and females are affected in equal proportions.

a. Make a claim stating the PoI you observe in Pedigree 1.

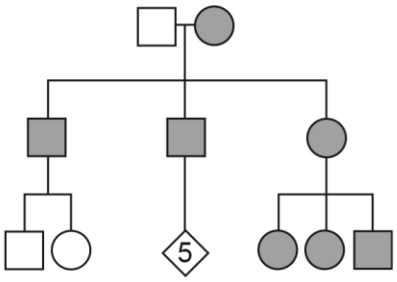
b. Support your claim with evidence. Describe specific aspects of Pedigree 1 that indicate the PoI you identified.

3. Create charts for Pedigrees 2 and 3. For each of these pedigrees, make a claim regarding the PoI and support each claim with specific evidence from the pedigree.

Pedigree 2



Pedigree 3



Analysis of Pedigree 2

PoI Claim	Evidence
X-linked Recessive	Only females are carriers, only males have the trait. The affected males have carrier mothers.

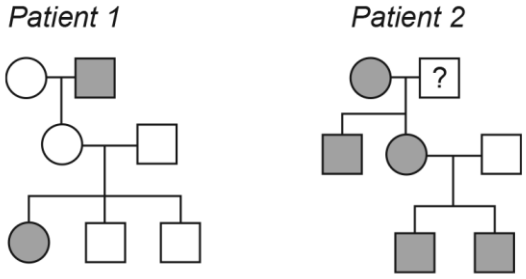
Analysis of Pedigree 3

PoI Claim	Evidence
Maternal	All affected mothers have 100% affected offspring. Males do not pass on the trait.

4. Discuss with your teacher and classmates the unusual pattern of inheritance seen in Pedigree 3. What types of genetic diseases would exhibit the PoI seen in Pedigree 3? Why would that happen?

This type of inheritance occurs only with DNA inherited from the mother. This is the case with mitochondrial DNA. All the mitochondria for a developing zygote and embryo are contained in the secondary oocyte. Therefore, all the mitochondria within an individual come from the mother. If the DNA in the mitochondria is mutated, then that DNA will be passed on to all of the mother's offspring. Affected males do not pass on the trait to offspring.

5. Construct a pedigree for Patient 1 based on the patient's family history (in the table in the Background section). Include all three generations. Shade any circles or squares that represent an individual who may have a mitochondrial disorder like MELAS.



Patient 1: The patient's maternal grandfather and the patient are the only members of the family to display symptoms that may indicate a mitochondrial disorder.

6. Construct a pedigree for Patient 2 based on the patient's family history (in the table in the Background section).

See diagram above.

Patient 2: The patient and his brother have symptoms that may indicate a mitochondrial disorder. Also, the patient's mother, uncle, and maternal grandmother suffer from conditions related to MELAS. It is unclear whether rheumatoid arthritis in the patient's maternal grandfather is related to this disorder.

Data Analysis

1. Which patient(s), if any, has a family pedigree consistent with the pattern of inheritance expected for the mitochondrial disorder MELAS? Use evidence from each pedigree to support your answer.

Analysis of patient histories

Claim	Evidence
Patient 2 has a pedigree consistent with MELAS (maternal PoI).	Patient 2's family history and pedigree indicates that both affected mothers (patient's mother and patient's maternal grandmother) had affected offspring. Male and female offspring were both affected. One hundred percent of the offspring were affected. The pedigree shows maternal inheritance, consistent with an mtDNA mutation. For Patient 1, the only affected relative was a maternal grandfather. "Skipping a generation" is not consistent with mtDNA mutations and maternal inheritance.

2. If a patient's family pedigree does not indicate a maternal pattern of inheritance, can MELAS be ruled out as a diagnosis? Explain your answer.

A diagnosis of MELAS should not be ruled out based on pedigree analysis alone. The patient may have a spontaneous mutation rather than an inherited mutation.

DNA Investigation

This investigation challenges students to use information about the Leu 1 (UUR) gene and restriction enzymes to predict the outcome of electrophoresis of a patient with the 3243A>G mutation, the mutation that is the most common cause of MELAS. Students run electrophoresis with provided DNA (from the patients, as well as control samples) and analyze the results for evidence of MELAS.

The DNA samples from Patient 1 and Patient 2 were produced through PCR amplification, making many copies of the 4,300 bp region of the mitochondrial genome. (Refer to the diagram in the Background Information Supplement.) The products were combined with restriction enzyme ApaL1, which recognizes the sequence GTGC***A***C within the normal gene but not the sequence GTGC***G***C present in the mutant form of the gene. If the mtDNA is normal, the 4300 bp PCR product is cut into fragments with lengths of 3000 bp and 1300 bp. If the mtDNA contains the 3243A>G mutation, the ApaL1 restriction site is absent and the enzyme does not cut the PCR product.

From the student handout:

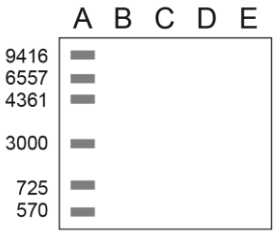
DNA samples from Patient 1 and Patient 2 were amplified via PCR to produce many copies of a 4,300 bp region of the mitochondrial genome. This PCR product contains the gene of interest, Leu 1 (UUR) as well as several neighboring genes. Because the Leu 1 gene is so small (44 bp) it is necessary to include more than just the gene of interest during DNA amplification. PCR was followed by digestion with restriction enzyme ApaL1, which recognizes the sequence GTGCAC within the normal gene, but not the sequence GTGCGC present in the mutant form of the gene. These DNA samples, as well as control samples, have been shipped to you for analysis.

Predict

1. Table 1 identifies the QuickStrip DNA samples. Copy this table and the gel diagram next to it into your lab notebook. Predict whether the restriction enzyme did or did not cut the DNA, and predict the number of bands you will see on the electrophoresis gel. Sketch your prediction of the results of the electrophoresis for samples B–E.

Table 1: Prediction of electrophoresis results

Sample	Contents	Restriction Enzyme Results	Number of Bands Expected
A	DNA Marker		
B	Normal DNA	Cut	Two bands
C	Mutated DNA	Not cut	One band
D	Patient 1	Answers will vary	Answers will vary
E	Patient 2	Answers will vary	Answers will vary



Load the Gel

2. Obtain a tray with the 0.8% agarose gel and take it to an electrophoresis chamber. Identify the *positive* (red cord) and *negative* (black cord) sides of the chamber. Place the gel, with the tray, in the chamber, positioning the wells on the negative side of the chamber (the side with the black cord).

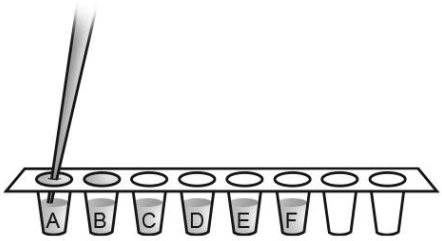
   NOTE: During electrophoresis, the DNA samples migrate through the gel towards the positive electrode. It is critical that you have the wells closest to the negative side of the chamber!

3. The gel should rest level and centered on the platform of the electrophoresis chamber and be submerged under the surface of dilute electrophoresis buffer. Add buffer to the chamber if the gel is not submerged.

4. Obtain one strip of QuickStrip DNA samples from your teacher. Tap the tubes gently on the table to ensure that the sample is at the bottom of the tubes.

5. Obtain an automatic micropipet and sterile tip. Place the sterile tip on the end of the micropipet. Set the pipet to 30 µL.

6. Pierce the protective overlay of the QuickStrip DNA samples container with the pipet tip, and draw 30 µL of sample A into the tip. Make sure there are no bubbles in the tip of the pipet after you have extracted your sample.



7. Carefully place the tip of the pipet halfway into the first well of the gel. *Slowly* press the plunger of the micropipet to expel the sample into the well.

   NOTE: You should see the DNA and loading dye drop into the bottom of the well. Do not push through the "soft stop" on the pipet. Leave your thumb at the soft stop, remove the pipet tip from the well, and eject the tip.

8. Using a clean pipet tip each time, load samples B–E into the wells in consecutive order.

Run the Gel

9. Place the lid securely on the electrophoresis chamber and connect the apparatus to the DC power supply.

10. Set the power source to the required voltage. Ask your teacher what voltage is recommended for your equipment.

11. Turn on the power supply. Check that the current is flowing properly: you should see bubbles forming on the two platinum electrodes.

12. Conduct the electrophoresis for the length of time instructed by your teacher. When instructed, turn off the power supply to stop the electrophoresis process.

Stain the Gel

13. Slowly pour 75 mL of water or electrophoresis buffer into the plastic tray for gel staining.

14. Put on disposable gloves. Carefully remove the gel from the electrophoresis chamber and place it in the plastic tray. (Slide the gel off the tray it was cast in.) The gel should be completely submerged in the liquid (add more liquid if necessary).

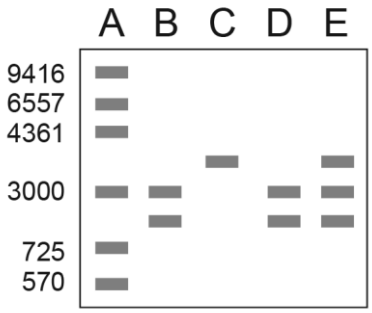
15. Place the blue dye side of the InstaStain card face down on the surface of the liquid, directly over the gel.

16. After 60 seconds, remove the card from the staining tray.

17. Cover the tray with plastic wrap and leave it undisturbed for at least 3 hours. (You can leave the gel in the tray overnight.)

18. Wearing the disposable gloves, remove the gel from the staining tray. If a light box visualization system is available, place the gel on the light box for an optimum view of the DNA bands.

19. Sketch a diagram in your lab notebook like the one shown below. Draw the banding patterns observed in your gel for each lane (sample).



20. Use the *molecular ruler* (the DNA Marker bands on the diagram), and the background information, to determine the sizes of the DNA bands in lanes 2 through 5 (samples B–E). Create a table in your lab notebook, or expand Table 1, to summarize the predictions and results of the DNA electrophoresis.

Electrophoresis results

Lane	Sample	Contents	Predicted Pattern[1]	Actual Pattern	Fragment Size(s) (bp)
1	A	DNA Marker	-----	-----	9416 bp, 6557 bp, 4361 bp, 3000 bp, 725 bp, 570 bp
2	B	Normal DNA	Two bands	Two bands	3000 bp, 1300 bp
3	C	Mutated DNA	One band	One band	4300 bp
4	D	Patient 1	Answers vary	Two bands	3000 bp, 1300 bp
5	E	Patient 2	Answers vary	Three bands	4300 bp, 3000 bp, 1300 bp

Data Analysis

1. Based on the evidence from the gel, which patient, if any, has the A>G point mutation in the Leu (UUR) gene—the mutation that is the most common cause of MELAS? Cite specific evidence from the gel to support your claim.

   It appears that Patient 2 has a point mutation in the Leu (UUR) gene. He has a band at the same place as the mutated control DNA. This band is 4300 bp and shows that some of the PCR products were not cut by the enzyme, which indicates an altered restriction site sequence.

2. Based on the evidence from the gel, does either patient appear to have a normal (non-mutated) Leu (UUR) gene? Support your claim with evidence.

   Both patients have a normal Leu (UUR) gene. The DNA of both patients resulted in bands of 1300 bp and 3000 bp, or the cut pattern, which is the same as the normal control DNA. The restriction enzyme cut within the 4300 bp PCR product, indicating that the restriction sequence was normal.

3. If the result of the mtDNA genetic test shows heteroplasmy, is the individual a carrier for the mitochondrial genetic disorder? Explain your answer.

   A person with heteroplasmy for a mitochondrial disorder is not a carrier of the disorder. In genetics, a carrier is a person who is not afflicted with the disorder but carries a recessive allele that may result in having an offspring with the disorder. In heteroplasmy, someone with both normal and mutated mtDNA is likely to suffer symptoms of the disorder due to having some abnormal mitochondria. For many, the symptoms are severe. This is much different than a person who is a carrier in a traditional, autosomal disorder.

4. If the genetic test is negative for the A>G mutation within the Leu (UUR) gene, can a diagnosis of MELAS (or other mitochondrial disorders) be ruled out? Explain your answer.

   MELAS can result from other mutations, not just the adenine–guanine substitution mutation at the 3243 bp locus. Even if the genetic test is negative for this mutation, MELAS cannot be ruled out; it may be due to a different mutation, or it may be a mitochondrial disorder that is not MELAS.

Synthesis Questions

1. Does the evidence from the case history, family pedigree, and DNA analysis for Patient 1 support or refute the original diagnosis of MELAS? Make a claim and then justify the claim with multiple lines of evidence.

   The evidence appears to refute the diagnosis of MELAS for Patient 1. Patient 1's pedigree did not show the maternal inheritance associated with a mitochondrial disorder. The only relative with a possible disorder was the patient's grandfather, but mitochondrial disorders are not passed on by males, only females. Also, the genetic test ruled out the 3243A>G mutation. Patient 1's DNA result was the same as the normal DNA sample.

2. Does the evidence from the case history, family pedigree, and DNA analysis for Patient 2 support or refute the original diagnosis of MELAS? Make a claim and then justify the claim with multiple lines of evidence.

   The evidence supports a diagnosis of MELAS for Patient 2. Patient 2's family history includes a number of relatives with symptoms associated with mitochondrial disorders: a maternal uncle died of stroke at a young age, the mother has diabetes, exercise intolerance, and ptosis. The pedigree shows a maternal inheritance pattern, consistent with MELAS.

   The genetic test confirms the presence of the 3243A>G mutation within the Leu (UUR) gene. Patient 2 had uncut DNA (a 4300 bp fragment), which shows that the substitution mutation is present (preventing the restriction enzyme from cutting the DNA).

3. If the diagnosis of MELAS cannot be justified for one or both of the patients, what is an alternate explanation for the symptoms present in the patient?

   Patient 1 could have MELAS caused by another mutation within the mitochondrial genome, or the patient could have a spontaneous mutation of another mitochondrial gene that causes symptoms similar to MELAS.

4. a. What does the tRNA Leu (UUR) gene do?

      The Leu (UUR) gene provides instructions to build a tRNA molecule that brings the amino acid leucine to the growing polypeptide chain during the synthesis of any of the thirteen ETC proteins coded for by mtDNA.

   b. How would a mutation in the Leu (UUR) gene affect the synthesis of protein complexes in the electron transport chain (ETC)?

      A mutation in the Leu (UUR) gene affects the synthesis of ETC proteins by halting or slowing the protein synthesis process at the ribosomes within the mitochondria. Ribosomes translate an mRNA molecule by bonding amino acids, delivered by tRNA molecules, in the order specified by the mRNA sequence. Each tRNA is specific to an amino acid, so if a tRNA built from a faulty Leu (UUR) gene cannot deliver leucine, no other tRNA can fulfill this role. Any protein of the ETC complexes that contains leucine would be missing this amino acid and its function would be significantly impaired.

   c. Why would one or more missing or nonfunctional proteins of the ETC result in insufficient ATP production?

      Missing or nonfunctional proteins of the ETC decrease the affected mitochondria's ability to carry out oxidative phosphorylation. The purpose of the ETC—the passing of electrons to oxygen molecules and ATP synthesis by chemiosmosis—depends on the efficient transfer of electrons along the proteins of the ETC complex. Without all of the proteins present and functional within the ETC, there will be an insufficient buildup of the hydrogen ion gradient that drives ATP synthesis.

5. MELAS is an acronym for "mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes." Copy the table below, provide the definition of each term, and identify the organ system(s) affected in each condition. Pick out two other symptoms exhibited by either patient (or the patient's family) and describe them also.

Table 2: MELAS symptoms

Condition	Definition	Affected Organ System
Myopathy	Fatigue and atrophy in skeletal muscle	Skeletal Muscle
Encephalopathy	The cerebral pathology of neurons and glial cells	Central Nervous System
Lactic acidosis	A surplus of lactic acid building up in cells and tissues caused by a decrease in oxidative phosphorylation	Widespread throughout organ systems
Stroke-like episodes	Memory lapses, temporary blindness, deafness, and paralysis	Central Nervous System
Other symptom 1	A stiff and unsteady gait caused by the uncoordinated contraction of antagonistic skeletal muscles	Skeletal Muscles
Other symptom 2	Drooping eyelids	Skeletal Muscles

6. Explain why cells and tissues that have high metabolic demands would be sensitive to mutations in protein complexes of the ETC.

   Cells with high metabolic demands need large amounts of ATP, which are generated from oxidative phosphorylation—a process carried out by proteins of the ETC and of the enzyme ATP synthase. If any of these proteins are missing (or are synthesized at a sub-optimal rate) because of a mutation within the mtDNA, ATP synthesis would be decreased; therefore the cells would not function, grow, or divide as well as normal cells.

7. What is heteroplasmy? Why might symptoms be more severe for one MELAS patient compared to another?

   Most mitochondria contain normal genomes, but some mitochondria may have mutated genomes. The condition of having more than one mitochondrial genome (both normal and mutated) within cells is known as *heteroplasmy*.

   Any given cell contains many mitochondria, and within each mitochondrion are numerous copies of the mtDNA genome. In the case of individuals with heteroplasmy, the symptoms presented may vary. One mitochondrion may be more "abnormal" than another, even if they both contain mutated mtDNA. Also, even if someone has the mutation associated with MELAS, some cells in this person may have many abnormal mitochondria while other cells contain mainly normal mitochondria.

   If a person inherits a large number of abnormal mitochondria that contain many copies of mutated mtDNA, that person will likely suffer from a severe form of MELAS, especially if they have many abnormal mitochondria in nerve or muscle cells. Another person with the same mutation in their mtDNA may have fewer symptoms if they have mostly normal mitochondria and fewer cells with mutated mtDNA, or if the abnormal mitochondria exist only in cells with lower metabolic demands.

Background Information Supplement – Understanding Inherited Mitochondrial Disorders

Most genetic disorders are caused by mutations in nuclear DNA (nDNA). Each disorder has a *pattern of inheritance* (PoI); this pattern describes how the mutation is passed down through a number of generations. Common patterns of inheritance are *autosomal recessive* and *X-linked* inheritance. A less common pattern of inheritance is known as *maternal inheritance*. Family pedigrees (which convey the pattern of inheritance) help scientists identify and understand how a disorder is inherited.

During human reproduction, each parent provides an equal contribution of nDNA; 23 chromosomes from the egg and 23 chromosomes from the sperm provide the zygote with its diploid genome. The genes (and mutations) located on these chromosomes follow common patterns of inheritance. However, nDNA is not the only DNA within a cell. During reproduction, the mother provides additional DNA to offspring in the form of mitochondrial DNA (mtDNA). The cytoplasm of the zygote is derived from the egg; therefore all of the mitochondria present in the offspring are maternal, as is the mtDNA contained within those organelles. If a mutation is present in mtDNA, a disorder caused by the mutation will display a maternal pattern of inheritance.

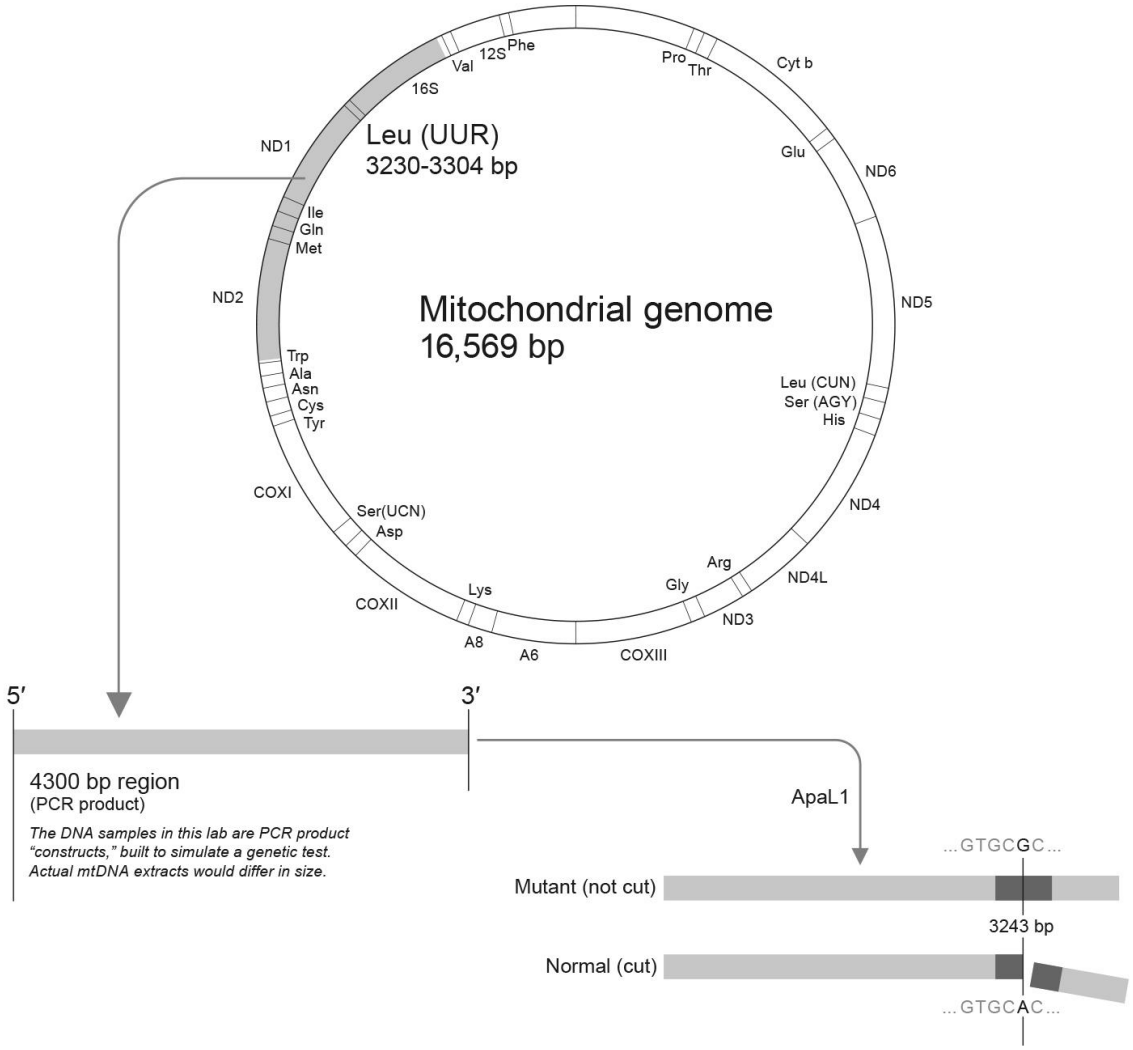
Mitochondrial DNA is evidence of the organelle's origin as a free-living, heterotrophic prokaryote. The genome is small, consisting of just 37 genes within a 16,569 bp loop of DNA. (Refer to the diagram of the mtDNA genome.) Thirteen of the genes code for proteins that form the complexes of the electron transport chain (ETC). (Additional ETC proteins are coded for by genes within nDNA.) The remaining mitochondrial genes code for rRNA and tRNA molecules that participate in the process of translation to build the proteins of the ETC. Mutations in these genes disable the protein synthesis process and cause the proteins of the ETC to be nonfunctional or not synthesized.

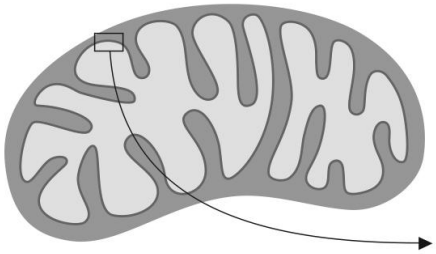
The electron transport chains within mitochondria are the critical structures that enable mitochondria to provide usable energy (ATP) to a cell. Mutations in the mtDNA genome can severely affect the functioning of the ETC, resulting in metabolic disorders due to insufficient production of ATP. Over 100 point mutations in the mtDNA genome have been identified and linked to genetic disorders. One of the most common is *MELAS* (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes). A point mutation in the Leu (UUR) gene is the cause of most MELAS cases. At the 3243 bp locus, adenine is substituted with guanine (symbolized by: 3243A>G). The Leu (UUR) gene is not a protein-coding gene, rather it codes for the tRNA molecule that brings leucine to a ribosome during protein synthesis.

Most cells are packed with hundreds, maybe thousands, of mitochondria. Often, most of these mitochondria have normal DNA; only some have the mutation. The condition of having both normal and abnormal mtDNA is known as *heteroplasmy* and results in differences in the severity of symptoms in affected individuals. Individuals with a small number of mitochondria containing mutated genomes may be asymptomatic or have low-level symptoms that are never officially linked to a diagnosis of MELAS. Individuals with a greater number of abnormal mitochondria are more likely to suffer symptoms that are debilitating or life-threatening, since the cells with “diseased” mitochondria are likely to function improperly or die.

Tissues that have high metabolic demands, needing a large quantity of ATP, have a low threshold for mitochondrial dysfunction and are more likely to show symptoms of mitochondrial disease. These tissues include neural, muscle, and renal tissues. Symptoms commonly associated with MELAS include: migraine-type headaches, brain lesions due to stroke-like events, vomiting, seizures, exercise intolerance, muscle weakness, hearing loss, diabetes, and short stature.

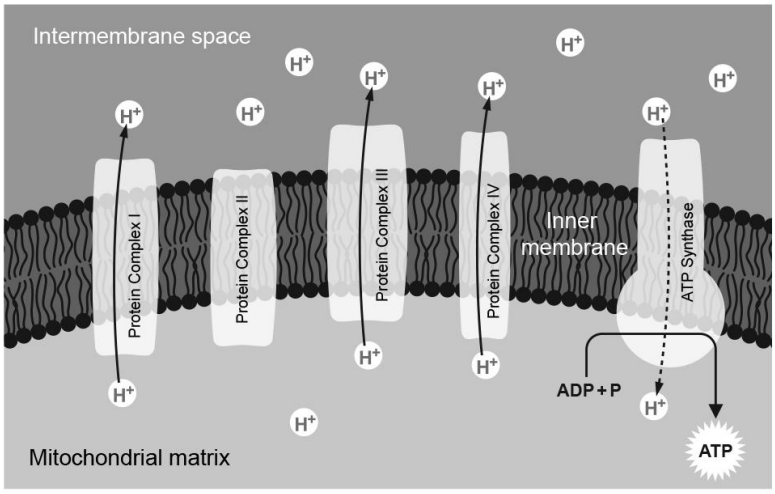
MELAS and other mitochondrial disorders can be difficult to diagnose. Typically, a doctor will evaluate the patient's family history and use the results of many tests, including a clinical exam, muscle biopsy, blood tests, and genetic tests to make a final diagnosis.





Mutations in coding regions of the mitochondrial genome can severely affect the functioning of the electron transport chain. Protein complexes are paramount to electron transfer and adequate ATP production. A mutation in the genetic "recipe" for a tRNA, such as Leu (UUR), is especially detrimental. Such a mutation affects the overall protein synthesis mechanism, and therefore affects the assembly of many proteins within these intermembrane complexes.

Electron transport chain



16. SICKLE CELL GENE DETECTION

Lab Overview

Many genetic disorders can be detected and diagnosed by a method employing polymerase chain reaction (PCR), restriction enzymes, and electrophoresis. Genetic tests often rely on digesting DNA with a restriction enzyme, which can cut within a normal gene sequence but not within the mutated sequence, resulting in different lengths of DNA. Electrophoresis separates DNA fragments based on the sizes of the fragments.

In this lab, students use electrophoresis to analyze DNA samples (previously treated with a restriction enzyme) from a child and the child's parents to determine if the child has inherited a mutation in the gene for hemoglobin B (*HBB*). The mutation causes sickle cell anemia, a common form of sickle cell disease, in homozygous individuals.

Pacing and Length of the Lab

Investigation	
Teacher Preparation Time	90 min
Investigation	60–80 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	3.A.1, 3.A.3, 4.A.1
Science Practices	1.4, 4.1, 4.3, 5.1, 6.1, 6.2, 7.2
Learning Objectives	3.1, 3.4, 3.6, 4.1, 4.2, 4.3

Materials and Equipment

For Each Student Station

- Horizontal gel electrophoresis apparatus
- DC power supply
- Automatic micropipet, 5 to 50 µL, with tips
- Tray with 0.8% agarose gel
- QuickStrip™ DNA samples
- InstaStain® Blue card
- Plastic tray for gel staining
- Plastic wrap
- Graduated cylinder, 100-mL
- Waste receptacles (for used tips)
- Disposable gloves
- Distilled water or buffer, 75–100 mL, for staining
- **OPTIONAL** (for preserving a record of the result)
- Camera (USB or other)
- Permanent marker
- Transparency film (for tracing the results)

One per Class

- DNA visualization system (white light)[1]
- Spatula (for handling the gel)

[1]A visualization system is not required, but will allow students to optimize the view of the gel.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

For Teacher Preparation

- Genetically Inherited Disease Detection Kit[1] (BP-6947)
- Erlenmeyer flasks, 500-mL (2)
- Large beaker or container, 3-L (to dilute buffer)
- Balance
- Microwave or hot plate
- Heat-resistant gloves
- Gel casting trays, 1 per group
- Scissors
- Plastic wrap or aluminum foil
- Distilled water, 3 L

[1]To prepare lab materials using the materials in the kit, refer to the Lab Preparation section.

Prerequisites

Students should be familiar with the following concepts:

- Hemoglobin is a protein found in red blood cells; it is composed of four polypeptide chains and binds oxygen molecules.
- Protein structure at the tertiary and quaternary levels involves interactions between the side chains of amino acids.
- Mutations can change the structure and function of a protein. Changes at the DNA level can lead to changes in one or more amino acids, which may affect the 3-dimensional conformation of a protein.
- Mutations in DNA can be detected by digestion with a restriction enzyme if the mutation occurs within the enzyme's restriction site. Gel electrophoresis can be used to detect cut (normal) and uncut (mutated) DNA samples.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times
- Make sure that all liquid reagents are safely stored and that areas are dry before plugging in and turning on electrophoresis equipment.
- Wear gloves when working with stain.

Lab Preparation

Use the components of the kit to prepare the materials and equipment for each student group or for the class prior to the lab:

Sickle Cell Gene Detection Kit

- Concentrated electrophoresis buffer, (50×) 60 mL
- Graduated transfer pipet
- Transfer pipets (10)
- UltraSpec-Agarose™ powder, 3 g
- InstaStain® Blue card (6)
- QuickStrip™ DNA samples (6 strips, 40 µL per well)

1. Dilute the concentrated electrophoresis buffer included in the kit.

   Add 60 mL of concentrated (50×) buffer to 2,940 mL of distilled water and mix. This makes 3 L of 1× buffer.

2. Prepare 0.8% agarose and cast the gels.

   a. Pour 250 mL of dilute buffer (1×) into each of the two 500-mL Erlenmeyer flasks. Add 1.5 g of UltraSpec-Agarose™ from the kit into the buffer in each flask and swirl.

   b. Heat the mixture to dissolve the agarose powder.

      - Microwave method: Cover the flasks with plastic wrap and heat on high for 1 minute. Swirl the mixture. Continue to heat on high for 30 second intervals until the agarose is completely dissolved. The mixture should appear perfectly clear.

      - Hot plate method: Cover the flasks with aluminum foil and heat the mixture to boiling. Swirl occasionally. Boil until the agarose is completely dissolved. The mixture should appear perfectly clear.

   NOTE: Use heat-resistant gloves when preparing the melted agarose for gels.

   c. Allow the agarose mixture to cool to 60 °C. Swirl the flask to promote even dissipation of heat.

   d. While the agarose is cooling, prepare the six gel casting trays (if more are needed, refer to Teacher Tip 1): Seal both ends of the casting trays using a rubber dam, lab tape, or masking tape. Place a gel comb in the first set of notches at the end of the tray. Make sure the comb fits firmly in the notches.

   e. When the agarose has cooled to 60 °C, cast the gel: Place the casting trays on a level surface and pour enough agarose mixture into each tray so the surface of the liquid is approximately halfway up the teeth of the gel comb. Do not allow agarose to overflow the tray (over the top of the dam or the tape). Leave the trays undisturbed until the agarose has completely solidified (approximately 20 minutes).

   f. After the gels have solidified, carefully remove the comb and the dam or tape from the ends of each casting tray. Store the gels (in the trays) in the refrigerator if they will not be used immediately.

3. Separate the block of QuickStrip™ samples into individual strips. Use scissors to cut carefully between the rows, and do not puncture the protective overlay.

4. Set up an electrophoresis station for each student group with the items listed in the Materials and Equipment list.

5. Pour 300–400 mL of dilute (1×) buffer into each electrophoresis chamber.

Teacher Tips

Tip 1 – Using the kit materials for more than six groups

While the kit is designed for six student groups, the amount of DNA provided in the QuickStrip samples and the amount of agarose provided are more than what is needed. The materials can be "stretched" to accommodate a larger class size, or two small classes.

1. The melted agarose mixture can be used to pour more than 6 gels if the gels are poured thinner. Be sure the comb is still submerged in the mixture to ensure that wells form properly. Also, agarose can be purchased separately from a supply company to provide enough gels for the number of groups in your class or classes.

2. The DNA in the QuickStrip DNA samples can provide the volume needed for two groups. While the lab procedures call for 30 µl per well when loading the gel, half of this volume will still provide good results. Instruct the students to set their micropipet to a smaller volume (15–20 µl) and to share the QuickStrip samples with another lab group.

3. If you prepare more than six gels, you will need to purchase additional InstaStain® Blue cards. These can be purchased in bulk (www.edvotek.com). Also, check for extra DNA stain in your chemical storage area, left over from previous DNA electrophoresis experiments or kits.

   NOTE: Edvotek also has InstaStain cards containing a few micrograms of ethidium bromide. If you already own equipment for visualizing DNA using a UV light system, you may wish to purchase these InstaStain Ethidum Bromide cards as an alternative. They provide excellent results.

Tip 2 – Using micropipets and loading gels

Loading the gel correctly requires students to be skilled in the use of a micropipet. The day prior to the DNA investigation, review micropipet use with students.

1. Without using the DNA samples (water with food coloring works well), review the steps of micropipet use with the students. They should practice putting a tip on the pipet, setting the volume of the pipet, drawing liquid into the pipet, and transferring and expelling liquid into a well. Science supply companies sell model gels for practice or you can make your own practice gels with agar and Petri plates.

2. Model for students the process of loading a DNA sample into the wells of a gel that is submerged under buffer solution. To keep the micropipet steady, both elbows can be placed on the table and both hands can be used to hold and position the pipet tip halfway into the well. Warn students that the well can be punctured if they put the tip too far down in the well. Explain that the DNA sample should be expelled from the tip by *slowly* pressing the plunger.

   It is also important for students to remember that they have to keep their thumb on the plunger, with the plunger depressed, as they remove the tip from the well to prevent drawing the sample back into the tip. Show students how to eject a tip and remind them that a new tip should be used for each sample.

NOTE: If your students have not loaded a gel or run electrophoresis before, you will need to provide additional instruction.

Tip 3 – Voltage and time recommendations

For most electrophoresis models and power supplies, best results are obtained by running the gel at 75–100 volts for 40–50 minutes. Refer to documentation from the manufacturer of your electrophoresis equipment for additional guidelines.

NOTE: Running at a higher voltage for quicker results can result in poor separation of DNA and blurry results without distinct bands.

Tip 4 – Preserving a record of the gel

Students will sketch a diagram of the gel results following electrophoresis. For additional analysis, students can preserve their results in one of the following ways.

- Trace the results by placing a piece of transparency film over the gel. Use a permanent marker to draw the position of each band on the gel onto the transparency. Be sure to include the outline of the gel and the locations of the sample wells, in addition to the DNA bands.
- Photograph the gel.

  a. Place a ruler next to the gel, with the edge of the ruler (0 cm) placed at the sample wells.

  b. Use a USB camera to capture and save an image in SPARKvue or on the SPARK Science Learning System.

Tip 5 – Connecting the DNA investigation to sickle cell disease at the protein and cellular levels

Here are two ways to help students visualize the changes in blood cells due to sickle cell disease.

- Use pop-beads to simulate hemoglobin molecules in a cell

  a. Put pop-beads into a small plastic bag or balloon. (The bag or balloon represents a red blood cell.) Explain to students that the normal Hb A hemoglobin proteins (with correct alpha and beta chains) exist as individual proteins in a red blood cell and do not bind or interact with each other. In this situation, a red blood cell will have the normal disc shape.

  b. Have students take out some of the pop-beads and link them together in chains of 5–10 beads per chain. Place the chains into the bag. Explain to students that if the abnormal *Hb S* protein is present, when oxygen molecules are released from the hemoglobin molecules, some of the hemoglobin proteins polymerize (form chains).

  NOTE: It is important to emphasize that each pop-bead represents a hemoglobin protein. (Students may get confused, thinking the pop-beads represent amino acids, rather than the entire protein molecule.)

  c. Have them remove additional pop-beads from the bag and connect them into additional chains. Place the chains into the bag. Explain that the lower the level of oxygen, the greater the amount of polymerization. Have the students describe how the shape of a "cell" with many polymerized hemoglobin molecules differs from a cell containing normal hemoglobin proteins. (The students are likely to see that the bag or balloon takes on a sickled shape, especially if the chains are long and stretch part of the bag.) You can also ask students to think about why red blood cells with abnormal hemoglobin are more likely to die (they die due to hemolysis).

- Use prepared slides (or pictures) of blood smears to compare normal and sickled red blood cells.

  Students can compare the appearance of blood samples from unaffected individuals, carriers, and affected individuals. For carriers, ask students to explain the ratio of normal to sickle cells. (Carriers will have both normal and sickle cells present in a blood sample, but a majority of the cells will appear normal. The sickle cells die, or are destroyed by the body, and are therefore present in smaller numbers.)

Investigation

Because of the complexity of this investigation, no student-designed experiment is included. Students gain an understanding of how to use electrophoresis to analyze DNA and detect genetic patterns.

From the student handout:

1. Compare the DNA sequence for normal hemoglobin B (*Hb A*) to the mutant DNA sequence for abnormal hemoglobin B (*Hb S*), described in Table 1.

   a. Identify the type of mutation that resulted in the Hb S variant.

   A point mutation, specifically, a substitution, resulted in the Hb S variant. Where the *normal sequence* contains an "A" (11 bp position within the given sequence), the *abnormal sequence has a* "T".

   b. Describe the effect the mutation has on the primary structure of the hemoglobin B polypeptide.

   Following transcription of this region of the *HBB* gene, the normal allele would code for the amino acid sequence Leu–Thr–Pro–Glu–Glu–Lys–Ser. The mutated allele would substitute the amino acid Val (valine) for the first Glu (glutamic acid).

   Table 1: Comparing the normal and mutant hemoglobin B alleles

Hemoglobin B	DNA Nucleotide Sequence (template strand for transcription) of the gene for Hemoglobin B (*HBB*)
Normal (*Hb A*)	C T G A C T C C T G A G G A G A A G T C T
Abnormal (*Hb S*)	C T G A C T C C T G T G G A G A A G T C T

2. Analyze each of the DNA sequences in Table 1 for the presence of one or more of the restriction site sequences listed in Table 2.

   Table 2: Recognition sequences for various restriction enzymes

Enzyme	Restriction Site Sequence[1]
*Bam*HI	GGATCC
*Mst*II	CCTNAGG
*Sac*I	GAGCTC
*Hinf*I	GANTC

[1]"N" can be any of the four nitrogen bases.

   a. Which enzyme or enzymes would cut within the normal *HBB* sequence? How does this compare to the effect the enzyme or enzymes would have on the abnormal *HBB* sequence?

   Both *Mst*II and *Hinf*I would cut the normal sequence. Only *Hinf*I will cut within the abnormal sequence.

   C T G A C T ***C C T G A G G*** A G A A G T C T (normal *HBB sequence*)
   C T ***G A C T C*** C T G A G G A G A A G T C T (normal *HBB sequence*)
   C T ***G A C T C*** C T G T G G A G A A G T C T (abnormal *HBB sequence*).

   b. In performing a diagnostic test for sickle cell disease, which enzyme would you choose to use in the digestion stage? Explain your reasoning.

   The restriction enzyme *Mst*II should be used to digest DNA samples to test for the *presence of the mutation*. If this enzyme is used, the *HBB* sequence will be cut, producing fragments. The *HBS* sequence will remain uncut. This difference can be detected with gel electrophoresis.

3. A diagnostic test for sickle cell disease will be performed using samples from a mother, father, and their child. To accurately determine the genotype of each person for the *HBB* locus, what control DNA samples are needed?

   There are three possible genotypes for the *HBB* locus: homozygous dominant (normal), heterozygous (carrier), and homozygous recessive (sickle cell disease). These three known gene samples should be run alongside the DNA samples from the mother, father, and child.

4. Put on your safety goggles.

5. Obtain the QuickStrip DNA samples A–F and a micropipet. Set the micropipet volume to 30 µL.

Load the Gel

6. Obtain a tray with the 0.8% agarose gel and take it to an electrophoresis chamber. Identify the *positive* (red cord) and *negative* (black cord) sides of the chamber. Place the gel, with the tray, in the chamber, positioning the wells on the negative side of the chamber (the side with the black cord).

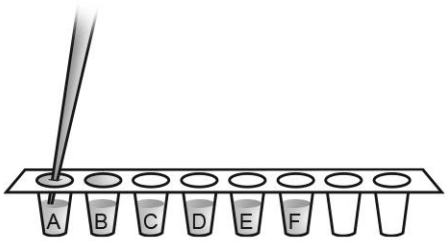
   NOTE: During electrophoresis, the DNA samples migrate through the gel towards the positive electrode. It is critical that you have the wells closest to the negative side of the chamber!

7. The gel should rest level and centered on the platform of the electrophoresis chamber and be submerged under the surface of dilute electrophoresis buffer. Add buffer to the chamber if the gel is not submerged.

8. Tap the QuickStrip DNA sample tubes gently on the table to ensure that the sample is at the bottom of the tubes.

9. Place a sterile tip on the end of the micropipet.

10. Pierce the protective overlay of the QuickStrip DNA sample container with the pipet tip and draw 30 µL of sample A into the tip. Make sure there are no bubbles in the tip of the pipet after you have extracted your sample.



11. Carefully place the tip of the pipet halfway into the first well of the gel. *Slowly* press the plunger of the micropipet to expel the sample into the well.

    NOTE: You should see the DNA and loading dye drop into the bottom of the well. Do not push through the "soft stop" on the pipet. Leave your thumb at the soft stop, remove the pipet tip from the well, and eject the tip.

12. Using a clean pipet tip each time, load samples B–F into the wells in consecutive order.

Run the Gel

13. Place the lid securely on the electrophoresis chamber and connect the apparatus to the DC power supply.

14. Set the power source to the required voltage. Ask your teacher what voltage is recommended for your equipment.

15. Turn on the power supply. Check that the current is flowing properly—you should see bubbles forming on the two platinum electrodes.

16. While you wait for results, copy Table 3 into your lab notebook and complete the "Expected Gel Pattern" section.

17. Conduct the electrophoresis for the length of time instructed by your teacher. When that time is up, turn off the power supply to stop the electrophoresis process.

Stain the Gel

18. Slowly pour 75 mL of water or electrophoresis buffer into the plastic tray for gel staining.

19. Put on disposable gloves. Carefully remove the gel from the electrophoresis chamber and place it into the plastic tray. (Slide the gel off the tray it was cast in.) The gel should be completely submerged in the liquid (add more liquid if necessary).

20. Place the blue dye side of the InstaStain® Blue card face down on the surface of the liquid, directly over the gel.

21. After 60 seconds, remove the card from the staining tray.

22. Cover the tray with plastic wrap and leave it undisturbed for at least 3 hours. (You can leave the gel in the tray overnight.)

23. Wearing the disposable gloves, remove the gel from the staining tray. If a light box visualization system is available, place the gel on the light box for an optimum view of the DNA bands.

24. Sketch a diagram, like the one shown below, in your lab notebook. Draw the banding patterns observed in your gel for each lane (each sample). Complete the “Actual Gel Pattern” column of Table 3.

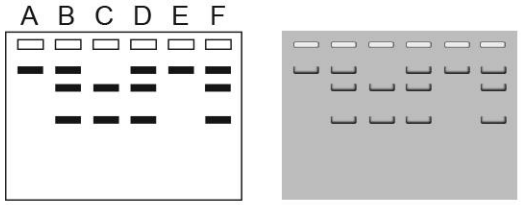


Table 3: Results of electrophoresis

DNA Sample	DNA Source	Expected Gel Pattern	Actual Gel Pattern	Genetic Status
A	Sickle cell control	1 band	1 band	
B	Carrier control	3 bands	3 bands	
C	Normal control	2 bands	2 bands	
D	Mother		3 bands	Carrier
E	Child		1 band	Sickle cell anemia
F	Father		3 bands	Carrier

Data Analysis

1. What is the genetic status of a person whose hemoglobin B DNA sample produces three bands on a gel? Explain why three bands are produced.

   If the DNA sample results in three bands on a gel, the person is a carrier for the sickle cell trait. The normal allele contains the restriction site that, after digestion, will become two DNA fragments. A carrier will also have the *abnormal* allele, which remains uncut. The presence of both forms of the allele results in three bands.

2. What is the genetic status of a person whose hemoglobin B DNA sample produces one band on a gel? Explain why only one band is present.

   If a DNA sample results in just one band on a gel, the person has sickle cell anemia. They have only the *abnormal* form of the gene. This allele lacks the restriction site for the *Mst*II enzyme and remains uncut. This large, uncut section of DNA will show up on the gel as a band near the sample wells (large pieces of DNA move slowly through the gel).

3. Why do the two fragments resulting from digestion with *Mst*II travel to different locations on the gel?

   The two fragments produced by the restriction enzyme are of two different sizes. The longer fragment (with a greater number of base pairs) moves more slowly through the gel. The shorter fragment moves more easily, and faster, through the gel so it ends up farther from the sample wells.

4. Consider the genetic status of the mother and father tested in this investigation.

   a. If the couple decides to have another child, what is the probability that their second child will have sickle cell anemia?

      Inheritance of the *HBB* gene follows Mendelian chromosomal inheritance. If both parents are carriers, there will be a 25% chance that their child inherits two copies of the *abnormal* allele.

   b. Is the probability of inheriting sickle cell anemia affected by the gender of the individual? Explain your reasoning.

      The gender of an individual does not influence the likelihood of inheriting sickle cell anemia. The gene for hemoglobin B is located on chromosome 11, an autosome. The disease is autosomal recessive and males and females have an equal chance of inheriting the mutations that cause the genetic disorder.

Synthesis Questions

1. The most common cause of sickle cell anemia is a mutation that results in the amino acid valine replacing glutamic acid in the hemoglobin B gene (*HBB*). This single amino acid difference leads to significant consequences on red blood cell structure and body physiology.

   a. Valine is a neutral and non-polar amino acid. Glutamic acid is an acidic and polar amino acid. Why do different amino acids have different chemical properties?

   The chemical properties of amino acids are determined by the chemical properties of their side-chains (R-groups). Some amino acids, such as glutamic acid, contain a hydroxyl group that makes it polar. Other amino acids contain $–CH_3$ as part of their side-chain and are non-polar and hydrophobic.

   b. Explain why the change from glutamic acid to valine affects the structure of the hemoglobin protein.

   The tertiary and quaternary levels of structure of a protein are dependent upon interactions between the side-chains of amino acids. These interactions are determined by the chemical nature of the side-chains. For example, a non-polar hydrophobic side chain will not have the same chemical interactions that a polar, hydrophilic side-chain has.

   The presence of valine in the abnormal hemoglobin B (or Hb S) polypeptide causes a change in the protein structure. Because valine is non-polar, it does not interact with neighboring amino acids in the same way that glutamic acid does in Hb A. In addition, side-chains from an amino acid in one hemoglobin B molecule can interact with a side-chain on a different hemoglobin molecule. This occurs when the hemoglobin chains contain valine (hemoglobin S or Hb S): it causes polymerization of hemoglobin when oxygen levels are low, giving red blood cells an abnormal shape. This interaction does not occur between Hb A chains, which contain glutamic acid.

2. Persons with sickle cell anemia are cautioned against participating in strenuous activity. The symptoms of the disease are most severe when the supply of oxygen is limited. Relate this characteristic of the disease to the location and role of hemoglobin in the body, and explain why exercise would amplify symptoms.

   Hemoglobin is present in red blood cells. These proteins bind oxygen molecules in the lungs and release the oxygen to the cells. (They also bind carbon dioxide and then release the carbon dioxide molecules in the lungs.)

   The release of oxygen results in polymerization of abnormal hemoglobin molecules. This distorts the shape of the red blood cells and makes it difficult for these cells to pass through capillaries, decreasing blood flow and oxygen delivery, and causing pain and tissue damage.

   During exercise, the increased demand for oxygen will cause more frequent polymerization and depolymerization, causing more damage to tissues and the increased death of red blood cells. The death of the red blood cells (hemolysis) leads to the anemia factor of sickle cell disease.

3. Scientists have discovered that a drug called *hydroxyurea* promotes the production of fetal hemoglobin, a form of hemoglobin present only in trace amounts after 1–2 years of age. Treatment with hydroxyurea causes many sickle cell patients to produce more fetal hemoglobin which, in turn, helps reduce polymerization of hemoglobin in red blood cells.

   a. Why would hydroxyurea be considered a treatment and not a cure for sickle cell anemia?

   The root cause of sickle cell anemia is a mutation in the DNA. Hydroxyurea does not fix this mutation; the mutated form of the *HBB* allele will continue to be expressed and Hb S hemoglobin protein will be produced and present in red blood cells. The treatment helps reduce polymerization and decreases the severity of symptoms, but if the treatment is removed, the symptoms return.

   b. To develop a cure for the disease, how might scientists induce the body to produce more fetal hemoglobin on its own, independent of a drug like hydroxyurea?

   The trace amounts of fetal hemoglobin present after 1–2 years of age must be due to reduced gene expression of the gene for Hb F. Researching gene expression in infants might lead to the design of a method to increase gene expression and production of Hb F in persons with sickle cell anemia.

17. ENERGY DYNAMICS

Lab Overview

Students set up a variety of simple detritus-based model systems to estimate energy flow and carbon cycling within an ecosystem. Students set up their ecosystem with a known detritivore, a known decomposer, or a combination of both detritivore and decomposer. The teacher provides students with data from two control systems to help interpret changes in the experimental systems.

Energy flux is estimated using gravimetric analysis and the carbon cycle is investigated using a carbon dioxide (CO_2) gas sensor. By tracking the movement of energy within these systems, students gain an understanding of the laws of thermodynamics as they relate to energy transfers in ecosystems. Following the Initial Investigation, students design an experiment around an abiotic factor or a biotic component of the system to manipulate or they simply monitor decomposition for a longer time period.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	25 min
Initial Investigation (over the course of 4 days)	60 min

Student-Designed Experiment	
Experiment Design	30 min
Experiment (may occur over days or weeks)	120 min
Data Analysis	45 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.A.1, 2.A.2, 2.A.3, 4.A.6
Science Practices	2.1, 2.2, 3.2, 4.1, 4.2, 4.3, 5.1, 5.2, 5.3
Learning Objectives	2.1, 2.2, 2.3, 2.22, 2.23, 4.14, 4.16

Materials and Equipment

For Each Student Station

- Data collection system
- Carbon dioxide gas sensor
- Sensor extension cable[1]
- EcoChamber™ container, with lid and stoppers
- Electronic balance, centigram (at least 1 per class)
- Weigh boat
- Plastic pipet, 1-mL
- Disposable gloves
- Small knife (for cutting fruit)
- Filter paper or coffee filter (9 cm diameter)
- Yeast suspension[2] or water, 5 mL
- Mealworms[2], 20
- Detritus (organic material such as apples and banana peels), approximately 60 g[3]

[1]Included with the carbon dioxide sensor.

[2]Refer to the Lab Preparation section for purchasing or preparation information. NOTE: Worms are not needed for all student groups, since the following three setups should be created and observed by different groups: System A—Detritus + yeast; System B—Detritus + *T.molitor* larvae; System C—Detritus + yeast + *T. molitor* larvae.

[3]Plastic wrap (30 cm) is needed for the teacher to prepare the detritus for Control Chamber 2. Refer to the Lab Preparation section for additional information.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Additional equipment recommended for the student-designed experiment:

- Additional sensors such as an oxygen gas sensor or temperature sensor
- Additional EcoChamber containers
- Different detritivores (earwigs, earthworms, crickets, ants, and similar organisms)
- Different sources of detritus (various fruit or vegetable scraps such as potato)

Prerequisites

Students should be familiar with the following concepts:

- The First and Second Laws of Thermodynamics
- The role of photosynthesis, cellular respiration, and fermentation in energy transfer and material cycling in ecosystems
- Trophic level pyramids and food web illustrations represent energy flow through ecosystems. Decomposers and detritivores are typically not shown in these illustrations because energy can enter this trophic level from any of the others.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times
- Use caution while handling decomposing organic matter and detritivores. Wear disposable gloves while handling ecosystem components and wash your hands immediately following ecosystem setup and measuring the mass of materials.
- Handle living organisms with care.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

NOTE: Based on your class size and the number of EcoChamber containers available, determine how many groups will set up System A, System B, and System C. Ideally, there will be at least two replicates of each system.

1. Prepare the detritus

   Purchase apples and bananas, one piece of each fruit per group. Other common fruits or vegetables can be used, but the directions of the Initial Investigation will need to be adjusted if other materials are used.

   Determine whether you prefer that students cut up the fruit or whether you will cut it in advance. For PASCO trials, apples were cut into cubes approximately 2 cm^3 and banana peels were cut into 2-cm strips. If you cut the fruit for students, place the fruit in a sealed container in a refrigerator until it will be used.

2. Obtain detritivores

   The Initial Investigation specifies using 20 mealworms *(Tenebrio molitor* larvae) for the detritivore population. Pet stores typically sell these organisms in tubs of 50 or 100. Based on the number of groups that will set up systems B and C in the Initial Investigation, estimate the number of tubs that need to be purchased from a pet store (or bait and tackle shop).

   NOTE: Crickets, earthworms, cockroaches, ants, and other animals that fit easily into the EcoChamber container can be used and are also available at many pet stores. These organisms can replace the mealworms in the Initial Investigation or can be provided to students for their student-designed experiments.

   NOTE: Rough handling is stressful to most animals and should be prohibited.

3. Prepare the yeast suspension

   Warm 150 mL of distilled water to approximately 43 °C, add a packet of active yeast (0.25 ounces or 7.8 grams), let the yeast sink into the water, and stir. Add 1 teaspoon table sugar (sucrose) to the yeast suspension and wait ten minutes until the yeast have activated (the volume of liquid will double, and yeast will produce a frothy head). Alternatively, follow the instructions on the package.

4. Set up the control EcoChamber containers

   - Control Chamber 1: A system with detritus only (no decomposers or detritivores)

     Place one piece of filter paper flat on the bottom of the chamber. Use a pipet to saturate the filter paper with 5 ml of distilled water. Place the detritus samples directly on the filter paper.

   - Control Chamber 2: A system with detritus only, covered to prevent dehydration

     Prepare the same setup as Control Chamber 1, but wrap the detritus in plastic wrap prior to placing the material on the filter paper.

     NOTE: The purpose of Control Chamber 2 is to help estimate how much of the loss of detrital mass is water loss and how much is loss resulting from consumption of detritus by detritivores or decomposers in the experimental chambers.

   The control chambers should be set up on the same day students begin the Initial Investigation so data collection for these chambers is simultaneous with data collection for the experimental chambers.

5. Number the electronic balances for student reference

   Students should use the same balance during the course of the investigation to minimize experimental error caused by classroom balances being out of calibration. If more than one balance is available to students, number the balances to ensure students use the same one each time they obtain the mass of the detritus components.

Teacher Tips

Tip 1 – Attempt to keep conditions stable during the 4-day Initial Investigation

Ecological data can be exceedingly variable, and data collected in the EcoChamber containers over the course of a number of days are not nearly as uniform as data collected in some other investigations, such as Enzyme Activity. Therefore, it is essential that all control chambers and experimental chambers are set up, handled, and monitored under similar conditions. Attention to detail will decrease variability between chambers (or "background noise"), and allow for relevant comparisons of each detritus-based ecosystem.

Regardless of the ecosystem components, all EcoChamber containers should be treated in the following manner: completely stopper EcoChamber containers while collecting CO_2 data, remove the gas sensor after four hours of data collection, and then stopper the open hole. Keep the system closed for an additional 20 hours. After 24 hours, allow the systems to remain open (no stoppers or lid) for an additional three days. During all PASCO trials, the EcoChamber containers were placed on a lab bench on the side of the classroom away from windows (direct light). The room was kept at normal room temperature (approximately 22 °C) and received approximately 13 hours of daylight and 11 hours of darkness.

Tip 2 – Handling and measuring the mass of the mealworms

T. molitor larvae are hardy, and can be handled gently with forceps. The mass of the larvae should be obtained collectively and the students should report the total mass of the population over the course of the experiment.

We advise keeping the *T. molitor* larvae after the investigation ends. They can be kept in a simple terrarium and used for taxis studies, fed to reptiles, or observed to better understand insect metamorphosis and insect life cycles.

Tip 3 – Handling and massing the detritus

The detritus may become covered in fungus, mushy, and difficult to handle after four days. In the PASCO trial, the mass was obtained of all the detritus together and then the apple pieces were removed and their mass obtained separately. The mass of the banana peels was then estimated by subtracting the mass of the apples from the mass of all detritus.

Because this is food waste, it can be placed in the regular garbage at the end of the investigation. If you keep a compost bin at school or at home, this material can be added to either of those and composted.

Tip 4 – Using the carbon dioxide gas sensor

Review the sensor manual before using the sensor and be sure to calibrate it before starting the lab.

The sensor can be affected by changes in temperature and ambient infrared radiation from incandescent lighting. For best results, use the sensor in a stable environment. The default sample rate for the sensor is 1 Hz; however, for investigations that measure CO_2 for many hours, the sample rate should be decreased. Data can be collected once every 5 or 10 minutes and still provide a good view of the trend over time.

After 4–6 hours, the calibration of the sensor may be affected and the trend may change. It is recommended to only measure carbon dioxide concentration for 6 hours or less. For multi-day investigations, the sensor can be recalibrated before each use and data can be collected for one to four hours each day, or every few days, to provide a "snapshot" of community respiration over long periods of time.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. If students are comfortable with probeware, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

Part 1 – System setup and carbon dioxide gas concentration monitoring

1. Put on your safety goggles and disposable gloves.

2. Copy Table 1 into your lab notebook and determine which of the following systems your group is responsible for.

   NOTE: At the end of the four-day investigation, you will need to gather data from other groups and your teacher to complete the table and make comparisons between all of the systems.

Table 1: Gravimetric analysis of EcoChamber container components

System Components		Initial Mass (g) of Detritus Components and Detritivore Population			Final Mass (g) of Detritus Components and Detritivore Population		
		Apple	Banana Peels	*T. molitor* larvae	Apple	Banana Peels	*T. molitor* larvae
A	Detritus + yeast	28.77	15.92		27.54	15.64	
B	Detritus + *T. molitor* larvae	29.47	13.57	2.11	28.91	13.34	2.13
C	Detritus + yeast + *T. molitor* larvae	29.97	16.50	1.97	28.06	15.60	2.12
Ctrl 1	Unwrapped detritus	29.75	17.31		29.18	16.89	
Ctrl 2	Wrapped detritus	29.07	13.78		28.55	13.52	

3. Place a piece of filter paper flat on the bottom of an EcoChamber container. Soak the paper with 5 mL of water or yeast suspension depending on the setup your group has been assigned.

4. Obtain the detritus material for the EcoChamber container and measure the initial mass of each component (record the number of the balance you use and always make your measurements on this balance):

   a. Place a weigh boat on the electronic balance and tare the balance. Add apple pieces to the weigh boat until you have approximately 30 grams of material. Record the collective mass of the apple pieces in your lab notebook.

   b. Add the apple pieces to the EcoChamber container on top of the filter paper.

   c. Obtain approximately 30 grams of cut banana peels. Record the collective mass of the material in your notebook and add the banana peels to the EcoChamber container.

5. Obtain the detritivore population for the chamber—20 mealworms is an adequate population. Measure and record the collective mass of the detritivore population before placing the worms in the chamber.

   NOTE: Add mealworms only to systems B and C.

6. Connect a carbon dioxide gas sensor to the data collection system and calibrate the sensor.

7. Create a display of the sensor measurement in ppm versus Time in minutes, and adjust the sample rate to one sample every 5 minutes. If possible, set an auto-stop condition for four hours. Place the sensor into one of the openings of the lid of the EcoChamber container.

   NOTE: Since data collection will occur over many hours, connect the data collection system to an AC power adapter.

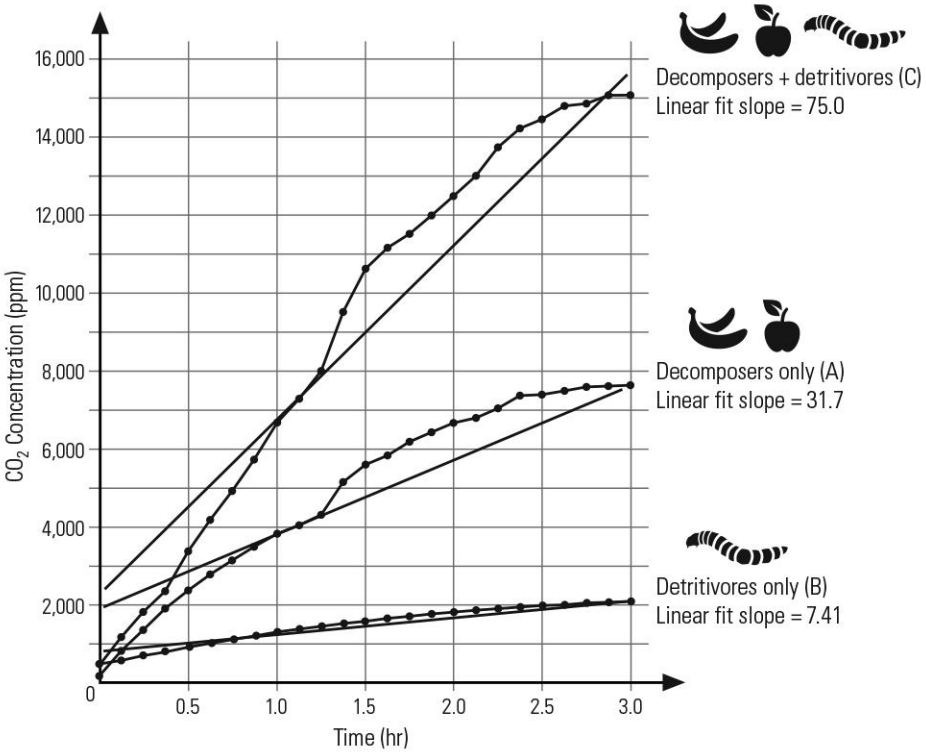
8. Secure the lid on the chamber and seal all openings with rubber stoppers. Place the chamber and data collection system in a location where it will not be disturbed and is away from direct sunlight. Begin recording data.

9. Describe aspects of the carbon cycle present within the model system. Name carbon storages as well as carbon fluxes (or movement) within the system. Do you expect the carbon dioxide gas concentration to change in the chamber? Why or why not?

   Carbon is stored within molecules of the detritus (apple and banana peel); for example, there are carbohydrates in the tissues of the fruit. These carbohydrate molecules are transferred from the detritus to the mealworms or to the yeast when the mealworms or yeast feed on the detritus or on microorganisms that live on the detritus. The organic molecules are then remineralized, that is, converted to the inorganic molecule carbon dioxide, by detritivores or decomposers when the organisms carry out cellular respiration. Additionally, some of this organic material is being egested as worm feces. The respiration of the living organisms in the system should cause the carbon dioxide concentration to increase in the chamber.

10. After 4 hours, stop data recording and save the data. Remove the carbon dioxide gas sensor and place a stopper in the open hole. Alternatively, if an auto-stop condition has been set, the gas sensor can remain in the lid until you return to the classroom after 24 hours.

11. Draw or print a record of the change in carbon dioxide concentration within the EcoChamber container.



System A: Carbon dioxide gas production in a detritus-based ecosystem with 5 ml of yeast.

System B: Carbon dioxide gas production in a detritus-based ecosystem with 20 *T. molitor* larvae present.

System C: Carbon dioxide gas production in a detritus-based ecosystem with 5 ml of yeast and 20 *T. molitor* larvae present.

Part 2 – Gravimetric Analysis and Estimation of Energy Transfers

12. After 24 hours, record observations of the components of your EcoChamber container.

    NOTE: If you have not already done so, disconnect the carbon dioxide gas sensor from the data collection system. For the next part of the investigation, keep the lid off the chamber; the chamber will remain open to the air.

13. Leave the chamber in a location away from direct sunlight for three additional days. Re-wet the filter paper with 5 mL of water each day.

14. Ecologists differentiate between "fresh" mass (total mass of material) and *biomass* (the mass of tissue present in an organism). The biomass is the collective mass of the organic compounds found in an organism's tissues: carbohydrates, proteins, and fats. These molecules store a certain amount of energy (measured as kilocalories, or kcal) and ecologists attempt to determine energy transfers by monitoring changes in biomass and relating these changes to the energy contained in that biomass.

    a. In general, if the apple pieces have a mass of 30 grams, the biomass of the apple pieces is approximately 5 grams. Explain why the total mass of apple pieces is not the same as the biomass of the sample.

       Apples contain a lot of water. Water is inorganic, is not a source of energy for organisms, and is not a molecule in which organisms store energy in their tissues. Therefore, water does not count as biomass.

b. Carbohydrates and proteins contain 4 kcal per gram and fats contain 9 kcal per gram. Which of these organic compounds is the primary component of apples? Estimate the energy, in kcal, contained in 30 g of apple pieces.

Apples primarily contain carbohydrates. At 4 kcal/g, the total energy content of 5 g of apple biomass is 20 kcal.

c. Mealworm larvae contain more kcal per gram of biomass than apples. Why would larvae biomass contain more energy than apple biomass?

Larvae contain more fat than apples. Since fats store 9 kcal/g, the energy contained in each gram of larvae biomass is greater than the energy in each gram of apple biomass.

15. Copy Table 2 into your lab notebook.

Table 2: Determining the energy content of detritus materials and detritivores

System C Components	Dry Matter (DM)[1] (%)	Gross Energy[1] (MJ/kg DM)	Energy Content per Gram of Fresh Mass (kcal/g)[2]	Total Energy Content of the Initial Mass of Each Sample (kcal)
Apple pieces			0.52	15.58
Banana peels	15.4	18.4	0.67	11.06
Mealworms[3]	42.2	26.8	2.69	5.30

[1]If the website www.feedipedia.org becomes unavailable, use a similar resource to obtain these values. Teachers: if the website becomes unavailable, you may choose to just provide these values to students.

[2]There are 238 kilocalories in every megajoule (MJ).

[3]If you set up Chamber A, this sample is not applicable to your setup. However, you will use the Energy Content per Gram of Fresh Mass for mealworms in a later question, so you should calculate that value here.

a. Use the information provided in the table for apple pieces to determine the energy content of the apples placed in the EcoChamber container on the first day of the investigation.

Energy content of apples = (energy content per gram) × (initial mass of apples)

Example calculation for System C: (0.52 kcal/g)(29.97 g) = 15.58 kcal

b. The website www.feedipedia.org provides nutritional information for a number of animal feed samples, including banana peels and mealworms. The energy content, in megajoules per kilogram (MJ/kg), is reported for the biomass (the dry matter) of the sample. The biomass can be determined by knowing the percentage of dry matter (DM) in the fresh sample. Use the information from the website to complete the table and then use dimensional analysis to determine the energy content per gram (kcal per gram of fresh mass) and the total energy content of banana peels and mealworms.

Example calculations for banana peels, System C:

Energy content per gram of fresh mass = 0.154 × 18.4 MJ/kg × 1 kg/1000 g × 238 kcal/MJ = 0.67 kcal/g

NOTE: Table 2 shows that the dry matter is 15.4% of the mass of fresh banana peels.

Energy content of the initial mass = 0.67 kcal/g × 16.50 g = 10.67 kcal

16. On Day 4 of the investigation, record observations of the chamber and measure the final mass of the detritus components and mealworms (if applicable). Record the data in Table 1.

17. For each item measured, calculate the percent change in mass that occurred over 4 days. Share this data with the class.

Example calculation for apples, for System C:

$$\text{Percent change} = \frac{\text{Final mass} - \text{Initial mass}}{\text{Initial mass}} \times 100$$

$$\text{Percent change} = \frac{28.06 - 29.97}{29.97} \times 100 = -6.4\%$$

18. Create a table to organize class data from the three different experimental systems (A–C), as well as the two controls set up by your teacher. Record the average percent change in mass for each component, and the rate of change in carbon dioxide during the first 4 hours of the investigation.

Change in mass of detritus and carbon dioxide concentration for various decomposition systems

System Components		Average Percent Change in Mass of System Components (%)			Rate of Change in CO_2 Gas Concentration, 0–4 hours (ppm/h)
		Apple	Banana peels	*T. molitor* larvae	
A	Detritus + yeast	–4.3	–1.8	NA	31.70
B	Detritus + *T. molitor* larvae	–1.9	–1.7	0.95	7.41
C	Detritus + yeast + *T. molitor* larvae	–6.4	–5.5	2.0	75.00
Ctrl 1	Unwrapped detritus	–1.9	–2.4	NA	2.89
Ctrl 2	Wrapped detritus	–1.8	–1.9	NA	5.28

19. Which contributes more to decomposition, decomposers (such as yeast) or detritivores (such as mealworms)? What evidence do you have to support your claim?

These sample data suggest decomposers contribute more to decomposition than detritivores. The apple lost more mass in the presence of decomposers than in the presence of detritivores: 4.3% mass lost compared to a 1.9% decrease, respectively. In fact, the apple decomposition rate in the presence of the mealworms was the same as the controls. Further evidence suggesting yeast contribute more to decomposition of this detritus than detritivores comes from the rate of carbon dioxide gas production. The yeast + detritus chamber generated 31.7 ppm CO_2/hour compared to 7.41 ppm CO_2/hour.

20. What purpose do the controls serve in this investigation? What can be concluded from a comparison of the controls and the experimental setups?

The controls serve two purposes in this investigation: First, they indicate the rate of detritus decomposition (or mass lost) as the material dehydrates. Second, the controls indicate the rate of CO_2 production from ambient/latent microorganisms present in the system. When comparing the controls and the experimental chambers, it is evident the apples and banana skins will decompose (or lose mass) when detritivores or decomposers are absent. These controls also indicate that detritivores and decomposers increase the rate of decomposition of this particular detritus.

21. Do the results of System C indicate that decomposers affect detritivores? What evidence supports your answer?

These sample data strongly suggest detritivores and decomposers interact and increase the rate of decomposition of detritus. Three lines of evidence support this claim. First, 48% more apple decomposed when mealworms were combined with yeast compared to yeast alone. Second, 58% more banana skin decomposed when mealworms were combined with yeast compared to yeast or mealworms alone. Third, the rate of CO_2 production in System C (mealworms combined with yeast) was 75 ppm CO_2/min; this was 92% greater than the sum of mealworm and yeast respiration in isolation.

22. Use data from one group's setup of System C (or an average from multiple setups of System C) and the conversion factors established in Table 2 to calculate the energy transfer in the system over the four days.

Table 3: Estimate of energy transfer in a model system containing detritus, decomposers, and detritivores

System C Ecosystem Component	Energy Content per Gram of Fresh Mass (kcal/g)	Change in Mass over 4 Days (g)	Change in Energy Content (kcal)
Detritus 1: Apple	0.52	–1.91	–0.99
Detritus 2: Banana skin	0.67	–0.90	–0.60
Detritivore: *T. monitor* larvae	2.69	0.15	0.40

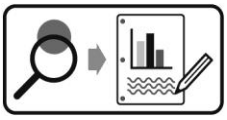
23. Calculate the ecological efficiency of the mealworms in System C during the Initial Investigation. Ecological efficiency can be calculated from the ratio of energy gained in the detritivore pool over energy lost from the detrital pool.

Student answers will vary, but based on the sample data included here, the mealworms have an ecological efficiency of 25.2% [that is, 0.40 kcal/(–0.99 kcal + –0.60 kcal)]. The detritus lost 1.59 kcal collectively and the mealworms gained 0.40 kcal in the biomass they accumulated. This is within the range of published ecological efficiencies for insects (10-44%).

Design and Conduct an Experiment

The Initial Investigation offers insight into the processing of detritus within a simple ecosystem. In actual ecosystems, a significant amount of biomass and energy from primary production is transferred to the detrital pool. This detritus serves as a trophic base for both detritivores and decomposers. A number of factors can affect this trophic level.

Students may choose to monitor the EcoChamber system for a longer period of time or change a biotic component of this detritus-based ecosystem, such as changing the type of detritivore, increasing the detritivore population, or changing the amount and type of organic matter in the ecosystem. Abiotic factors such as temperature can also be investigated.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- How will detritivore and detritus mass change if the initial experiment continues? (In other words, can detritus sustain detritivore growth over a longer period?)*
- Is the rate of decomposition affected by the presence of more than one type of detritivore in the system?
- How much energy is transferred as heat to the environment during decomposition?
- How quickly do leaves, or other "natural" detritus, decompose?
- Does increased temperature speed up decomposition of detritus?
- Do other types of detritus material (for example, raw potato, oats, corn meal, radishes) sustain mealworm larva growth better than apples and bananas?
- Do earthworms facilitate the decomposition of detritus in soil-based systems?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the Suggested Inquiry Question: "How will detritivore and detritus mass change if the initial experiment continues over a longer period?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they plan to analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected decomposition. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

   The independent variable was time, monitoring decomposition for 14 days instead of only 4 days. My hypothesis that the detritivores would eventually lose mass was supported. On Day 14, the mass of the detritivores was 8% less than it was when the investigation began. Also, as expected, the detrital pool was almost gone by the end of the investigation, with a 92% loss of mass for apples and 90% loss of mass for banana peels.

   b. Based on the evidence you collected, explain why the results occurred.

   The majority of detrital biomass was converted to carbon dioxide by decomposers and detritivores, and much of the water content of the food samples was lost to the environment as the detritus dehydrated. The decomposing material was covered with fungus by Day 7, contributing to "community respiration" in the chamber and contributing to the loss of mass in the detrital pool. The detritivores lost mass due to using more energy than they were acquiring from the detritus.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   There is no evidence that experimental error or other uncontrolled variables affected the results. The data are reliable.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. How do the Laws of Thermodynamics relate to the model decomposition systems that were set up in the Initial Investigation?

   The First Law of Thermodynamics is, "Energy is neither created, nor destroyed, but merely transformed." The Second Law of Thermodynamics follows the first, simply put, "No energy transformation is 100% efficient." With each energy transformation, energy is lost to the environment. Another way of stating the second law is, "With each energy transformation, the entropy of the system increases."

   Both laws of thermodynamics relate to this model system. The carbohydrates present in the detritus contain energy (chemical potential energy). During consumption or decomposition, this energy is not destroyed but converted to other forms as the complex organic molecules are converted to the lower-energy molecule CO_2. The energy originally present in the carbohydrates is transferred to molecules of ATP. Further, some of the energy in the carbohydrates is transferred out of the living system to the environment as heat.

2. With regard to measuring energy dynamics, describe at least three limitations of the model systems used in the Initial Investigation.

   One, water loss from the fruit was difficult to measure. The loss of mass in the detritus could be caused by dehydration rather than decomposition.

   The gain in mass of the *T. molitor* could be caused by the beetle larvae consuming water from the detritus or the wet filter paper. *T. monitor* feces (frass) were not collected. Collecting and determining the mass of the *T. molitor* feces in both chambers with mealworms could help account for the mass lost in the detritus.

   The yeast were activated prior to adding them to the ecosystem and there is no way to distinguish between yeast respiration from the initial medium and the yeast respiration on the detritus contained within the EcoChamber container. Adding dry yeast to the system would likely delay decomposition of detritus, but would more accurately reflect yeast contribution to energy dynamics in this model ecosystem.

3. Movement of matter and energy occurs in all ecosystems. Often a model of this transfer is presented as a diagram, a food web for example.

   a. Describe the movement of matter and energy in ecosystems. Include in your description how different types of organisms acquire the free energy they need to sustain life, and why an ecosystem requires a constant input of free energy.

      All living things are carbon based, and are therefore composed of organic matter that contains mainly carbon, nitrogen, oxygen, and hydrogen. All of this organic matter has chemical potential energy; energy stored in the bonds that maintain the arrangement of the atoms in the molecule. When one organism eats another, the consumer gains both matter and energy. The matter can be stored in the body of the consumer, or used to provide energy for its cells. Therefore, the energy gained by the consumer may remain stored within organic compounds, or it may be transferred to ATP and used, or lost to the environment as heat.

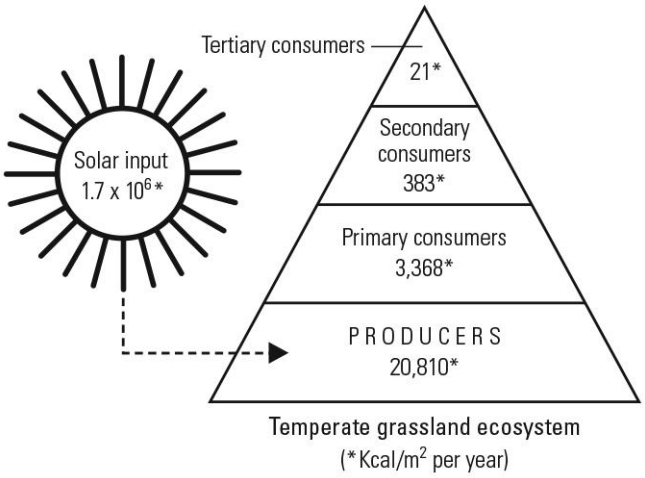
      In ecosystems, energy flows first from producers to primary consumers. Producers capture energy from the sun by carrying out photosynthesis. Primary consumers feed on producers, secondary consumers feed on primary consumers, and so on. Much of the biomass at the producer level isn't consumed by primary consumers and becomes food for decomposers and detritivores when the producers die. At each trophic level, energy is lost to the environment and ecosystems depend on producers to continually capture free energy and make it available to other organisms through the compounds they make with the captured energy.

   b. Food webs and trophic level pyramids typically do not include decomposers or detritivores in the illustration. Propose an explanation for why this is the case.

      Detrital pathways are typically excluded for two main reasons: 1) Most trophic pyramids explain annual productivity across trophic levels. The detrital pool turns over at a much slower rate, therefore the mass in the detrital pool represents several years of productivity, as opposed to, for example, primary producers or primary consumers that represent an annual standing crop or biomass. 2) All trophic levels provide matter for decomposition, so the energy transfer to the decomposer trophic level is not “linear” as it is for other trophic levels.

4. The diagram below provides information about the amount of solar energy entering an ecosystem, the net primary productivity of the ecosystem's producers, and the amount of energy at each successive trophic level.

   a. Calculate the ecological efficiency of energy transfer between each successive trophic level and explain why ecological efficiency is often less than 10%.



Ecological efficiency was calculated by dividing the energy of a certain trophic level by the energy of the trophic level below it on the pyramid.

For example, the ecological efficiency of the primary consumer is calculated by dividing the annual energy of the primary consumer trophic level (3,368 kJ/m^2) by net productivity of the primary producer trophic level (20,810 kJ/m^2).

Ecological efficiency in a temperate grassland ecosystem

Trophic Level	Ecological Efficiency
Primary Producer	1.2 %
Primary Consumer	16.2 %
Secondary Consumer	11.3%
Tertiary Consumer	5.7%

Ecological efficiency is low for two reasons. First, some of the living biomass at each trophic level dies and moves to the detrital pool where it is unavailable to the consumers at the next trophic level. Second, most of the energy the producers and consumers take in is then used for metabolism and ultimately lost as heat to the environment. For producers, there is a saturation of light; much more light hits earth than can be captured by the plants.

b. Identify a biome that would have a higher net primary productivity (NPP) than a grassland ecosystem and a biome that would have a lower NPP. For each of your choices, explain biotic or abiotic aspects of the biome that affect its NPP. How would decomposition rates compare for these biomes?

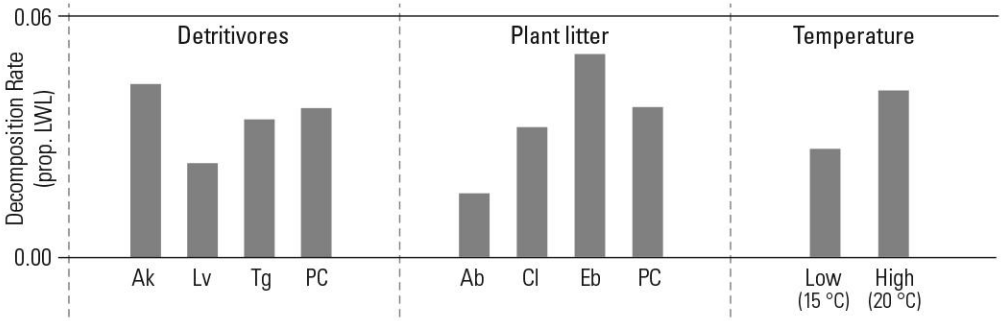
Ground biomass [(gC/m^2)/year] such as grasslands contain (600 gC/m^2)/year. Temperate deciduous forests typically have NPP two times higher, (1200 gC/m^2)/year, than grassland ecosystems. Tundra biomes are usually less productive than grasslands, typically producing only (200 gC/m^2)/year.

In general, biome productivity is driven by temperature and precipitation, as well as day length. Temperate forests and temperate grasslands experience similar temperature regimes, but forest biomes, being closer to the Atlantic coast, receive more rainfall annually. This drives net primary productivity up. Tundra biomes, on the other hand, are much colder than temperate grasslands. They also receive much less solar energy on an annual basis, and the cold Arctic air holds less moisture. Therefore, tundra biomes receive less annual precipitation. All these factors limit net primary productivity in polar biomes.

Warmer, wetter climates (for example, forest biomes) have a higher rate of decomposition and remineralization of organic matter in the soil than drier climates (such as grasslands). This fast decomposition rate returns nutrients to primary producers quickly and contributes to the high primary productivity rates measured in this biome.

5. Researchers carried out numerous experiments designed to study decomposition rates under a variety of conditions.[16] The experiments were designed to model the decomposition that occurs in an Australian stream. Three different leaf-shredding detritivore species were taken from the stream for use in the study, leaves from three native riparian tree species were collected for use as plant litter, and the researchers carried out experiments in model stream habitats at two different temperatures (typical stream temperature and 5 °C warmer). In total, almost 200 experiments were performed by the researchers.

The following diagram illustrates the results of these experiments. The bars show the mean detritivore-mediated decomposition rates measured as a proportion of leaf weight loss per detritivore (prop. LWL). Detritivores were present in all trials; the species identity or richness of detritivores was manipulated for some trials, as was the species identity or richness of plant litter. Each manipulation of detritivores or plant litter species was tested at two temperatures. Researchers performed a variety of statistical analyses to the data and the resulting probability values *p* are provided below each graph.



Ak = *A. kirramus*
Lv = *L. varians*
Tg = *T. gonetalus*
PC = "polyculture", all three species of detritivore are present

In each trial, 6 larvae were placed into the model stream.

Ab = *A. brachystyllis*
Cl = *C. leucophylla*
Eb = *E. bessaphila*
PC = "polyculture", all three species of tree leaves are present

In each trial, 3 leaf pieces were placed in the model stream.

The 5 °C difference was chosen based on predictions of the possible impact of climate change on stream temperatures and also because it was within the tolerance range for the detritivore species.

[16] Boyero, L.; Bradley, J.C.; Bastian, M; Pearson, R.G. Biotic vs. Abiotic Control of Decomposition: A Comparison of the Effects of Simulated Extinctions and Changes in Temperature. *PLoS ONE* (Impact Factor: 3.73). 01/2014; 9(1):e87426. DOI:10.1371/journal.pone.0087426 http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0087426 (accessed July 3, 2014).

Table 4: Statistical analysis of decomposition rates under different conditions of detritivore, leaf litter, and temperature

	Statistical Comparisons	
	Species Identity: ***compared decomposition rate by Ak, Lv, and Tg and compared rates of decomposition of Ab, Cl, and Eb***	**Species Richness:** ***compared the polyculture decomposition rates to the single-species decomposition rates***
Detritivores	$p < 0.001$	$p = 0.79$
Plant litter	$p < 0.001$	$p = 0.83$
	Compared decomposition rates in low temperature and high temperature stream water	
Temperature	$p < 0.001$	

a. Identify two scientific questions that the researchers were likely investigating with these experiments.

Student answers will vary. Examples include:

- Do different species of detritivores contribute equally to decomposition of plant litter in the stream?
- Do leaves from different plant species decompose at different rates?
- Is decomposition rate increased in the presence of three different species of detritivores?
- Does temperature affect decomposition rates of plant litter in the steam?

b. For one of the scientific questions you describe, propose an experimental design for the experiment used to test the question. Be sure to make clear the independent and dependent variables, as well as the constant variables for the proposed experimental design.

Student answers will vary. A possible response:

To test the question, does temperature affect decomposition rates of plant litter in the stream, I would create a model stream habitat by adding water to a small glass aquarium and placing leaf pieces at the bottom of the aquarium. The water would be sourced from the same stream the detritivores are collected from. Leaves would be collected from *E. bessaphila* trees that live along the bank of the stream. For each trial, the mass of three leaves would be obtained. The leaves would be cut into 4-cm square pieces and placed into the aquarium. Six detritivores would be present in the aquarium for each trial: 2 larvae of each of the 3 species (*A. kirramus, L. varians, T. gonetalus).*

The stream water in the aquarium would be maintained at 15 °C in the control group of the experiment. This is the typical temperature of the stream in which the detritivores live. The aquarium would receive the same number of hours of light and darkness as the outside environment. After 5 days, the leaf pieces would be removed and the mass obtained to determine the leaf weight loss per capita.

The temperature is the independent variable of the experiment. In the experimental group, the stream water would be maintained at a temperature of 20 °C. All other variable would be the same in the experimental group and control group: number of hours of light and dark, type and number of leaves and sizes of leaf pieces, type and number of detritivores, size of the aquarium and volume of water added to the aquarium. After 5 days, the leaf weight loss per capita would be determined for the higher temperature condition.

Three trials would be run for each temperature.

c. What can be concluded from the provided probability values?

For both detritivores and plant litter, the identity of the species was more important than whether there were multiple species present. When *p* is greater than 0.05, the variable cannot be said to definitively affect the outcome. The decomposition rate when 3 different species of detritivores were present was not significantly different than when a single species, such as "Ak," was present. This was also the case for the plant species. The decomposition rate was significantly affected when changing the identity of the species used for plant litter, but when all 3 species were used for litter it did not result in a significant change in decomposition rate. Because the *p* values were less than 0.001 when comparing decomposition across species, the *p* value indicates that the species identity is significant. Similarly, the *p* value for the temperature experiment indicates that a change of 5 °C significantly affects decomposition rate.

d. For either detritivores or plant litter, identify a characteristic of the organism or leaves that may contribute to faster or slower decomposition.

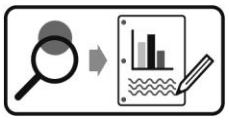
Body size may be a factor that affects detritivore-mediated decomposition. If they are larger organisms, they require more energy and may consume a greater mass of material.

The method of eating may differ between detritivores. The ones in the study were described as “leaf-shredding,” but other detritivores may use other feeding methods.

Some leaves may be bitter or in other ways less palatable to detritivores and would be less likely to be eaten. Others may contain a thick epidermis or other material difficult for detritivores to eat.

Design and Conduct an Experiment Key

The Initial Investigation offers insight into the processing of detritus within a simple ecosystem. In actual ecosystems, a significant amount of biomass and energy from primary production is transferred to the detrital pool. This detritus serves as a trophic base for both detritivores and decomposers. A number of biotic or abiotic factors can affect this trophic level. How can you change a component or condition of the model system to test factors that affect decomposition in ecosystems?



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of energy dynamics and decomposition, what environmental factors (abiotic or biotic) could affect this process?

   Environmental factors include time, water availability, type of detritus, type of detritivore, type of decomposer, and temperature.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   How will detritivore and detritus mass change if the initial experiment continues? (In other words, can this amount of detritus sustain detritivore growth over a longer period?)

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   To understand the detritus trophic level, it is meaningful to know how much detritus is needed to support a population of detritivores over a period of time.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The Independent Variable (IV) will be time, comparing decomposition rates and changes in biomass over 4 days and 14 days. The setup will be the same as System B, but monitored for a longer time than the Initial Investigation.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variables (DV) will be the change in mass over two weeks for all biotic components in the ecosystem (apples, bananas, and mealworm larvae), and the rate of CO_2 production over 4 hours in each chamber on Day 0, Day 7, and Day 14.

   The change in mass will be converted to percent change in mass. The rate of respiration will be determined by calculating ppm/min from the total change in carbon dioxide over 4 hours.

6. Write a testable hypothesis (If…then…).

   If the detritus loses much of its mass over a longer period of time, then the mass of the detritivores will decrease as well.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   All of the following will be kept constant in the trials: types of detritus (apples and banana peels), initial mass of the detritus samples (30 g, and 20 g, respectively), the type and population size of the detritivore (20 *T. molitor* larvae), amount of water added to the system, temperature of the room where the chambers are placed (22 °C), amount of light and dark (continuous cycle of 13 hours light and 11 hours dark), the same balance used to obtain the mass of all samples, the same carbon dioxide gas sensor used for all respiration measurements.

8. How many trials will be run for each experimental group? Justify your choice.

   Given the time requirement of this experiment, two trial groups will be run simultaneously.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   To get a "snapshot" of the energy transfers in the system over time, the mass of each ecosystem component will be measured every 72 hours for two weeks. The mass change will be converted to percent change in mass. In addition, CO_2 production over 4 hours will be measured at the beginning, middle, and end of the investigation. The rate of carbon dioxide production will be determined in ppm/min to compare the activity of detritivores during these times.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    Some of the mealworms may die. The mass of these dead organisms will still be obtained, but their desiccation will indicate greater mass loss from the detritivore population than actually occurred.

    Also, as detritus decomposes and becomes less solid, separating out each type of detritus for accurate measurement will become difficult.

    And, as detritus dehydrates, it will lose mass and this mass loss is difficult to distinguish from the mass loss due to decomposition.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

    Set up the system as described in the Initial Investigation. Record the initial mass of each component and measure carbon dioxide production over the first four hours of Day 0. Remove the lid after completing the measurement of carbon dioxide.

    72 hours later, on Day 3, carefully remove the filter paper with detritus and mealworms from the chamber and measure the mass of each component. If the filter paper is dry, wet the paper with 5 mL of water. Return the components to the system.

    On Day 6, measure the mass of the components again.

    On Day 7, place the lid on the container and measure carbon dioxide production over 4 hours. Then remove the lid.

    On Days 9, 12, and 14, measure the mass of the system components.

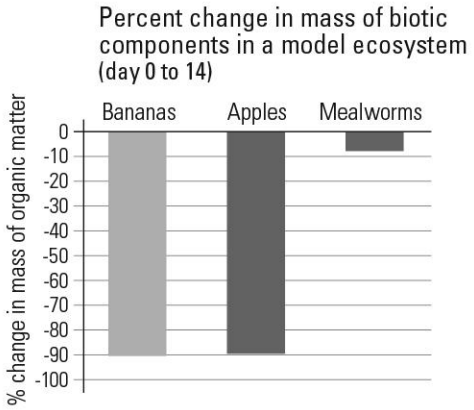
    Repeat carbon dioxide measurements for 4 hours on the final day, Day 14.

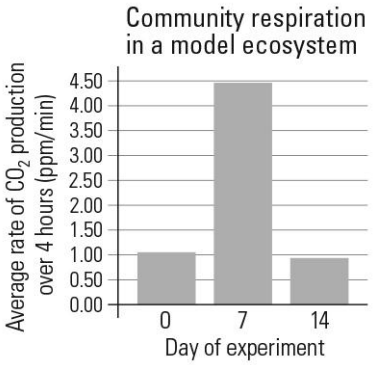
12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

Fourteen day study of System B (Detritus + *T.molitor* larvae)

Day	Measurements of the Decomposition System		
	Change in Detritus Mass (g)		Change in Detritivore Mass (g)
	Apple	Banana	Mealworms
0–3	–7.39	–9.24	0.58
3–6	–2.63	–3.34	–0.03
6–9	–5.91	–13.25	–0.29
9–12	–1.46	–1.98	–0.15
12–14	–0.43	–0.68	–0.04





The results shown above are from the sample inquiry described in the Design and Conduct an Experiment Key: How will detritivore and detritus mass change if the initial experiment (System B) continues?

18. ARTIFICIAL SELECTION

Lab Overview

Students follow the growth and development of Wisconsin Fast Plants® (a variety of *Brassica rapa*) through two generations. These plants have a short life cycle, can be grown easily in the classroom, and offer an assortment of traits students can readily observe. Quantitative traits, such as height, trichome hairs, and number of true leaves, offer students an opportunity to apply statistics to describe the range and variation in these traits in a population. Unlike discrete traits, such as flower color, quantitative traits are continuous in a population and are typically represented in histograms (frequency graphs).

In the Initial Investigation, students measure the height and one additional quantitative trait of their choice. They then select a small proportion of plants to cross-pollinate and determine if the selection of plants based on a certain trait affects the frequency of the trait in the subsequent generation. The study of a real population, and the time invested in tending and growing two generations of plants, is invaluable in helping students develop true science process skills, especially the application of descriptive statistics to data. This lab is less about students obtaining a predicted set of results than it is about students engaging in the process of scientific inquiry. After they gain skills in growing the Fast Plants® and using statistics to make meaning of data, students can design and carry out an experiment of their own design.

NOTE: The Fast Plants themselves are a great example of artificial selection, the result of selecting the fastest breeding plants in each generation. As students research these plants on the Wisconsin Fast Plants website, they will likely read about different varieties, such as the "hairy" plants. Talking about how these varieties are the result of scientists selecting for particular traits over many generations can make for a good classroom discussion.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	50 min
Initial Investigation: Setup	30–45 min
Over the course of 8 weeks	180 min

Student-Designed Experiment	
Experiment Design	30 min
Experiment: over many weeks	90 min
Data Analysis	40 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	1.A.1, 1.A.2, 1.C.3
Science Practices	1.5, 2.2, 5.3, 7.1
Learning Objectives	1.1, 1.2, 1.3, 1.4, 1.5

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Materials and Equipment

For Each Student Station

- Wisconsin Fast Plants seeds (18), standard
- Seed-starting soil or germinating mix (such as Jiffy Mix®)[1,2]
- Fertilizer, Osmocote™ pellets (24) or a water-soluble fertilizer
- Wicking material (3), #18 nylon mason twine
- Recycled plastic bottles (3), 0.5 L to 1 L
- Soda bottle cap with hole (3)[3]
- Plant vermiculite[2]
- Labeling tape and markers
- Black plastic to cover the water reservoir (3) (optional)
- Water in a rinse bottle
- Lighting system with fluorescent lights (shared by the class)
- Bee sticks or cotton applicators (3)
- Plastic plant labels (3)
- Scissors
- 12-inch ruler
- Stakes and holders, as needed (wooden splints and plastic straws)
- Dechlorinated water or nutrient solution (for the reservoir)
- Hand-held plastic magnifier
- Petri dish lid
- Paper envelope, small

[1]This type of soil mix is *not* potting soil.

[2]Purchase enough of these materials to set up the growing systems twice: once for the first generation and again for the second generation.

[3]Several layers of aluminum foil (with a hole poked in it, once it is arranged) can be placed across the opening and held in place with a rubber band.

Additional equipment recommended for the student-designed experiment:

- Data collection system
- pH sensor
- Transfer pipets
- 1 M nitric acid (HNO_3)
- 1 M sulfuric acid (H_2SO_4)

Prerequisites

Students should be familiar with the following concepts:

- Genotype gives rise to phenotype. Only traits that are heritable can be reliably transmitted to the next generation.
- Where genetic variation exists, species are mutable over time.
- Artificial selection is a type of selection where the grower chooses a trait of interest and cultivates those plants that exhibit a particular trait.
- Under natural selection, environmental conditions influence the differential reproductive success of populations best suited for a particular environment.
- Natural selection over a long period of time is a mechanism of evolution.
- Fossil fuel contaminants such as carbon dioxide, sulfur oxides, and nitrogen oxides react with water in the atmosphere to yield acidic precipitation.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- When handling scissors or other tools to cut plastic bottles, use them with care and work on a surface that can support sharp instruments.
- Keep water away from electrical outlets and all electronic equipment.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. A fluorescent lighting system

   Lighting systems can be purchased or built from easy-to-acquire materials. Refer to the Wisconsin Fast Plants website for additional information: http://www.fastplants.org/how_to_grow/growing_lighting/

2. Components of the growing system

   Growing systems are easy to make from plastic bottles and should be designed with water reservoirs to irrigate the soil in the upper compartment.

   NOTE: Familiarize yourself with the setup of the Bottle Growing System using the numerous resources available on the Wisconsin Fast Plants website.

   - Plastic bottles: Ask students to bring empty bottles to class for this purpose.
   - Bottle lids: Drill holes in the bottle lids to allow wicking material to be threaded through the top.
   - Acquire wicking material, fertilizer, seed-starting soil, vermiculite, and plastic plant labels from a local hardware store or plant nursery.
   - Nutrient solution or water: If the fertilizer is water soluble and will be used in the reservoir of the growing system, prepare the nutrient solution for the systems following the directions provided with the fertilizer.

3. Wisconsin Fast Plants seeds (Standard seed stock) and bee sticks

   A population of 120–180 plants is typical for a class working in groups of 2–4 students. Order enough seeds for each class and store the seeds in a dry, dark location at room temperature until use. Be sure to order the seeds well enough in advance to allow for the time for the seeds to be shipped to your school.

   If you expect students may want to explore a different variety of Fast Plants for their student-designed experiment, order one or more additional seed stocks such as high-anthocyanin or dwarf plants.

   If you plan to have students use bee sticks for pollination, purchase these when you purchase the seeds.

Teacher Tips

Tip 1 – Plan for the activity well in advance: schedule a 6-week period from seed planting to harvesting.

A six-week period should be chosen that will not be interrupted by extended school vacations. Seeding on Monday or Tuesday is recommended so that students can perform the activities during the weekdays and activities on the weekend can be avoided. Week 1 consists of seed planting and germination. Week 2 marks the first sign of flowers; students should make general observations about plant development. This is a good opportunity to introduce the tools of statistical analysis. During Week 3, selection decisions should be made and cross-pollination carried out.

The majority of classroom time needed for the investigation occurs during the first three weeks, during which time students will measure the quantitative trait of interest, make selection decisions, and perform cross-pollination. Thereafter, there is minimal effort needed to maintain the plants: the water reservoirs will need to be replenished, distance from lighting adjusted, and plants may need to be supported on wood sticks, as they will grow rapidly. The grower's calendar below serves as a guide to help you plan the investigation.

NOTE: If it is not convenient to plant the 2nd generation right away, the seeds can be stored in paper envelopes and planted at a later time.

Grower's calendar: due to variation in the plants or growing conditions, the appearance of flowers and other events may occur a few days before or after the day indicated on the calendar.

Mon	Tue	Wed	Thurs	Fri	Sat	Sun
0 Plant seeds	1	2	3 Two cotyledons appear	4	5 True leaves develop	6
7	8 Flower buds appear	9	10 Stem elongates	11	12	13
14 Flower buds open	15 Cross-pollinate for 3 consecutive days	16	17	18 Embryo development	19	20
21 Water throughout the week	22	23	24	25	26	27
28 Water throughout the week	29	30	31	32	33	34
35 Remove water source and allow plants to dry	36	37	38	39	40	41
42 Remove pods and harvest seeds.	43 Plant seeds to obtain the 2nd generation	44	45	46	47	48

NOTE: Shaded boxes indicate days that require classroom time to conduct the lab investigation.

Tip 2 – Estimate the number of seeds for each class size

A population of 120–180 plants for one classroom provides ample variation. Order enough seeds for each class. Seeds can be stored in a dry, dark location at room temperature until use. Be sure to order the seeds well enough in advance that there is time for the seeds to be shipped to your school.

Tip 3 – Organize the requirements for each lab group and the class as a whole

Prior to beginning the investigation, arrange an area of the classroom that will provide sufficient space for the number of bottles needing to be set up by students. All bottles will need to be under a lighting system that provides a continuous cool-white fluorescent light positioned very close to the plants (5–10 cm).

NOTE: Leave a note near your lighting system to inform the custodian or others of the need for the light to remain on 24 hours a day.

Ask each group of students to bring in three empty 1-L plastic bottles that will be used for their growing systems. Have students begin to research this growing system and learn more about Fast Plants on the Wisconsin Fast Plants website (www.fastplants.org). There are a number of growing systems possible, for example, plastic deli dishes can substitute for plastic bottles. Fertilizer pellets can be added to the substrate, or fertilizer can be dissolved in the water of the system reservoir. This lab provides a good opportunity to put students in charge of the design and setup of the Initial Investigation, leaving you as a facilitator of their inquiry.

As the investigation begins, keep a record for yourself of what is being done by each group and the class as a whole in the form of a table like the one shown below (numbers can be adjusted depending on class size). Each lab group will prepare 3 growing bottles. A single 1-L growing container can hold 6–8 seeds. Keep track of how many total plants will be grown per class.

Group tracking

Group Number	Student Names	Bottles	Seeds	Observations and Notes
1		3	18	
2		3	18	
3		3	18	
4		3	18	
5		3	18	
6		3	18	
7		3	18	
8		3	18	
8 groups		24 bottles	144 seeds	

Tip 4 – Stakes and holders

Fast plants characteristically grow very rapidly and sometimes collapse under their own weight. In the second week, most plants will need to be supported. Wooden splints or long sticks should be placed gently in the soil next to the plant. Then wrap a piece of a straw (~1 cm in length), cut open, around both the wooden splint and the plant stem. Gently close the straw in place. This can be done at multiple points along the length of the growing plant.

Tip 5 – Choosing a quantitative trait for selection

Choosing a quantitative trait to study is an important aspect of scientific inquiry. A quantitative trait should demonstrate wide variation within a population and be easy to measure by students. For each trait, the precise day for taking measurements should be decided in advance. All student groups will measure height on Day 15. Students are asked to choose one additional trait to keep a record of. For their trait selection decision, they may choose either height or a different quantitative trait they've chosen to measure. Here are some examples of quantitative traits that can be easily studied with Wisconsin Fast Plants:

- Number of trichomes or trichome density
- Number of true leaves, flowers, pods, or seeds
- Length of pods
- Time to flower
- Size of cotyledons

Initial Investigation

The Initial Investigation is designed to familiarize students with the materials and techniques necessary to design their own experiment.

From the student handout:

1. Put on your safety goggles.

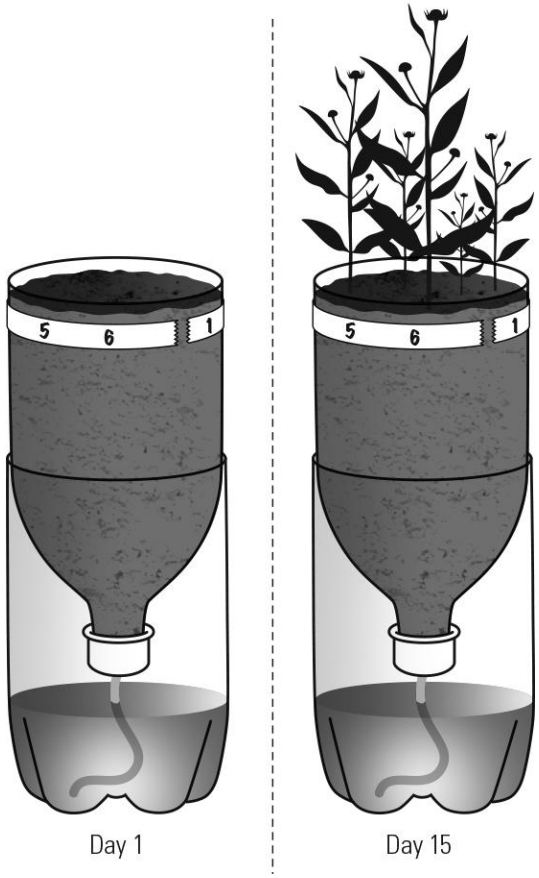
2. Before preparing the growing systems from reused plastic bottles, refer to the Wisconsin Fast Plants website (www.fastplants.org) for full instructions. A search of the website for "bottle growing system" will direct you to numerous relevant resources. The digital resource library also offers resources, such as this video: https://www.youtube.com/watch?v=eEOCRz0j6iA&feature=youtu.be.

3. Prepare the three growing systems, using recycled bottles that have been cut:

   a. Tie a knot in the wicking material, thoroughly wet the wick, and thread it through the hole of the bottle top. Screw the top onto the bottle and check that the wick will touch the bottom of the reservoir when the top piece of the growing system is placed (inverted) into the reservoir.

   b. Following instructions from the Wisconsin Fast Plants website resources—or your teacher's instructions, add starting soil, vermiculite, fertilizer (if not added to the reservoir), water, and seeds to each growing system. As you add the contents, be sure the wick remains in the center of the soil and does not extend above the soil surface.

      NOTE: **Before planting seeds** *in the growing system, wrap a piece of labeling tape around the cut edge of the top piece of each growing system. For the first one, write the numbers 1–6 on the tape, equally spacing the numbers around the circumference of the bottle. The numbers will be used to identify the seeds (plants) throughout the investigation.*



      Repeat the process, labeling the top piece of the second bottle with the numbers 7–12 and of the third bottle with 13–18.

   c. After adding the seeds, cover them with vermiculite and use the rinse bottle to wet the contents until water begins to drip from the wick.

   d. Add dechlorinated tap water or nutrient solution (water with fertilizer) to the water reservoir of the growing system. Place the top piece of the growing system into the reservoir to complete the system.

   e. Label three plastic plant labels with your group identification and the planting date; insert one label into the soil of each system.

4. Place your growing systems under the lighting system set up by your teacher.

   NOTE: The distance between the light source and the plants will need to be adjusted as the plants grow. Also, top off the water reservoir as needed due to water loss through evaporation.

5. Copy Tables 1 and 2 into your lab notebook to organize your observations (which are *not* limited to the "expected events") of the plants over the next several weeks of data collection. Detailed observations and careful measurement are an important aspect of this lab, and a full page or more of your notebook should be devoted to each data table.

   Notable events are likely to occur daily in the first part of the plants' life cycle. In the later weeks you may only need to make observations once or twice a week. Be sure to record specific seed (plant) ID numbers for certain observations.

Table 1: Observing growth and milestones in the Fast Plant life cycle

Week	Expected Events	Observation Date	Observations
1	Opening of cotyledons Emergence of true leaves		
2	Significant growth Development of flowers		
3	Select traits and plants for breeding Cross-pollination		
4–5	Appearance of seed pods		
6	Plant drying and seed harvesting		

Table 2: Quantitative trait measurement and breeding chart

Seed/Plant Number	Day 15 Height (cm)	Additional Quantitative Trait[1]: Time to Flower	Selected Trait: Height Cross-pollinated?	Number of Pods	Number of Seeds Harvested
1	19	16 days			
2	21.5	17 days	yes	3	8–12
3	21.5	15 days	yes	6	8–12
4	15.5	17 days			
5	21	16 days			
6	25	16 days			
7	16	17 days			
8	16.5	16 days			
9	5	16 days			
10	9	16 days			
11	12.5	17 days			
12	11	17 days			
13	15	17 days			
14	21	16 days	yes	6	8–12
15	14.5	17 days			
16	16.5	17 days			
17	20	16 days	yes	5	8–12
18	16	17 days			
19[2]	23.5	17 days	yes	3	8–12
20[2]	23.5	17 days	yes	5	8–12

[1]Additional quantitative traits can include: germination time, size of cotyledons, time of appearance of true leaves, flowers, or seed pods, trichome density, number of leaves or distance between leaves (internode length), plant height at first flower or plant height at first seed pods, length of pods, and number of seeds per pod.

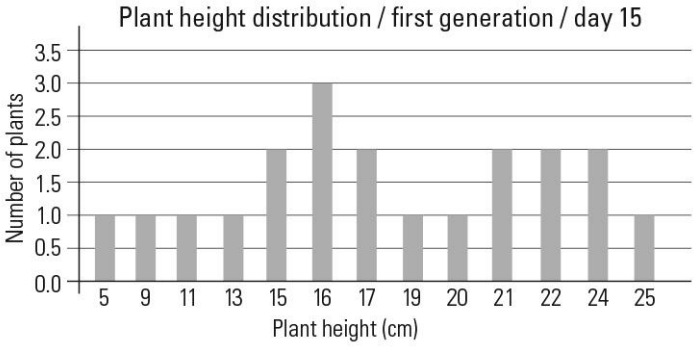
[2]For the PASCO trial of the investigation, 20 seeds were planted rather than 18.

6. After observing the growth and development of the plants for two weeks, describe the variation you observe in the traits of the plants in your growing systems.

   Student answers will vary. Answers should include detailed observations of the variation in the population for numerous traits such as: the time needed for germination or emergence from the soil, leaf color, stem color, plant height, flowering time, and number of flowers.

7. Measure the height of each of the individual plants on Day 15.

8. Create a histogram (frequency graph) that shows the height distribution at Day 15 for your population of Fast Plants.

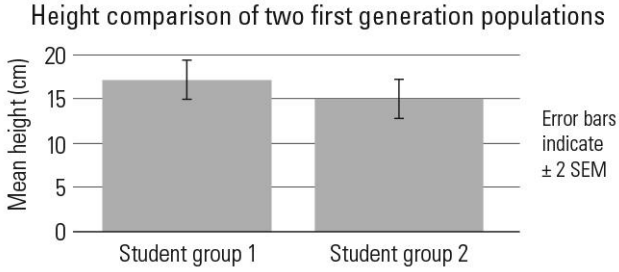


9. In your lab notebook, calculate and organize in a table the appropriate descriptive statistics about plant height for the first generation: mean, median, range, standard deviation, and standard error.

Descriptive Statistics for Day 15 Plant Height in Generation 1

Statistic	Value for the Plant Population
Mean	17.2 cm
Median	16.5 cm
Range	5–25 cm
Standard deviation (SD)	5.2
Standard error (SEM)	1.1

10. Plotting the means of two populations along with ±2 standard errors of the mean (SEM) is a good starting point to determine if any difference in the means is significant. Create such a graph in your lab notebook. Include the mean of your group's population of plants and the mean of a population of plants grown by another group in class.



11. Are the means of the two populations the same? If not, is the difference significant? Provide evidence to support your claim.

Answers will vary. The means of the two populations are not likely to be identical; they are expected to be very similar. Given the same seed stock, lighting, and other growing conditions, the plant populations of two student groups are not expected to have significantly different means. In other words, the SEM bars are expected to overlap when the two means are graphed ±2 SEM.

12. By Day 15 you need to make a selection decision, that is, you need to determine which trait to use as a criterion for selecting certain plants for cross-pollination. These selected plants will serve as the parents for the second generation. For example, one might choose to cross-pollinate plants that are 21 cm or taller to see if breeding the tallest plants changes the mean plant height in the second generation.

    Once you determine the trait you will select for, record this, as well as which plants you will keep to use for cross-pollination, in Table 2. To prevent unwanted pollination, remove and discard the non-selected plants from the growing systems.

13. When several flowers are present on each of the selected plants, cross-pollinate the plants with a single bee stick or a cotton applicator. Transfer pollen from the anthers of one plant to the stigma of another plant. Collect and distribute pollen from every flower on every selected plant. Repeat the cross-pollination procedure for 3 consecutive days.

    NOTE: Once the seed pods start to develop, trim away any additional flowers that grow on the plants. This will allow resources to be directed to the developing seeds. Replenish the water reservoirs as the seeds develop.

14. At Day 35, pour out the water from the reservoirs and allow the plants to dry for 3–5 days.

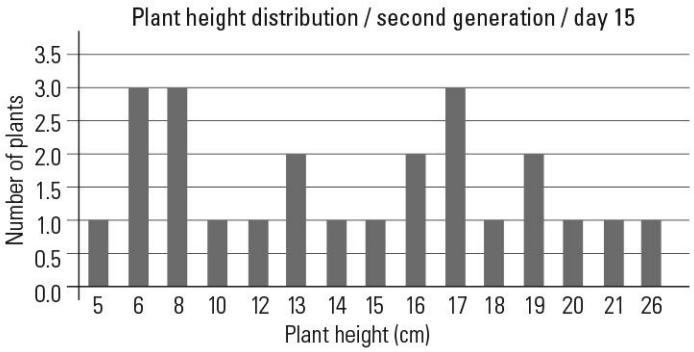
15. After the plants and seedpods have dried, harvest the seeds by breaking open the seedpods into the lid of a small Petri dish. Store the seeds in a small paper bag or envelope, labeled with your group identification and the date of harvest.

    NOTE: Remove the material from the growing systems and rinse the bottles. Keep the bottles to use for growing the second generation plants.

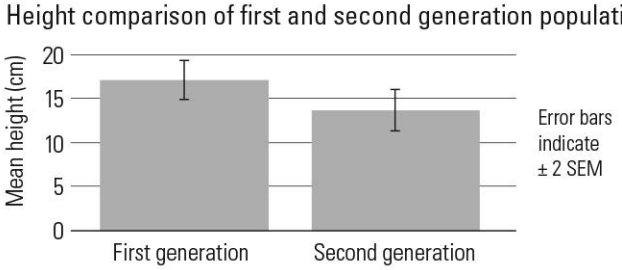
16. You now have a population of second-generation seeds. Plant the seeds as you did before, using fresh starting soil and other materials, and monitor the growth of the 2nd generation, keeping detailed records of your observations and measurements. Create an appropriate histogram for the data and calculate the appropriate descriptive statistics regarding the selected trait in the 2nd generation.

Descriptive statistics for Day 15 plant height in the second generation

Statistic	Value for the Plant Population
Mean	13.83 cm
Median	14.35 cm
Range	5–26 cm
Standard deviation	5.54
Standard error	1.18



17. Did the selection process result in a significant change in phenotype distribution in the second generation compared to the first generation? Provide evidence to support your claim.



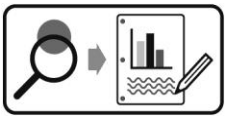
Student results will vary. It is likely that there will not be a significant change in only one generation. For the sample data provided, the second generation does differ significantly from the first generation. While the SEM bars do overlap (typically indicating that the difference is not significant) a more rigorous t-test results in a p value of 0.04, which is less than the $p = 0.05$ needed to indicate a significant difference.

18. What factors were controlled over the duration of the 6-week experiment?

The factors controlled were: constant room temperature, constant humidity level, continuous and equally distributed light, continuous and equally distributed hydration, equivalent soil quantity, and equivalent spacing for growth.

Design and Conduct an Experiment

Once students are experienced in the process of growing, pollinating, and harvesting seeds from Fast Plants they may want to continue the selection experiment over multiple generations. Alternatively, students may want to test whether environmental conditions, such as acid rain, affect the results of the selection experiment. Another option is for students to explore whether there is a correlation between two plant traits, such as seedpod size and number of seeds.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- How does water acidity affect plant height?*
- How does water acidity affect seed germination?
- How does plant height reflect biomass production?
- Is germination rate a predictor of plant height?
- Is the rate of appearance of true leaves a predictor of plant height?
- What effect does temperature have on plant growth rate?
- Is seedpod size an indicator of seed number?
- Is plant height an indicator of seed number?
- Can different varieties of Fast Plants interbreed and produce offspring?
- What are the stem colors of the offspring if purple-stem plants are crossed with non-purple stem plants?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "How does water acidity affect plant height?" Results of this experiment are shown in the Sample Data section.

1. From your observations and your data:

   a. Is your hypothesis for the driving question of your experiment supported? Justify your claim with evidence from your experiment.

   My hypothesis that predicted that simulating acid rain conditions would negatively affect plant height in both generations is not supported by the evidence. In the first generation, the differences in mean height for groups A (control), B (nitric acid), and C (sulfuric acid) were not significant according to the results of an unpaired t-test (Student's t-test). All p values were greater than 0.05. In the second generation, there was no significant difference between groups A and C nor B and C. However, the difference between groups A and B was significant; the plants exposed to nitric acid were significantly taller than control plants ($p = 0.003$).

b. Based on the evidence you collected, explain why the results occurred.

Fast Plants are hardy plants, developed specifically for classroom use, and seem to be mostly unaffected by growing in acidic conditions. Height is a polygenic trait influenced by a number of genes and the expression of these genes seem to be unaffected by a low pH. In fact, the plants grown with a source of water containing nitric acid had the greatest mean height. The nitrogen from nitric acid may have had an effect similar to nitrogen-containing fertilizers, resulting in greater growth of these plants.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

The data is reliable enough to determine that the hypothesis is not supported. There is no evidence that experimental error or other uncontrolled variables affected the results.

3. Identify any new questions that have arisen as a result of your research.

Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. What do descriptive statistics such as the mean, median, range, standard deviation, and standard error tell the experimenter?

Descriptive statistics allows the experimenter to determine the extent of variation of a quantitative trait within a population.

- Sample mean: the average value of a certain variable measured in a population
- Median: the value at the center of the spread of the measured variable; one half of the population measure less than the median and one half of the population measures greater than the median.

The mean and median both provide an experimenter with information about the central tendency of their measured population. Using the median is advantageous to using the mean if there is a small population or if there are a number of outliers in the population.

- Range: this indicates the lowest and highest values for the measured trait in a population. It provides an experimenter a quick look at the variation that exists in a population for the trait of interest, but it does not give any information about the central tendency of the data.
- Standard deviation (SD): a value that indicates the spread in the data; the dispersion of the values on either side of the mean. In other words, it helps an experimenter understand the extent of variation in the quantitative trait being measured for a population. Two populations of Fast Plants may both have a mean height of 17 cm, but one population's SD might be 10 and the other's SD might be 2, indicating that the second population varies little in height; that is, most plants have a height close to the mean.
- Standard error or standard error of the mean (SE or SEM): a value that helps the experimenter identify a range above and below the measured mean for which there is a high level confidence that the true mean of a population falls inside of that range (since the true "parametric" mean cannot actually be realistically measured). The formula for SE takes into account the population size; the larger the sample size the smaller the SE. The mean of a population ±2 SEM gives an experimenter 95% confidence that the true population mean falls inside of this range. This statistical calculation is often used to compare two populations. Plotting the means of two groups with corresponding SE bars provides information about how different the two means are.

2. Refer to the graphs below. The data is from a study during which the investigators used artificial selection, selecting fish for breeding on the basis of brain size to create two distinctly different F_2 generations.[17] Figure 1 displays the change in brain size as a result of artificial selection, and the average brain size of four populations created for the purpose of the study: smaller-brained and larger-brained females, and smaller-brained and larger-brained males. The researchers developed a "learning test" to measure the cognitive ability of the fish and compared females of different brain sizes and males of different brain sizes.

Figure 1 / Artificial selection for small and large brain size

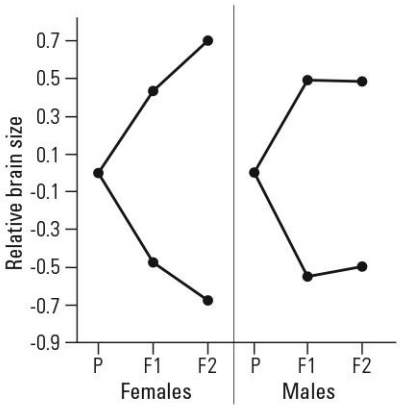


Figure 2 / Cognitive ability test

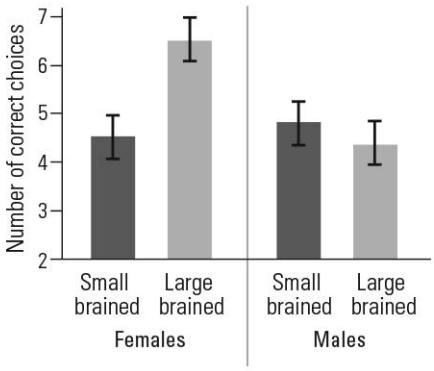
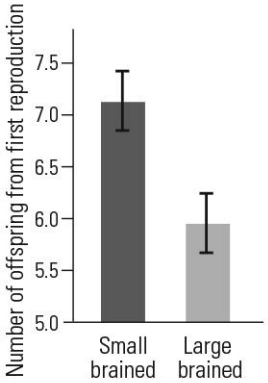


Figure 3 / Female fecundity



a. The scientists concluded that there is a correlation between brain size and cognitive ability for female guppies but that the correlation does not hold true for male guppies. Describe evidence from Figure 2 that supports the scientists' conclusion.

The mean number of correct choices is significantly greater for large-brained females than for small-brained females. The small-brained males, however, had a greater average number of correct choices than the large-brained males. The non-overlapping error bars for the two female categories indicate that the difference in the means is significant. Because the error bars overlap for the male means, the difference in cognitive ability is not significant for small and large brained male guppies.

b. The scientists also compared the mean number of offspring produced by each category of female at first reproduction. The data are shown in Figure 3. The scientists used artificial selection in the laboratory to increase brain size in females. Do you predict natural selection will favor large-brained females? Provide evidence to support your prediction.

Natural selection will not favor large-brained females. These females produced fewer offspring, indicating a lower reproductive fitness than small-brained females. Natural selection favors individuals with the greatest reproductive fitness.

3. Quantitative traits in Wisconsin Fast Plants include plant height, number of seeds produced per seedpod, and time to flower.

a. For humans, which is a quantitative trait, blood type or blood cholesterol level? Explain your reasoning for your answer.

Blood cholesterol level is a quantitative trait in humans. If the cholesterol level of 1000 people is measured, the data would likely have a normal distribution. Blood type is a discrete trait: it is Type A, Type AB, Type B, or Type 0. Data for blood type will not show a normal distribution.

[17] Kotrschal et al., Artificial Selection on Relative Brain Size in the Guppy Reveals Costs and Benefits of Evolving a Larger Brain, Current Biology (2013), http://dx.doi.org/10.1016/j.cub.2012.11.058.

b. Many quantitative traits are polygenic. Explain the concept of a polygenic trait.

A polygenic trait is one that is determined by two or more genes, for example, skin color. The trait itself would be reflected in the population as a normal distribution. Thus, a greater number of individuals would express the trait near the average value, rather than at the extremes. This means that most individuals would have medium skin color, as opposed to light or dark color.

4. Since the advent of agriculture in ancient civilizations, humans have modified crops and domesticated animals through selective breeding, or more recently through biotechnology (genetic modification).

a. Compare and contrast artificial selection and recombinant DNA technology.

Similarities – both have as their goal the expression of a particular trait of interest. The desirable traits are heritable, thus determined by genes. Both entail human intervention.

Differences – in artificial selection, no prior knowledge of the gene is required; rather, selection relies simply on the phenotype. In contrast, recombinant DNA technology requires extensive knowledge of the gene of interest. That gene can be further propagated by means of an expression vector, such as a plasmid used in bacterial transformation.

b. Considering the advantages and disadvantages of each technique, identify two instances in which artificial selection would be advantageous and two instances in which genetic modification technology would be advantageous for producing certain desired traits in organisms.

Artificial selection is useful in situations where a number of genes are likely involved in the traits that are considered desirable or in situations where similar species can interbreed:

- Creating new fruit or vegetable varieties (pluots for example)
- Enhancing an existing trait of an organism, such as trichome density to make the "hairy" variety of Fast Plants

Recombinant DNA technology would be useful for traits that can be easily influenced by a single gene, or to introduce a trait from an organism that is a distant ancestor of the modified organism, preventing interbreeding of varieties.

- Creating genetically modified bacteria that produce pharmaceuticals, such as human insulin or growth hormone
- Creating goats that produce and secrete a spider silk protein in their milk

5. Natural selection works much like artificial selection.

a. Explain how Darwin's observations of artificial selection influenced his proposed theory of natural selection.

Darwin was familiar with bird fanciers and dog breeders who specialized in selecting desirable traits in these animals. A breeder who wanted to accentuate a particular trait—feathers, crown, height, coloration—would choose individuals bearing those desirable traits from among a large population and selectively breed them. Darwin proposed that nature will also select for desirable traits from the variation in a population, giving populations the ability to adapt as desirable traits become more common in the population.

b. Identify 3 abiotic factors and 3 biotic factors that influence natural selection. State a hypothesis for how EACH abiotic or biotic factor would have an effect on natural selection.

3 abiotic factors: temperature, precipitation, sunlight

Hypothesis: If temperature influences the course of natural selection, then organisms adapted to colder climates should share characteristics that benefit life in the cold.

Hypothesis: If precipitation influences the course of natural selection, then organisms adapted to areas of low annual precipitation should share characteristics that benefit life with infrequent precipitation.

Hypothesis: If sunlight influences the course of natural selection, then organisms adapted to a specific number of hours of sunlight should share adaptations that benefit life with such sunlight.

3 biotic factors: food sources, predators, disease

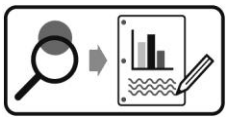
Hypothesis: If food sources influence the course of natural selection, then organisms adapted to a specific food source should share adaptations that are useful for feeding on that food source.

Hypothesis: If predators influence the course of natural selection, then organisms adapted to survive against a predator should share adaptations that support that predator–prey relationship.

Hypothesis: If disease influences the course of natural selection, then organisms adapted to evade a disease should share adaptations that are useful for survival in the presence of that foreign pathogen.

Design and Conduct an Experiment Key

Once you are experienced in the process of growing, pollinating, and harvesting seeds from Fast Plants, you may want to continue the selection experiment over multiple generations. Alternatively, you may want to test whether environmental conditions, such as acid rain, affect the results of the selection experiment. Yet another option is to explore if there is a relationship between two plant traits, such seedpod size and number of seeds.



Develop and conduct your experiment using the following guide.

1. Create a driving question for an extension activity or additional investigation you will carry out using Fast Plants.

   What is the impact of acidic conditions on plant height over two generations?

2. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   Acid rain is a known consequence of industrialization. It is important to understand the impact of the addition of air pollutants on agriculture. Furthermore, photosynthetic organisms like plants are at the base of the worldwide food chain. Height correlates with biomass production. If acid conditions negatively impact biomass, this phenomenon should be better understood.

3. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable will be the conditions for growth, specifically, comparing plant growth in water versus plant growth in acid. A second independent variable will be the successive generations of plants.

4. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable that will be measured is plant height. Height will be measured on day 15 of the plant life cycle.

5. Write a testable hypothesis (If…then…).

   If acid conditions negatively impact plant growth, then a reduction in the average height of plants will be observed. The difference will be statistically significant compared to normal conditions of growth in water.

6. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   Constant room temperature, constant humidity level, continuous and equally distributed light, continuous and equally distributed hydration, equivalent soil quantity, and equivalent spacing for growth.

7. How many trials will be run for each experimental group? Justify your choice.

   Two generations of Fast Plants will be grown with three replicates for each condition. Three replicates will provide a sufficiently large sample size but is a small enough number of systems to allow for all bottles to be placed under the same light source.

8. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   Average height will be compared. The Student's t-test will be applied as a measure of statistical significance. A *p*-value of less than 0.05 will be considered significant.

9. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

   Sources of error might include incorrect preparation of the acid solutions, making them too acidic or not acidic enough. The plants may not get equal exposure to light. If water reservoirs are allowed to dry out periodically over prolonged period, the height measurements could be affected due to poor growth or a change in the concentration of the solutions as water evaporates.

10. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

    Set up 9 growing systems: 3 controls, containing water in the reservoir, 3 systems that contain diluted nitric acid in the reservoir, and 3 systems that contain diluted sulfuric acid in the reservoir. Plant six standard-stock seeds in each growing system.

    - Germinate the seeds and grow the plants under continuous light and measure the height of all plants on Day 15.
    - When flowers begin to develop, determine which plants will be cross-pollinated. The tallest five plants from each group (control and experimental groups) will be used as parents for the second generation.
    - When several flowers are present, cross-pollinate 5 flowers within each group (not across groups). After seedpods develop, harvest the seeds.
    - Plant the seeds and monitor the growth of the second generation. Measure the height of all plants on Day 15 of the life cycle of this generation.

11. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

Comparison	Probability (*p*) Obtained from an Unpaired t-Test (* indicates a significant difference)	
	First Generation	Second Generation
Group A to B	0.5361	0.0027*
Group A to C	0.6319	0.1467
Group B to C	0.7808	0.1906

The results shown above are from an inquiry testing the effect of acid rain on plant growth over two generations. The second generation was grown from seeds from the tallest plants in the first generation.

19. BLAST BIOINFORMATICS

Lab Overview

In this activity, students employ the same tools used by research scientists all over the world: online databases that allow comparison of DNA and protein sequences across species. Students compare 100-nucleotide sequences of the Hemoglobin B gene (*HBB*) to determine the relatedness of five different mammals. *HBB* codes for the beta chain of the hemoglobin protein; this chain is also referred to as *beta globin*.

In just the first decade of the 21st century there has been an exponential increase in the number of species that have had their gene and protein sequences identified, and this information is readily available and searchable online. One such database is managed by the National Library of Medicine (NLM), part of the National Center of Biotechnology Information (NCBI). Using search tools such as BLAST® (Basic Local Alignment Search Tool), researchers can identify, from an unknown segment of DNA or a polypeptide, the precise genus and species from which it originated.

Importantly, BLAST can locate regions of genomes or proteomes similar to a given segment of interest, thus providing information about the "alignment" of species, that is, their evolutionary relatedness. Other databases can be accessed from NCBI to determine what is known about the structure of the protein, and embedded links provide easy access to the related scientific literature.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	10 min
Initial Investigation	60 min

Student-Designed Experiment	
Experiment Design	15 min
Experiment	60 min
Data Analysis	15 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	1.A.4, 1.A.3, 1.B.2, 1.C.3
Science Practices	1.1–1.3, 3.1, 3.3, 4.1–4.3, 5.1, 5.3, 6.1, 6.2, 7.2
Learning Objectives	1.9, 1.11–1.13, 1.15, 1.17–1.19

Materials and Equipment

For Each Student Station

- Computer with Internet access
- DNA Sequences Worksheet
- ABI BLAST Sequences.docx
- Highlighter
- Scissors (optional)
- Ruler or large index cards

NOTE: No additional equipment is needed for the student-designed experiment.

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- DNA structure and function, including a basic understanding of exons and introns.
- Protein structure and function, including familiarity with hemoglobin.
- Evolutionary relationships are inferred by comparing physical features as well as molecular sequences between species.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Never eat or drink around computer equipment.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Make a class set of copies of the DNA Sequences Worksheet (at the end of the student and teacher versions of this lab) and gather the needed materials for manually comparing sequences.

2. Make the digital copy of the BLAST Sequences Worksheet ("ABI BLAST Sequences.docx") easily accessible to students. Saving the file to Dropbox, Google Drive™, or a school website are all good options.

3. If your students will not be able to use computers in the classroom, reserve the school computer lab for two or three class periods.

Teacher Tips

Tip 1 – Manually comparing sequences

- In the first part of the initial investigation, students manually determine the number of differences in the DNA sequences of six species. This activity does not require computer access and can be assigned as homework to reduce the class time needed for this lab.
- All species will be compared to Species A. To make comparisons easier, students can fold the paper or cut sequence A into a strip that can be placed above or below each of the other sequences for side-by-side comparison.

Tip 2 – Using the NCBI website and tools

- If you, or students, create user accounts for the NCBI website, your searches can be saved for future reference. This could be helpful for class discussions, allowing you to quickly bring up a search result to reference.
- NCBI has produced numerous brief tutorials that can be viewed on their YouTube channel: https://www.youtube.com/user/NCBINLM/. Another tutorial from Johns Hopkins University is available at: https://www.youtube.com/watch?v=HXEpBnUbAMo.
- A complete guide to the NCBI website can be downloaded at: http://www.ncbi.nlm.nih.gov/books/NBK21101/.
- To find genes or proteins and species to use in the student-designed investigation, you can search the NCBI databases with general terms such as "beta globin mammals," or "alligator," rather than specific species names. Students can then find a representative group of species, appropriate to their question, that all have entries in the database for a particular gene or protein.

Initial Investigation

The Initial Investigation is designed to familiarize students with the NCBI and BLAST websites so they gain the necessary skills to design their own investigation.

From the student handout:

Manual comparison of mammalian DNA sequences of the HBB gene

1. Obtain a copy of the DNA Sequences Worksheet. This worksheet contains nucleotide sequences for the beta globin gene (*HBB*) of five mammalian species. Compare Species A, the chimpanzee (*Pan troglodytes*), to four other species, as follows.

   To complete the comparison of Species A to the other species, first copy the following data table into your lab notebook.

Table 1: Manual and computer database gene and protein comparison of chimpanzees to other mammals

Species	Common Name	Scientific Name	Number of Nucleotide Differences	BLAST Ident[4] (%): *HBB* Gene Comparison[1]	BLAST Ident[4] (%): Beta Globin Protein Comparison[2]
A	Chimpanzee	*Pan troglodytes*			
B	Pig-tailed macaque	*Macaca nemestrina*	2	96	not available[3]
C	Dog	*Canis familiaris*	14	81	90
D	Cattle	*Bos taurus*	12	82	85
E	Norway rat	*Rattus norvegicus*	23	77	82

[1]Gene accession number: FJ788228.1

[2]Protein accession number: P68873.2

[3]The full beta globin sequence has not been published for this species.

[4]"Ident" refers to the percentage of similarity of aspects of the sequences (nucleotide or protein).

2. Determine the number of nucleotide differences between Species A and Species B.

   a. Use a ruler or index card to move along the sequences of the two species one letter (one nucleotide) at a time, or one codon at a time.

   b. If Species B has a nucleotide that differs from A, highlight that letter in the sequence of Species B.

   c. Continue to compare sequences for each row of nucleotides, until you reach the final "A" (adenine), the 100th nucleotide). Then count and record the total number of differences present in the DNA sequences.

      NOTE: A dash instead of a letter in a species' sequence indicates an unknown base and should ***not*** *be counted as a difference.*

3. Repeat the steps above to compare Species A to Species C. Then continue with the remaining comparisons: A to D, and A to E. You may fold the paper or cut out sequence A to make the comparisons easier.

4. Confirm the number of differences you found with your classmates and reconcile any variations in the counts. Adjust the numbers recorded in the data table if necessary.

5. The four "unknown" species (B–E) on the worksheet are, in no particular order: cow, Norway rat, pig-tailed macaque, and dog. Which species do you predict has the least number of differences in the *HBB* gene compared to chimpanzees? Which species do you predict has the greatest number of differences? Provide an explanation for each of your predictions.

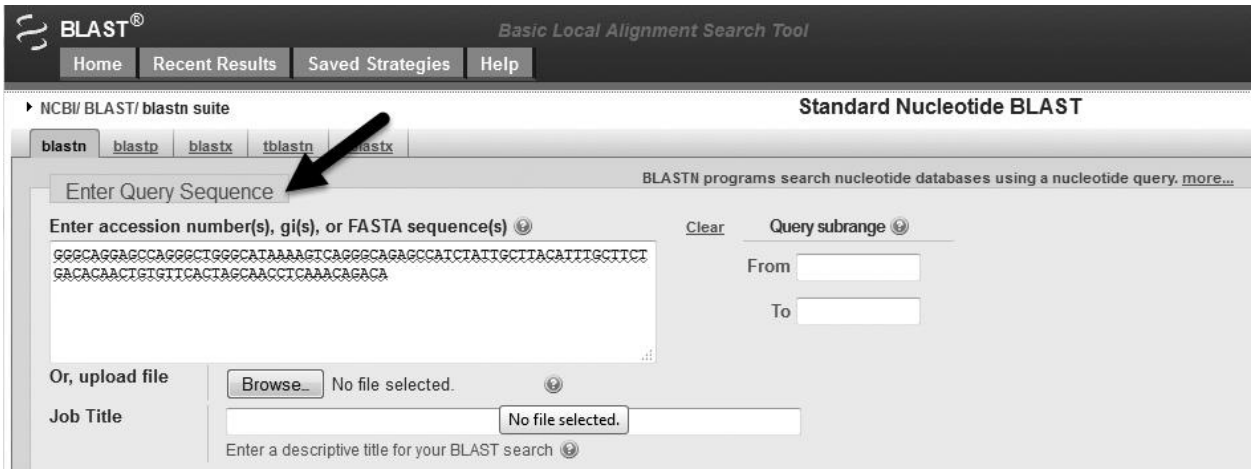
   Student answers will vary. Students are likely to predict that the macaque, being a primate, will have the least number of differences. It is expected that students may struggle to decide which of the other species is more closely related to the chimpanzee. They may predict that cattle have the greatest number of differences because it is an herbivore with a unique four-chambered stomach.

BLAST comparison of mammalian DNA sequences of the HBB gene

6. Go to the BLAST website: http://blast.ncbi.nlm.nih.gov/Blast.cgi. Select "nucleotide blast" from the "Basic BLAST" menu in the middle of the page.

IDENTIFYING SPECIES A

7. Open the digital copy of the BLAST Sequences Worksheet (ABI BLAST Sequences.docx). Copy the *HBB* sequence for Species A and paste the sequence into the query box of the nucleotide BLAST page.



8. Scroll down to "Program Selection" and select the option to optimize for "Somewhat similar sequences." Then click the "BLAST" button.

   NOTE: The BLAST program searches through thousands of sequences contained in a database for the best species match for the partial HBB *sequence you entered into the query.*

9. In the BLAST report generated from the search, scroll past the graphic summary to the "Descriptions" table. Note that *Homo sapiens*, *Gorilla gorilla*, and *Pan troglodytes* all have alignments with a "100%" Ident value, meaning that these species have no nucleotide differences in their *HBB* genes. From an evolutionary perspective, provide an explanation for this fact.

   These three species share a recent common ancestor and mutations have not occurred in the *HBB* gene sequences between the time of evolutionary divergence to the present.

10. Click on the accession number for the first *Pan troglodytes* link: FJ788228.1. This takes you to a page with a wealth of information regarding the gene of interest, such as the number of base pairs and the scientific article where the gene sequence was originally published.

11. Scroll down to "FEATURES."

    - Click on "gene" and observe the section highlighted in the nucleotide sequence at the bottom of the page (under "ORIGIN").
    - Click on "mRNA" and observe the change in the nucleotide selection that is highlighted.
    - Click on the first "exon" link. Then click on the second "exon" link.

12. a. Why would the gene have a different number of nitrogen bases than mRNA?

    A gene contains both exons and introns. Introns are spliced out during mRNA processing, leaving only exons in the final mRNA product, so the mRNA will have fewer nucleotides compared to the gene used for transcription.

    b. What do you observe when you compare the highlighted regions for the exons to the highlighted region for mRNA? What is the relationship between exons and mRNA?

    Exons are regions of a gene that code for the amino acids to be included in the polypeptide. Exons are present in the final mRNA product that is translated during protein synthesis. In the *HBB* sequence, when exon 1 and exon 2 are looked at together, they are the same as the mRNA highlighted region—the mRNA is comprised of the two exons (the sequence between them was the intron that is spliced out; its sequence is not present in the final mRNA).

IDENTIFYING SPECIES B THROUGH E AND COMPARISON WITH SPECIES A

13. The browser page for the nucleotide sequence for *Pan troglodytes* opened on either a new tab or a new window within the Internet browser. Return to the NCBI BLAST search report and click the "Edit and Resubmit" link at the top left of the page.

14. Copy and paste the nucleotide sequence for Species B from the BLAST Sequences Worksheet into the query box of the nucleotide BLAST page. Click "BLAST" to initiate a new search.

15. Scroll to the Descriptions table and click on the first accession number. Find the "SOURCE" line on the gene information page that opens after clicking the accession number. In Table 1 in your lab notebook, record the common name and scientific name of Species B.

16. On the gene information page for Species B, under "Analyze this sequence" (on the right side of the page), choose "Run BLAST." The query box will appear with the accession number of Species B. Now you can compare the entire *HBB* gene of Species A and Species B.

    Click the check box "Align two or more sequences" and type the accession number for the chimpanzee gene, FJ788228.1, into the "Enter Subject Sequence" area that appears. Click "BLAST" to generate the report.

17. Find the Ident value which indicates the amount of similarity in the two sequences being aligned. Record this value in Table 1.

18. To begin the comparison with the next species, click "Edit and Resubmit" and uncheck the "Align two or more sequences box." Copy and paste the nucleotide sequence of Species C from the BLAST Sequences Worksheet into the query box and initiate the BLAST search to find the identify of Species C.

19. Click on the first accession link and record the common name and scientific name of Species C. Then choose "Run BLAST" to align the sequences of Species A and C, as you did for the Species A and B comparison. Record the Ident value.

20. Repeat the process to identify Species D and E, and perform the alignments of each of these species with Species A.

    NOTE: For Species D, click on the 2nd accession number for the "adult beta globin gene."

21. In the comparison you did manually, you compared sequences 100 nucleotides in length; these sequences were just part of the *HBB* gene. The BLAST program compared over 1000 nucleotides of the *HBB* genes in these species. Do the results of your manual comparison agree with the results of the computer-generated alignment? Explain your answer.

    The results of the manual comparison and computer alignment are in agreement. The macaque, Species B, had the least number of differences and the greatest percent similarity and Species E, the Norway rat, had the greatest number of differences and the least percent similarity.

22. What are the advantages of using a computer program over manual comparison of DNA sequences?

    The computer program is more accurate and much faster than manual comparison. When students compare their manual comparison differences with their classmates, it is likely that one or more students will have made an error and will have different results than other students. The manual comparison takes at least 15 minutes for only a portion of a gene for just five species. The computer program can compare a given sequence to thousands of others in the database in less than one minute.

23. Now that you know the identities of Species B–E:

    a. Which of the four species is most closely related to the chimpanzee? Was your prediction correct? Do the results make sense based on other factors, such as morphology? Explain your answer.

       Student answers will vary. Sample response: My prediction was correct. The pig-tailed macaque is most closely related to the chimpanzee, having a gene sequence that is 96% similar to that of the chimpanzee. This makes sense, given that these species are both primates and have many similar features: shortened snouts (flat face), several types of teeth, grasping feet, mobile thumbs, ability to stand and walk on two legs, and stereoscopic vision.

    b. Which of the four species is least closely related to the chimpanzee? Was your prediction correct? Do the results make sense based on other factors? Explain your answer.

       Student answers will vary. Sample response: My prediction was incorrect; I thought that cattle would be the least similar to the chimpanzee. However, the results indicate that the Norway rat is the most distant relative of the chimpanzee. It is somewhat surprising that other mammals, such as cattle and dogs, are more closely related to the chimpanzee. Given their use in medical research, I expected that the rat would have fewer nucleotide differences than some other mammals.

24. For the protein comparison Ident values provided in Table 1, BLAST was used to compare the amino acid sequence of chimpanzee beta globin to the amino acid sequences of beta globin proteins in the other species. Which have a greater similarity between two species: gene sequences or protein amino acid sequences? Explain why the percent similarity is not the same for genes and proteins.

    The protein sequences had greater similarity than the gene sequences. The dog and chimpanzee only had 81% similarity in their *HBB* genes, but there was 90% similarity in the amino acid sequences of the proteins. A mutation changes one or more nucleotides in the gene, but it does not always change the amino acids coded for. For example: CGU, CGC, CGA, and CGG all code for arginine. Some mutations are "silent" and the difference is only noticed when comparing DNA sequences rather than protein sequences.

COMPARING PROTEINS AND VISUALIZING EVOLUTIONARY RELATIONSHIPS

25. Use the NCBI website to find the beta globin sequences for two additional species: Atlantic salmon and minke whale, and then compare them to the sequence for the chimpanzee. For each of the additional species:

    a. Go to the NCBI homepage: http://www.ncbi.nlm.nih.gov/. In the search dropdown menu at the top, change "All Databases" to "Protein."

    b. In the search field, type "beta globin" and the species name and choose "Search."

    c. On the search results page, click on the FASTA link to see the amino acid sequence. Copy and paste the sequence into the space provided on page 2 of the digital copy of the BLAST Sequences Worksheet, making sure the letters are adjacent to each other, that is, they should not be separated by spaces or "returns" and are in a single paragraph.

    >Atlantic salmon

    MVDWTDAERSAIVGLWGKISVDEIGPQALARLLIVSPWTQRHFSTFGNLSTPAAIMGNPAVAKHGKTVMHGLDRAVQNL
    DDIKNAYTALSVMHSEKLHVDPDNFRLLADCITVCVAAKLGPTVFSADIQEAFQKFLAVVVSALGRQYH

    >minke whale

    VHLTAEEKSAVTALWAKVNVEEVGGEALGRLLVVYPWTQRFFEAFGDLSTADAVMKNPKVKAHGKKVLASFSDGLKHL
    DDLKGTFATLSELHCDKLHVDPENFRLLGNVLVIVLARHFGKEFTPELQAAYQKVVAGVANALAHKYH

    d. Find the "Run BLAST" option under "Analyze this sequence." Click on the "Align two or more sequences" box, as before, and enter this protein identification number for the chimpanzee into the Subject Sequence box: P68873.2. Click "BLAST." Record the Ident value for the comparison.

    The Ident value for the chimpanzee–salmon beta globin comparison is 50%. For the chimpanzee–minke whale comparison it is 90%.

26. Which of the two species is least similar to chimpanzee, based on the beta globin comparison? Is this surprising? Explain your answer.

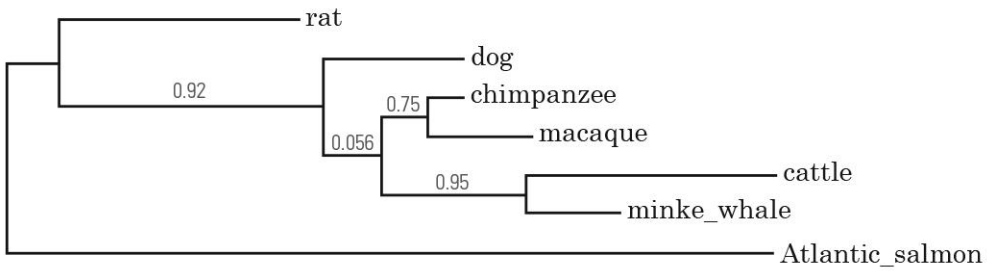
    Atlantic salmon is least similar. This is not surprising given that the minke whale is a mammal like the chimpanzee, while salmon are fish, more distantly related to mammals.

27. Ident values are useful for comparing species and inferring evolutionary relationships. However, *phylogenetic trees* or *cladograms* provide a more complete picture. Programs used to create these diagrams compare all selected species to one another, not just one species to others. To create a phylogenetic tree, go to www.phylogeny.fr.[18] Choose the "One Click" phylogenetic analysis option.

[18] Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M., Gascuel O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*. **2008** Jul 1; *36* (Web Server Issue):W465-9. Epub 2008 Apr 19. PubMed: http://www.ncbi.nlm.nih.gov/pubmed?cmd=Retrieve&list_uids=18424797 (accessed May 28, 2014).

28. Copy and paste the entire text of beta globin sequences from the worksheet into the space provided on the phylogeny website. Then click "Submit." Save your results—the phylogenetic tree—as a PNG or PDF (select the "Download the tree" option just below the phylogenetic tree) and print a record for your lab notebook.

    NOTE: Be sure to include the " >[name]" *in addition to the letters symbolizing the amino acids when you copy and paste. Also, the sequence for each species must be separated by a blank line.*



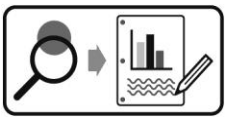
29. What can you conclude regarding the evolutionary relationships between the Atlantic salmon, minke whale, and Species A–E?

    The salmon does not share a recent common ancestor with the other species. It is distantly related. The minke whale is most closely related to cattle and shares a more recent ancestor with the chimpanzee than it does with the rat or dog.

Design and Conduct an Experiment

Now that students are familiar with the tools available for comparing gene and protein sequences, they can investigate a question of their own related to the evolutionary relationships among species. Students can choose one or more of the following proteins to use for the investigation (or you can allow students to choose proteins not listed). Students can compare gene sequences, amino acid sequences, or both.

- ATP synthase
- Catalase
- Myosin
- Cytochrome b
- Cytochrome c
- GAPDH
- Rubisco (RuBP)
- Peroxidase
- Ubiquitin



Students can design their experiment using the Design and Conduct an Experiment Worksheet.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Are the great apes more closely related to New World monkeys or Old World monkeys?*
- Are marine mammals more closely related to one another than to a terrestrial mammal?*
- How similar are proteins of the mitochondria in plants, animals, and fungi?
- Are crocodiles and alligators close relatives?
- Are penguin species of different continents more closely related to one another than they are to other types of birds that live on the same continent?*

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Are the great apes more closely related to New World monkeys or Old World monkeys?" Results of this research are shown in the Sample Data section.

1. Describe the evolutionary relationships between the species you investigated. Does the data support your hypothesis? Justify your claim with evidence.

   The hypothesis from the Design and Conduct an Experiment Key is not supported. If Old World monkeys and New World monkeys diverged from the ape lineage at the same time, then they would have the same percent similarity when compared to apes. However, for the cytochrome b gene, the New World monkeys showed less similarity (75–77%) with the apes compared to the Old World monkeys (78–79%).

   The phylogenetic tree built from a comparison of catalase protein sequences supports the conclusion that Old World monkeys shared a more recent common ancestor with apes and that New World monkeys had diverged earlier from the ape lineage.

2. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. Differences in the nucleotide sequences of a gene in different species are the result of mutations that occur over time.

   a. Identify and describe three different types of mutations.

   Substitution, deletion, and insertion are three types of mutations. In a substitution mutation there was incorrect base pairing during DNA replication, resulting in one nitrogen base being changed in the gene sequence. For example, where there was a G in the original sequence, there may be a T present in the mutated sequence.

   In a deletion mutation, there is the loss of a base from the sequence and in an insertion mutation, an extra base is added to the sequence.

   b. Describe the possible consequence(s) of each type of mutation.

   Some mutations are "silent" and do not have an effect. For example, a substitution mutation that changes the DNA sequence from CAG to CAC will result in the same protein, since the resulting mRNA codons GUC and GUG both code for the amino acid valine. Also, even if the change to DNA results in a change to one amino acid, this may not affect a polypeptide made of hundreds of amino acids, especially if the original and substituted amino acids have similar chemical properties. However, many substitution mutations do affect a protein's structure and function, such as the mutation that changes the structure of beta globin and causes sickle cell disease.

   Deletion and insertion mutations are called "frame shift" mutations because they cause a change to all of the codons "downstream" of the mutation. The 3-base codons are affected by the removal or addition of a nitrogen base in the sequence and the affected protein contains many incorrect amino acids. It cannot fold correctly and its function is affected.

   c. Although genes from *Homo sapiens* (humans) and *Drosophila melanogaster* (fruit fly) differ significantly, there are "conserved" regions, that is, nucleotide sequences within genes that have not changed much over time. Why would conserved regions be of particular interest to scientists?

   Natural selection selects against genetic changes that are detrimental to an organism's ability to survive. If a mutation negatively affects a protein's function, the mutation will be selected against so is less likely to persist through many generations and through evolutionary lineages.

   The most critical amino acids in a protein, such as those in the active site of an enzyme, are the ones most likely to be the same across taxa. By determining the DNA sequences most similar between species, scientists can identify which amino acids are most important within a protein. They can then analyze the placement of these amino acids within the protein's structure to better understand how those amino acids facilitate the protein's task.

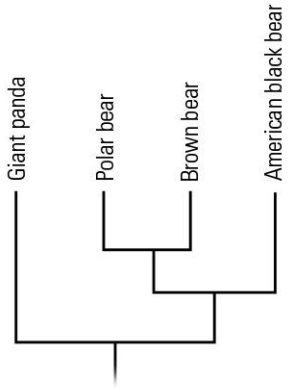
2. A student is interested in the evolutionary history of kingdom Fungi. For her investigation, she plans to use the NCBI protein database and BLAST to compare a number of fungal species to a variety of species from kingdom Plantae and kingdom Animalia. Of the following proteins, which one would you recommend the student use for her investigation: catalase, rubisco (RuBP), or hemoglobin? Provide an explanation for your choice.

   The student should compare catalase sequences. Rubisco is an important enzyme of the Calvin cycle which is part of photosynthesis. This protein is not present in yeast or animals, so it is not a good choice. Hemoglobin is found in animals, but not in yeast or plants, so it is also a poor choice. Catalase is universal and present in all three taxa, so it is the best choice for determining the relationship between fungi and other taxa.

3. The table below lists the first twenty four amino acids of the ATP synthase protein in each bear species. Analyze the data and complete the cladogram. Provide an explanation for your placement of each species on the cladogram.

Table 2: Comparing the ATP synthase proteins of bears

Common Name	Scientific Name	Amino Acid Sequence
Brown bear	*Ursus arctos*	MNENLFTSFITPTMVGIPIVLLII
American black bear	*Ursus americanus*	MNE**S**LFTSFITPTM**M**GIPIV**V**LII
Giant panda	*Ailuropoda melanoleuca*	MNENLF**A**SF**T**TP**M**M**M**G**V**PIV**V**LII
Polar bear	*Ursus maritimus*	MNENLFTSFITPTMVGIPIV**P**LII

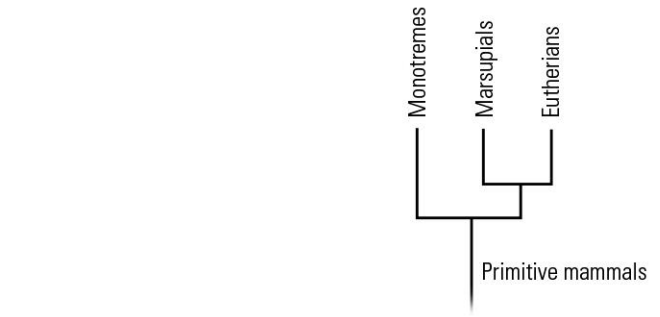


The polar bear is the closest relative to the brown bear, since the amino acid sequence of these species only differed by one amino acid. This indicates that these species diverged recently from a common ancestor and should be placed closest together on the cladogram. The giant panda, however, shares a distant ancestor with the brown bear. The giant panda and brown bear sequences differed by 6 amino acids, the greatest number of differences for the species compared. So the giant panda is placed furthest from the brown bear. The American black bear had 3 amino acid differences, indicating it is more closely related to the brown bear than the giant panda is, but it is not as closely related as the polar bear is to the brown bear.

4. Table 3 shows the results from a BLAST comparison of the NADH dehydrogenase protein of the gray wolf with the common mouse and Tasmanian wolf (pictured below). The cladogram provides information regarding the evolution of three mammalian clades.

Table 3: Results from a BLAST comparison

Species	BLAST Ident (%): Similarity to the Gray wolf	Classification
Gray wolf		Eutherian
Mouse	55	Eutherian
Tasmanian wolf	47	Marsupial



a. Provide an evolutionary explanation for the level of similarity between the gray wolf and the mouse.

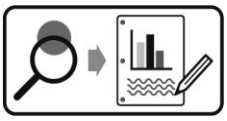
While the gray wolf and mouse look quite different, the 55% similarity in their NADH dehydrogenase genes indicates that they are more closely related to each other than the gray wolf is to the Tasmanian wolf. The gray wolf and mouse both evolved from a placental mammal ancestor. Since the Tasmanian wolf is a marsupial, it diverged from the placental mammal lineage prior to the divergence of the mouse and grey wolf lineages. Since the grey wolf and mouse diverged more recently than the gray wolf and Tasmanian wolf, there has been less time for genetic mutations to accumulate.

b. Provide an evolutionary explanation for the level of similarity between the gray wolf and the Tasmanian wolf.

The physical similarities between the gray wolf and Tasmanian wolf are the result of convergent evolution. Their similarities are due to natural selection. The two species have similar niches, similar "ways of life" in their geographic locations and over time they developed similar adaptations that made each successful in its niche.

Design and Conduct an Experiment Key

Identify a set of species you are interested in investigating with regard to their evolutionary history.



Develop and conduct your experiment using the following guide.

1. List the species you are interested in comparing.

   Great apes: gorilla, chimpanzee, and orangutan

   Old World monkeys: macaque, baboon

   New World monkeys: squirrel monkey, marmoset

2. Create a driving question: develop a testable question for your experiment.

   Are the great apes more closely related to New World monkeys or Old World monkeys?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   People typically look at all primates as "monkeys." I know that monkeys and apes are different, but I'm interested in the relationship between apes and the two categories of monkeys. It will be interesting to know which are closer relatives to the apes, including *Homo sapiens*.

4. What gene or protein sequence(s) do you plan to use for the investigation? Describe the function of the gene or protein and indicate why you chose it.

   I am going to use sequences of the catalase protein for building a phylogenetic tree with these species. Catalase breaks down hydrogen peroxide into water and oxygen, and is an enzyme present in many species across many taxa. I think I will be able to find enough catalase sequences for the primates to be able to make a good comparison.

   The gene for cytochrome b is often used in evolution studies to determine relatedness between species. So to compare the primates, I will compare cytochrome b nucleotide sequences to see if the data is consistent with what is discovered by analyzing catalase protein sequences. Cytochrome b is one of the proteins of the electron transport chain of the mitochondrion.

5. What data will be collected, and how will it be collected, to build a phylogenetic tree for the species you're investigating?

   Catalase comparison: the NCBI protein database will be searched to find the amino acid sequences of catalase in the different primate species. The sequences will be copied and pasted into www.phylogeny.fr to build a phylogenetic tree.

   Cytochrome b comparison: the NCBI gene database will be searched to find the nucleotide sequences for the cytochrome b genes of the different species. The accession numbers for these genes will be recorded and BLAST will be used to run alignments between the genes of the New World monkeys and apes and between Old World monkeys and apes.

6. Write a testable hypothesis (If…then…).

   If Old World monkeys and New World monkeys diverged from the ape lineage about the same time, then the protein and gene sequences of these groups will show the same level of similarity when each group is compared to apes.

7. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

   For comparison of catalase proteins:

   a. Set the NCBI search field to "protein" and search "catalase chimpanzee." Use the FASTA link to acquire the amino acid sequence of the protein. Copy and paste this sequence into a document.
   b. Repeat the catalase search for: gorilla, orangutan, macaque, baboon, squirrel monkey, and marmoset.
   c. Copy all sequences into the "One Click" phylogeny analysis at www.phylogeny.fr. "Submit" to generate the phylogenetic tree.

   For comparison of cytochrome b gene sequences:

   a. Set the NCBI search field to "gene" and search "cytochrome b chimpanzee." Find and record the accession number for this sequence.
   b. Repeat the cytochrome b search for: gorilla, orangutan, macaque, baboon, squirrel monkey, and marmoset.
   c. Go to the nucleotide BLAST search page and enter the accession number for the macaque into the query box. Check "Align two or more sequences" and enter the accession number for the chimpanzee gene into the subject box. Click "BLAST" and find and record the Ident value.
   d. Choose "Edit and Resubmit" and change the accession number in the subject box from the one for the chimpanzee to the one for the gorilla. Run the BLAST. Repeat this process to compare the macaque sequence to the orangutan sequence.
   e. Repeat the process to compare each monkey to each of the apes.

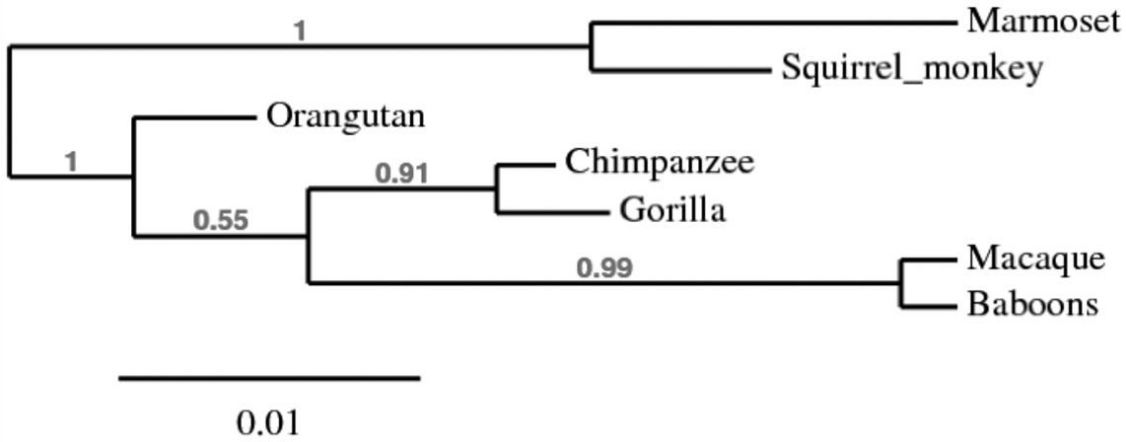
8. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

In a phylogenetic tree, the length of a horizontal segment relates to the amount of genetic change that has occurred in that lineage. The scale below the phylogenetic tree provides a reference for the amount of genetic change that occurred in the specific set of lineages being compared.

The numbers next to each node of a phylogenetic tree indicate a confidence value; the values fall between 0 and 1. A value of 1 indicates that there is very strong evidence that the species clustered to the right of the node are appropriately clustered, to the exclusion of other species included in the tree.[19]

The main point for students to note on a phylogenetic tree, however, is the overall appearance of the tree and the clustering of species that helps them determine close and more distant relationships.



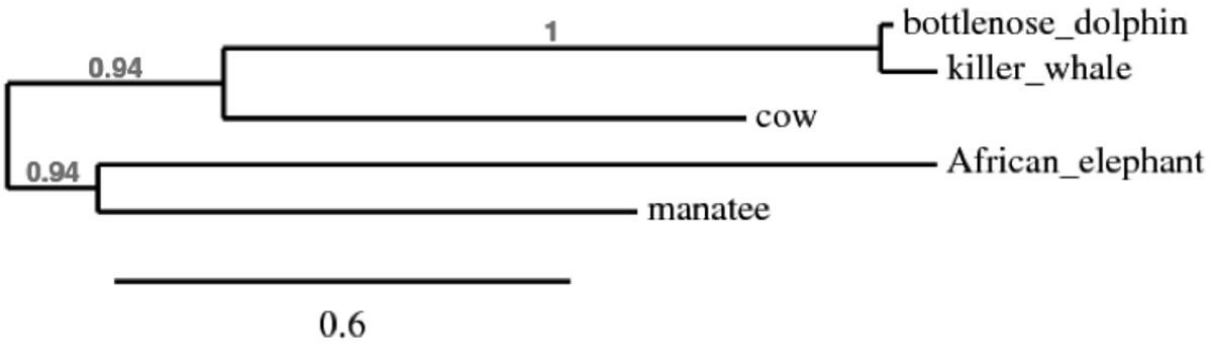
The results shown above are from an inquiry comparing the amino acid sequence of the catalase enzyme of Old World monkeys, New World monkeys, and apes. Sequences were acquired from NCBI. Comparison of the sequences and generation of the phylogenetic tree were done through computer automation at www.phylogeny.fr.

Comparison of cytochrome b nucleotide sequence

Monkeys Being Compared to Great Apes		BLAST Ident (%): Cytochrome b Gene Comparison		
		Great Apes		
		Chimpanzee	Gorilla	Orangutan
New World Monkeys	Marmoset	77	76	76
	Squirrel monkey	76	76	75
Old World Monkeys	Macaque	79	79	79
	Baboons	79	79	78

The results shown above are from an inquiry comparing gene sequences of the cytochrome b gene in Old World monkeys, New World monkeys, and apes. Sequences were acquired from NCBI. BLAST was used to determine the Ident values (% similarity) between the genes of these species.

[19] Rambaut, A. How to read a phylogenetic tree. http://epidemic.bio.ed.ac.uk/how_to_read_a_phylogeny (accessed June 3, 2014), Institute of Evolutionary Biology, University of Edinburg.



The results shown above are from an inquiry comparing the complete mitochondrial genomes in terrestrial mammals and marine mammals. Sequences were acquired from NCBI. Comparison of the sequences and generation of the phylogenetic tree were done through computer automation at www.phylogeny.fr.

Comparison of the complete mitochondrial genome

Marine Mammals Compared to Terrestrial Mammals		BLAST Ident (%): Mitochondrial Genome		
		Marine Mammals		
		Bottlenose Dolphin	Orca	Manatee
Terrestrial Mammals	Cow	82	82	81
	African elephant	78	78	79

Comparison of the complete mitochondrial genomes of marine mammals

Marine Species	BLAST Ident (%): Mitochondrial Genome		
	Bottlenose dolphin	Orca	Manatee
Bottlenose dolphin		93	80
Orca			79
Manatee			

The results shown above are from an inquiry comparing complete mitochondrial genomes in terrestrial mammals and marine mammals. Sequences were acquired from NCBI by initially searching for cytochrome c oxidase genes. BLAST was used to determine the Ident values (% similarity) between the mitochondrial genomes of these species.

Comparison of cytochrome b gene sequences

Penguins Compared to Other Bird Species		BLAST Ident (%): Cytochrome b Gene Comparison			
		Penguins			
		Emperor	Adelie	Little Blue	Rockhopper
Antarctic birds	Great-winged petrel	90	93	91	92
	Albatross	89	91	90	92
	Antarctic tern	91	92	92	92

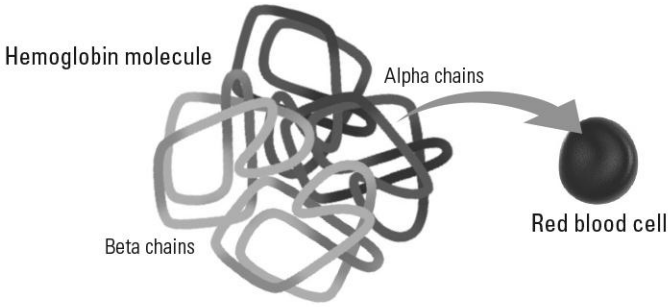
Comparison of cytochrome b gene sequences of penguin species

Penguin Species	Emperor	Adelie	Little blue	Rockhopper
Emperor		94	93	93
Adelie			96	96
Little blue				97
Rockhopper				

The results shown above are from an inquiry comparing cytochrome b sequences from penguins that live in various locations (Antarctica, New Zealand, and South America) to three species of Antarctic birds. Sequences were acquired from NCBI. BLAST was used to determine the Ident values (% similarity) between the mitochondrial genomes of these species.

DNA Sequences Worksheet

Beta Globin Gene Nucleotide Sequences



Species A	GGGCAGGAG GAGCCATCT TGTGTTCAC	CCAGGGCTG ATTGCTTAC TAGCAACCT	GGCATAAAA ATTTGCTTC CAAACAGAC	GTCAGGGCA TGACACAAC A
Species B	GGGCAGGAG GAGCCATCT TGTGTTCAC	CCAGGGCTG ATTGCTTAC GAGCAACCT	GGCATAAAA ACTTGCTTC CAAACAGAC	GTCAGGGCA TGACACAAC A
Species C	GGGCAAGAT GGGACAGCT CGTGTTCAC	C–AGGGCTG GCTGCTTAC TAGCAACCA	GGCATAAAA ATTTGCTTC CAAACAGAC	GGAAGAACA TGAAACAAC A
Species D	GGGCAGGAG GGGCCAGCT CGTGTTCAC	GCAGGGCTG GCTGCTTAC TAGCA––CA	GGCATAAAA ACTTGCTTC CAAACAGAC	GGAAGAGCT TGACACAAC A
Species E	––GCAGGAG GGATCAGTC TGTGTTGAC	CCAGG–CAG GCTCCTCAC TCACAAAC–	AGCATAAAA ATTTGCTTC –AAACAGAC	GGTGGGGCG TGACATAGT A

20. POPULATION GENETICS

Lab Overview

Students use class data for the PTC (phenylthiocarbamide) tasting trait to derive allele frequencies for a population. Based on their phenotype and predicted genotype, students use "allele cards" to simulate the next generation and determine if allele frequencies change over time in the absence of selection, non-random mating, mutations, and migration. Students may or may not observe genetic drift in the simulation due to the small population size of the class. Student-designed experiments focus on determining the effect of violating one of the Hardy–Weinberg conditions on allele frequencies in a gene pool.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	15 min
Initial Investigation	40 min

Student-Designed Experiment	
Experiment Design	20 min
Experiment	40 min
Data Analysis	20 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	1.A.1, 1.A.2, 1.A.3, 1.A.4
Science Practices	1.1, 2.1 2.2, 2.3, 3.1, 4.2, 4.3, 6.1, 7.2
Learning Objectives	1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.13

Materials and Equipment

For Each Student

- PTC (phenylthiocarbamide) paper
- Control paper *(optional)*
- Calculator with square root function
- Allele cards from the gene pool (2)[1]
- Class data page (1 per class)

[1]Refer to the Lab Preparation section for preparing allele cards from 3 × 5 index cards.

Additional equipment recommended for the student-designed experiment:

- Beads, (100 or as needed) 2 or more contrasting colors
- Large cups (2 or more)

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- Genetics vocabulary: alleles, heterozygous, homozygous, genotype, phenotype
- Evolution by natural selection: natural selection acts upon phenotypes, but only variation that is heritable can be acted upon by selection forces
- Evolution by genetic drift
- New alleles are introduced into a gene pool through mutations or migration
- Basic math skills: percentages and frequencies, solving for an unknown, taking a square root of a number or squaring a number

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- PTC, or phenylthiocarbamide, is safe for consumption at the concentrations provided on the PTC paper.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Purchase PTC paper

   The supply companies commonly used by science teachers offer PTC paper for purchase. Be sure to purchase a quantity sufficient for the number of students you have. Although not required, you may want to purchase control paper as well so students can confirm that they taste the chemical compound and not just paper.

   NOTE: PTC, or phenylthiocarbamide, is safe for student consumption at the concentrations provided on paper that is purchased from science education supply companies.

2. Prepare the allele (gamete) cards for the class population gene pool

   To reduce the number of index cards needed, cut 3 × 5 index cards in half. For a typical class size, cutting 40 cards in half will provide a sufficient number of allele cards for the gene pool. You'll need to prepare more cards than will actually be used since you won't know the allele frequencies in your class until data is collected in the Initial Investigation.

   Use a bold permanent marker to write “T” on 50 of the half-size cards and “t” on 20 of the half-size cards. Leave the remaining 10 cards blank so you can write a “T” or “t” on additional cards if needed, based on the class data.

   Determine a location in the room to designate the “gene pool.”

3. Class data page and offspring container

   Find an empty container for the index cards and place a label on the outside with the word “Offspring.”

   Create a t-chart on a blank piece of paper and label one side “Tasters” and the other side “Non-tasters (tt).” Then split the “Tasters” column into two columns, one labeled “TT” and the other “Tt.”

   Place both items at the front of the room so students can add their cards to the container and mark the result of their “mating.”

Teacher Tips

Tip 1 – Facilitating the Initial Investigation

The Initial Investigation is a teacher-directed activity. Be sure to review the directions of the investigation carefully so you are prepared to guide students through the steps and calculations.

Prior to the investigation, determine how you would like students to report their PTC phenotypes. Depending on class size, you may wish to just verbally poll the class. Alternatively, you can create a chart on the front board and ask students to come to the front and record a tally mark in the correct column. Another option is for a group leader to go to the front board to report the phenotypes for all members of that group.

Before directing students to acquire allele cards from the gene pool, you will need to assign some of the tasters to be homozygous in genotype (TT) and others to be heterozygous (Tt). For example, if you have 18 students who are tasters and five of these should be of the homozygous genotype, you can ask all tasters to raise their hands and then point to five students who have their hands raised and ask that they be homozygous.

You may need to help the class determine the total number of T and t alleles in the gene pool, which students are directed to do in the Initial Investigation. Each homozygous dominant individual has 2 T's, so the number of T alleles for this genotype is 2 × number of persons of that genotype. Each heterozygote has 1 T and 1 t, so the T alleles and t alleles for this genotype are equal to the number of individuals with the genotype. Lastly, the number of t alleles from the homozygous recessive individuals is 2 × the number of person of that genotype.

Tip 2 – Random mating

You can simply ask students to walk randomly around the room and wait for a signal from you, at which point they turn to a person near them, or you can have students arrange themselves in two concentric circles and have the inner circle rotate while music plays. (Think about musical chairs.) When the music stops, the inner circle students pair up with the person next to them in the outer circle.

Tip 3 – Different genders

Emphasize to students that in the “mating” procedure, they are representing an individual of a certain genotype, not of a certain gender. It does not matter if two females or two males pair up to produce an offspring.

Initial Investigation

The Initial Investigation is designed to familiarize students with deriving allele frequencies to determine if allele frequencies in a population change over time. With this skill, students can carry out their own investigations of Hardy–Weinberg conditions on allele frequencies in a gene pool.

From the student handout:

1. Obtain a piece of PTC paper and place it on your tongue. Record your phenotype for the PTC tasting trait as "taster" or "non-taster."

   NOTE: If it tastes bitter, you are a "taster" and if it is tasteless, you are a "non-taster." If control paper is available, you can use this paper to confirm your ability to taste or not taste the PTC. If the control paper and PTC paper taste the same, you are a non-taster.

2. Based on your phenotype, can you identify your genotype? Explain the reasoning for the answer you provide.

   Student answers will vary. Students with the non-taster phenotype know that their genotype must be tt, since the non-taster allele is recessive to the taster allele. Students with the taster phenotype may be homozygous in genotype (TT) or heterozygous (Tt). While research has shown that heterozygotes can be less sensitive to the taste than homozygotes, "taster" students won't know if they are intermediately sensitive or highly sensitive to the taste of PTC, so anyone who is a taster has a T allele but the other allele is unknown.

3. Follow your teacher's directions to report your phenotype to the class. Copy Tables 1 and 2 into your lab notebook and use the compiled class data and the directions below to complete Table 1.

Table 1: Determining the phenotypic and allelic frequencies for the PTC tasting trait

Phenotype	Class Population Phenotype and Allele Frequencies			
	Number of Students	Phenotype Frequency	Allele Frequencies	
Tasters	18	69%	p	0.44
Non-taster	8	31%	q	0.56

Table 2: Determining the number of dominant and recessive alleles in the gene pool

Genotype	Class Population Gene Pool				
	Frequency of the Genotype		Number of Students of This Genotype	Number of T alleles	Number of t alleles
Homozygous dominant	p^2	0.19	5	10	0
Heterozygous	$2pq$	0.49	13	13	13
Homozygous recessive	q^2	0.31	8	0	16
			Total number in the gene pool	23	29

a. Determine the frequency of each phenotype in the class population.

b. Use the frequency of non-tasters and the following equation to find the frequency q of the recessive allele for the population.

   $p^2 + 2pq + q^2 = 1.0$, where q^2 is equal to the frequency of the recessive genotype.

   Answers will vary based on the frequencies of the class population. For the sample data, q is equal to the square root of 0.31, which is 0.56.

c. Derive the frequency p of the dominant allele in the population.

Since there are only two possible alleles, T and t, the value of p is calculated in the following way:

$p = 1.0 - q$

$p = 1.0 - 0.56 = 0.44$

4. Why did you determine the value for q first, rather than determining the value for p?

The T allele is present in both homozygous dominant and heterozygous genotypes. It is not known which genotype each taster has, so the value of p cannot be calculated from the phenotype frequency. On the other hand, it is known that all non-tasters must be tt, so q can be calculated from the frequency of homozygous recessive individuals.

5. To model the gene pool of the class population, the number of homozygous dominant genotypes and heterozygous genotypes need to be determined. The frequencies of these genotypes can be derived from the Hardy–Weinberg equation and the size of the class population.

a. Since the frequency of the T allele is known, p^2 can be easily calculated. Use p^2 to estimate how many students in the class population have the TT genotype. Record these values in Table 2.

For the sample data:

$p^2 = 0.44^2 = 0.19$

If 19% of the class population is expected to have the homozygous dominant genotype, then 19% of 26 students, or 5 students, have the TT genotype.

b. How many students are likely to have a Tt genotype? Record this number in Table 2.

For the sample data:

$2pq = 2(0.44)(0.56) = 0.49 \approx 0.5$

Alternatively,

$2pq = 1.0 - p^2 - q^2 = 1.0 - 0.19 - 0.31 = 0.50$

50% of the class population, which is 13 students, is expected to have the heterozygous genotype.

c. If you have not already done so, record the frequency and number of students in Table 2 for the homozygous recessive genotype.

$q^2 = 0.31$; $0.31 \times 26 = 8.06 \approx 8$ students have the homozygous recessive genotype.

6. The gene pool will be simulated using index cards with letters written on them to symbolize alleles. Each card represents a gamete and will therefore have only one allele written on it.

a. Work together as a class to determine the total number of T alleles and the total number of t alleles that should be in the gene pool. Your teacher will then place the correct number of "T" and "t" cards in a designated area.

b. Based on your genotype for the PTC tasting trait, pick up two appropriate cards from the designated area.

NOTE: If you are a taster, your teacher will use the information from Table 2 to assign tasters to be either homozygous or heterozygous.

7. Follow your teacher's directions to find a "mate" at random. Hold the two cards behind your back and shuffle them. Pull out one gamete card and show it to your mate. Observe the combination of alleles present on your card and your mate's card and determine the phenotype inherited by the offspring.

8. On the class data page, record a tally mark next to the genotype and phenotype that describes your offspring and place the two gamete cards into the class "Offspring" container provided by your teacher.

9. Copy Table 3 and record the compiled data for the offspring produced in the simulation.

Table 3: Determining the number of dominant and recessive alleles in offspring generation

Phenotype	Number of Offspring	Genotype	Number of Offspring	Number of T alleles	Number of t alleles
Taster	8	TT	2	4	0
		Tt	6	6	6
Non-taster	5	tt	5	0	10
Total number of alleles in the offspring				10	16

10. What are p and q for the offspring population?

For the sample data:

$$q^2 = \frac{\text{5 non-tasters}}{\text{13 total offspring}} = 0.38$$

$$q = \sqrt{0.38} = 0.62$$

Alternatively, there were 16 t alleles out of 26 total alleles in the offspring and q is the frequency of the recessive allele, which is:

$$q = \frac{16}{26} = 0.62$$

$$p = 1.0 - 0.62 = 0.38$$

Or, determining the frequency by the number of T alleles, p is equal to $\frac{10}{26} = 0.38$

11. Did the allele frequencies change in the second generation? If yes, propose one or more reasons for the change. If no, propose one or more reasons for the frequencies remaining stable.

Answers will vary. It is likely that genetic drift occurs due to the small size of the class population. In the sample data provided, the allele frequency of q changed from 0.56 to 0.62, indicating genetic drift. However, in other instances of this simulation the allele frequencies may remain the same or almost the same because there is no selection against either of the phenotypes; the mating occurs randomly, and there is no migration.

12. Studies have been performed which analyzed DNA from over 300 human populations and found the mean frequency for q for the PTC trait is 0.48.[20]

a. How do these reported frequencies compare to your class population and the offspring population produced by the class?

Answers will vary based on the frequencies observed in each class of students and the results of the offspring simulation. For the sample data provided, the frequency of q is greater in the class population than the reported mean, 0.56 compared to 0.48, respectively.

b. Why might the class or offspring population not be representative of an actual human population used for genetic research?

If the class frequency is different than these reported frequencies, it may be due to the small size of the class. Due to the small sample size, the class population may not be representative of a larger sample. Human populations used for genetic research are a much larger sample size.

[20] Wooding, S.; Kim, U.; Bamshad, M.J.; Larsen, J.; Jorde, L.B.; Drayna, D. Natural Selection and Molecular Evolution in PTC, a Bitter-Taste Receptor Gene . *American Journal of Human Genetics*. Apr 2004; 74(4): 637–646. doi: 10.1086/383092

13. Imagine that a teacher devises a similar lab activity for her students, but has students use colored beads to represent the two alleles for the PTC tasting trait and has them start with a gene pool of 100 beads placed into a large cup. The number of dark-colored beads is equal to the frequency of the T allele in the class population; the number of light-colored beads is equal to the frequency of the t allele. The cup is shaken to mix the beads and one person blindly pulls out two beads at a time to randomly determine the alleles inherited by an offspring.

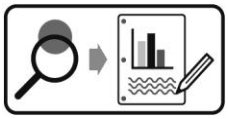
    For the first four offspring produced, the two beads inherited by the offspring are placed into a cup labeled "Survived." For the 5th offspring produced, a coin is flipped to determine if that offspring survives to reproductive age. If the coin lands heads up, the beads go into the "Survived" cup; if it lands tails up the beads go into a "Died" cup. The process is repeated, flipping the coin every 5th offspring produced. After all beads have been removed from the "gene pool," the beads from the "Survived" cup are poured back into the gene pool cup. After 10 generations of offspring have been produced, the data is analyzed to determine if the p and q frequencies changed over time.

    The activity you performed with cards and the activity described above with beads are both meant to model reproduction in a population and determine if a population evolves over time, that is, to determine if allele frequencies change over time. What are some advantages to the beads model described above?

    The model described, using beads, allows for the study of a larger population. If the gene pool begins with 100 beads, a population size of 50 is represented. This is more representative of an actual population, compared to an Advanced Biology class that may have 20–30 students. The bead model also includes a method of eliminating some genes from the gene pool, simulating the fact that in actual populations not all individuals survive to reproductive age. Additionally, instead of generating one offspring generation, the bead model follows the alleles through 10 generations. Lastly, it is difficult for students to truly carry out "random mating" in a simulation and not pair up with friends or others they are more familiar with. Using beads takes human behavior out of the simulation.

Design and Conduct an Experiment

The Hardy–Weinberg theory states five conditions that must be met by populations for allele frequencies to remain in equilibrium over time. In actual populations, these conditions are rarely met. Students are asked to devise a way to model the effect of violating one of the Hardy–Weinberg conditions in a simulated population and determine if the population remains in equilibrium or whether the population evolves under the conditions of your model.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If they are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Does the recessive allele eventually disappear if the homozygous recessive genotype is fatal?*
- Does a small population always experience genetic drift?
- How small does a bottleneck event or founder effect population have to be to experience genetic drift?
- Does a heterozygote advantage affect allele frequencies over time?
- What effect does non-random mating have on allele frequencies over time?*

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Does the recessive allele eventually disappear if the homozygous recessive genotype is fatal?" Results of this experiment are shown in the Sample Data section.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected allele frequencies in the population over time. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

      Introducing a condition in which the homozygous recessive phenotype is lethal did cause allele frequencies to change over time. As predicted in my hypothesis, *q* decreased over time. In five generations, the T allele frequency increased from 0.50 to 0.85, and the frequency of t decreased from 0.50 to 0.15.

   b. Based on the evidence you collected, explain why the results occurred.

      When there is differential survival in a population, the alleles in the most-fit organisms are passed on to offspring and increase in frequency while alleles in individuals who die and do not reproduce are not passed on. Only surviving heterozygotes pass on the fatal recessive alleles so the frequency *q* decreases over time.

c. Describe a real-life population or situation in which a species has experienced or would experience the conditions modeled in your simulation.

Some genetic conditions, such as Tay-Sachs, are 100% fatal. All individuals with the homozygous recessive phenotype die at an early age. The fatal allele is not eliminated as long as heterozygous individuals remain in the population and successfully mate and produce offspring.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

There is no evidence from my observations and data that experimental error or other uncontrolled variables affected the results. Similar results were obtained in all three trials in which the independent variable was altered.

3. Identify any new questions that have arisen as a result of your research.

Answers will vary based on the student's knowledge, experience, and results.

Synthesis Questions

1. Phenylketonuria (PKU) is a severe form of mental retardation caused by a rare autosomal recessive genotype. Parents typically have no idea they carry an allele for PKU until they have an affected child. In North America, approximately 1 in 10,000 Caucasian babies are born with the disease. Estimate the number of persons who are carriers of the PKU allele in North America.

$q^2 = 0.0001$, so $q = 0.01$

Given that $p = 1.0 - q$, p is equal to 0.99.

In the Hardy–Weinberg equation, the frequency of heterozygotes is represented by $2pq$:

$2(0.99)(0.01) = 0.0198$

The percentage of carriers of the PKU allele is approximately 1.98% of the population.

2. A population of 900 students was surveyed to determine the frequency of *positive* and *negative* blood types. This characteristic of red blood cells is called the *Rhesus factor* and the allele that codes for the factor is dominant over a mutated form of the gene that results in the factor being absent from red blood cells.

Ninety-three percent of the students are Rh-positive, that is, they have the Rhesus factor. If the population is in Hardy–Weinberg equilibrium, how many students would have each of the following genotypes: homozygous dominant, heterozygous, and homozygous recessive?

Since 7%, or 63 of 900 students, are homozygous recessive for the Rh factor, $q^2 = 0.07$ and $q = 0.26$.

If $q = 0.26$, then p is equal to 0.74 and $p^2 = 0.55$. So 495 (that is, 55%) of the students are predicted to be homozygous dominant for the trait.

The remaining 342 students are heterozygous for the Rh factor ($2pq = 0.38$, or 38%).

3. Analyze the following situations and explain the evolutionary mechanism that results in significantly different allele frequencies in the populations.

a. Sickle cell disease is an autosomal recessive disease caused by the inheritance of a mutated gene that results in abnormal hemoglobin. Northern European populations, living in an area with a low incidence of malaria, have a q between 0 and 0.005. In Africa where malaria is a cause of many deaths, q is much greater, between 0.020 and 0.181.

Natural selection is the reason for the allele differences in these populations. In the African populations, there is an advantage to being heterozygous, having one normal copy and one abnormal copy of the hemoglobin gene; persons with this genotype are least susceptible to malaria. The higher death rate of homozygous dominant individuals from malaria causes p to be lower in African populations than European populations. The reproductive success of heterozygotes keeps q higher in African populations.

b. Tay-Sachs is an autosomal recessive disease that results in death typically before age five. Affected individuals lack a vital enzyme and are unable to break down a fatty substance found in the brain. In Ashkenazi Jews (Jews of Eastern European Jewish decent), 1 in 27 people are carriers of the fatal recessive allele. In the general non-Jewish population, the carrier rate is 1 in 250.

The founder effect (a form of genetic drift) is the reason for the higher incidence of the carrier genotype in Ashkenazi Jews. People of Ashkenazi Jewish decent are descended from a small group of people. For religious reasons, there was little interbreeding with the general population. The mutation was originally present in one of the founders and was maintained at an abnormally high frequency in the population due to the lack of migration. The gene pool remained isolated over many generations. Parents were typically carriers and they passed the gene on to their offspring. In the general non-Jewish population, it is rare for two parents to both be carriers for the disease.

4. DNA profiling has become an important crime-solving tool in the 21st century. The FBI maintains a Combined DNA Index System (CODIS) that contains over 10 million DNA profiles. A person's DNA profile is comprised of the person's genotypes for 13 STR (short tandem repeats) loci. Scientists have analyzed these STR loci in DNA from thousands of people in hundreds of populations to determine allele frequencies. The Hardy–Weinberg equation is then used to help determine the frequency with which a particular genotype is observed in a population. DNA analysis, combined with probability calculations, can provide compelling evidence of guilt or innocence.

The diagram below illustrates part of a person's DNA profile. The table provides allele frequencies for the STR loci shown in the diagram as well as other STR loci.

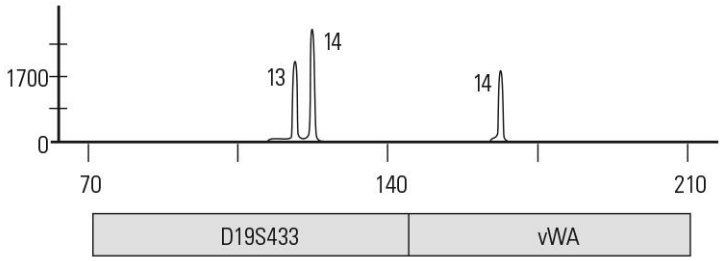


Table 4: Allele frequencies of several STR loci

STR Locus	STR Loci Data Compiled from Unrelated U.S. Population Samples[21]			
	Allele	Frequency	Allele	Frequency
D19S433	13	0.2471	14	0.3041
vWA	13	0.0034	14	0.0956
FGA	17	0.0014	20	0.0883
D21S11	28	0.1646	29	0.2042
THO1	6	0.1959	9.3	0.2056

a. The person is heterozygous for the D19S433 locus, having alleles 13 and 14 at this site. Based on the information provided and the Hardy–Weinberg equation, what is the frequency of the (13, 14) heterozygous genotype for this locus?

The frequency is equal to 2*pq*.

2(0.2471)(0.3041) = 0.15, or 15%

[21] Hill, C.R.; Duewer, D.L.; Kline, M.C.; Coble, M.D.; Butler, J.M. U.S. population data for 29 autosomal STR loci. *Forensic Sci. Int. Genet.* 7(2013): e82-e83 (Supplemental Material Table 2).

b. What is the frequency of the person's genotype for the vWA locus in the U.S. population?

The person is homozygous for allele 14, so the frequency is equal to $(0.0956)^2$, which is 0.0091, or 0.91%.

c. How can the person's genotype be identified as homozygous or heterozygous in the DNA profile diagram?

If the person is heterozygous, there will be two peaks in the profile due to the two different alleles. If the person is homozygous, there will be only a single peak.

d. What is the frequency of the following genotype in the population: D19S433 (13, 14), vWA (14, 14), FGA (17, 20), D21S11 (28, 29), and THO1 (9.3, 9.3)?

The frequency is equal to the product of the frequencies of the genotypes:

$(0.15)(0.0091)(0.00025)(0.67)(0.042) = 9.6 \times 10^{-9}$

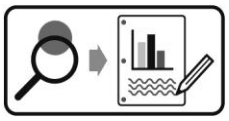
e. Do the frequencies of the two alleles shown in the table for the various STR loci add up to 1.0? If yes, explain why this is the case. If no, propose a reason for this.

No, the frequencies do not add up to one. The Hardy–Weinberg equation that states that $p + q = 1.0$ assumes that only two alleles exist for a given trait. Often a DNA locus has more variation than that; in other words, there are more than two alleles for that locus.

For STR loci there are numerous alleles for each locus. The table provides information about two of the alleles, but there are many more possible. For example, the D19S433 locus has 16 alleles.

Design and Conduct an Experiment Key

The Hardy–Weinberg theory states five conditions that must be met by populations for allele frequencies to remain in equilibrium over time. In actual populations, these conditions are rarely met. Devise a way to model the effect of violating one of the Hardy–Weinberg conditions in a simulated population and determine if the population remains in equilibrium or whether the population evolves under the conditions of your model.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of evolution and the Hardy–Weinberg theory, what factors (abiotic or biotic) could affect gene frequencies in a population?

   Factors include population size, mating rituals, female preference for mates with certain phenotypic characteristics, appearance of new mutations, migration into or out of a population, and natural selection pressures.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   Does the recessive allele eventually disappear if the homozygous recessive genotype is fatal?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   A number of genetic disorders result in death before reproductive age. One example is Tay-Sachs. It will be interesting to model this to determine whether the allele that causes this disorder is likely to be eliminated from the human population over time.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable in the experiment will be the survival rate for different genotypes. All offspring with the dominant phenotype will survive and all offspring with the recessive phenotype will not; when beads are picked from a cup, they will be placed into one of two other cups (“Survived” or “Died”) based on this criterion.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable will be allele frequencies. As beads are picked two at a time from the gene pool, each offspring's phenotype and genotype will be recorded. The surviving genotypes will be totaled after removing all beads from the cup and the frequencies of *p* and *q* will be calculated and compared for numerous generations.

6. Write a testable hypothesis (If…then…).

   If a lethal recessive allele can eventually be removed from a population, then *q* should become zero after some number of generations.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   The beads of contrasting colors will be the same size so they have an equal probability of being picked from the cup. The person taking beads from the cup will be blindfolded to ensure that the selection of beads is random. Each trial will begin with the same total number of beads and the same proportion of colors as the other trials. The dominant phenotype will have a 100% survival rate in all trials.

8. How many trials will be run for each experimental group? Justify your choice.

   There will be one experimental group, a group in which the survival of the dominant phenotype is 100% and survival of the recessive phenotype is 0%. Three trials will be run for the experiment. Three trials will ensure repeatability in results.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   For each trial, the allele frequencies in the parent population will be compared to the allele frequencies in the 5th generation (the final generation), that is, p and q will be compared to see if these changed over time.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    A starting gene pool of 100 beads may still be too small to prevent genetic drift from affecting the outcome. Also, the population size decreases over time due to the death of some individuals, which again may result in genetic drift. The beads in the original gene pool or the "Survived" or "Died" cups may be miscounted, leading to inaccurate allele frequency calculations.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

    Place 50 red beads and 50 yellow beads into a cup and shake the cup to mix them.

    Have a blindfolded person pick out two beads at a time. Record the phenotype and genotype of the offspring. Based on the phenotype, determine if the offspring survives. If it does, place the beads into the "Survived" cup.

    Repeat the process until all beads have been removed from the original cup. Count the beads in the "Survived" cup and determine the allele frequencies of the first generation.

    Place the beads from the "Survived" cup back into the original cup.

    Repeat the process for four more generations.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

Change in allele frequency due to a fatal homozygous recessive genotype

Generation	Allele Frequencies					
	Trial 1		Trial 2		Trial 3	
	p	*q*	*p*	*q*	*p*	*q*
1	0.65	0.35	0.66	0.34	0.64	0.36
2	0.73	0.27	0.75	0.25	0.76	0.24
3	0.80	0.20	0.83	0.17	0.85	0.15
4	0.81	0.19	0.82	0.18	0.88	0.12
5	0.83	0.17	0.83	0.17	0.88	0.12

The results shown above are from an inquiry investigating the effect of a lethal recessive allele on allele frequencies over five generations. The procedure for the inquiry is described in the Design and Conduct an Experiment Worksheet key. Parent generation starts with $p = 0.5$ and $q = 0.5$.

Non-random mating effect on allele frequency

Generation	Allele Frequencies					
	Trial 1		Trial 2		Trial 3	
	p	*q*	*p*	*q*	*p*	*q*
1	0.48	0.52	0.48	0.52	0.44	0.56
2	0.42	0.58	0.39	0.61	0.45	0.55
3	0.40	0.60	0.36	0.64	0.49	0.51
4	0.35	0.65	0.31	0.69	0.38	0.62
5	0.28	0.72	0.27	0.73	0.33	0.67

The results shown above are from an inquiry investigating the effect of non-random mating on allele frequencies over five generations. Female preference in mate selection was modeled by adjusting the number of offspring produced from males with different genotypes. Parent generation starts with $p = 0.5$ and $q = 0.5$.

In the simulation, females preferred the recessive phenotype to the dominant phenotype. Two cups were set up, one with beads representing female gametes and a second one with beads representing male gametes. The initial frequencies were the same in the cups. First a female gamete was selected, then a male gamete. If the male gamete had the dominant allele, one offspring was recorded. If the male gamete had the recessive allele, a coin was flipped to determine if it was from a heterozygous male (“heads”) or a homozygous recessive male (“tails”). If the coin indicated heterozygous, then one offspring was recorded (the heterozygote has the dominant phenotype). If the coin indicated homozygous recessive, then three offspring were recorded to indicate that females preferentially mate with individuals of that phenotype.

21. MATHEMATICAL MODELING OF EVOLUTION

Lab Overview

Students work with a mathematical model and computer simulation to explore how inheritance patterns and gene frequencies change in a population. The model lets students explore parameters that affect allele frequencies including population size, selection, and initial allele frequency.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	10 min
Initial Investigation	20 min

Student Designed Experiment	
Experiment Design	15 min
Experiment	30 min
Data Analysis	20 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	1.A.1, 1.A.2, 1.A.3, 1.C.3
Science Practices	1.2, 1.4, 2.1, 2.2, 5.3
Learning Objectives	1.1, 1.25, 1.26, 1.3, 1.4, 1.6, 1.7

Materials and Equipment

- Computer
- Mathematical model spreadsheet file: ABI Mathematical Modeling Spreadsheet.xlsx
- Spreadsheet program (such as Microsoft Excel®, Numbers®[22], or Google Docs™[23])

No additional equipment is needed for the student-designed experiment.

Prerequisites

Students should be familiar with the following concepts:

- Mendelian genetics
- Hardy–Weinberg equation and conditions
- How natural selection can alter the allele frequencies in a population
- Basic spreadsheet use and common spreadsheet functions (such as IF, COUNT, and RANDOM)

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

[22] Numbers is a trademark of Apple Inc., registered in the U.S. and other countries.

[23] © 2012 Google Inc. All rights reserved. Google Docs is a trademark of Google Inc.

Lab Preparation

Prior to doing the lab with students, open the mathematical model spreadsheet file, found on the electronic storage device that accompanies this manual, to make sure it functions correctly. This file was created in Microsoft Excel but can also be opened in Google® Spreadsheets, as an Apache OpenOffice™ spreadsheet, in Numbers, and in other spreadsheet programs. If these do not function correctly, we recommend using Microsoft Excel.

Teacher Tips

Tip 1 – Mathematical modeling spreadsheet

Having students produce their own spreadsheet instead of using an existing one allows them to become more familiar with creating mathematical models and understanding the model's limitations. This exercise will take two 45-min periods to complete and requires the teacher to be familiar with the creation and modification of the modeling spreadsheet. There are several excellent resources that can help:

- *AP Biology Investigative Labs: An Inquiry-Based Approach, Teacher Manual*. The College Board. 2012.
- *A Biologist's Guide to Mathematical Modeling in Ecology and Evolution* by Sarah P. Otto and Troy Day, 2007, Princeton University Press.

Tip 2 – Alternative modeling for extended inquiry

Student inquiry questions may push the limits of spreadsheet programs as well as students' (and teachers'!) programming ability. Fortunately, very sophisticated ready-to-use models, available on the Internet, allow students to ask and answer more complex questions. Here is a list of suggested simulators:

- http://bioquest.org/esteem/esteem_details.php?product_id=193

  NOTE: Search for terms such as "Deme 2.0 excel" if the link is no longer active
- http://faculty.washington.edu/herronjc/SoftwareFolder/AlleleA1.html

  NOTE: Search for terms such as "Allele A1 simulator" if the link is no longer valid
- http://learndat.tech.msu.edu/resources/hardy-weinberg-simulator

  NOTE: Search for terms such as "Hardy-Weinberg Simulator Michigan State" if the link is no longer valid

Tip 3 – Spreadsheet errors

If unintended edits or changes are made that affect the function of the spreadsheet provided, try to undo those edits. If the changes were saved, please redistribute the original file found on the digital storage device. The original file may also be downloaded from the "Advanced Biology through Inquiry Manual" page on www.pasco.com.

Initial Investigation

The Initial Investigation is designed to familiarize students with the equipment and techniques necessary to design their own experiment. In this investigation, students will use a computer simulation to model the changes of a population's genetic makeup from generation to generation when altering the initial allele frequencies, changing population size, and introducing selective pressure. If students are comfortable with the procedure, and have a strong understanding of the concepts, this section may be removed, placing more responsibility on the students for developing an experiment.

From the student handout:

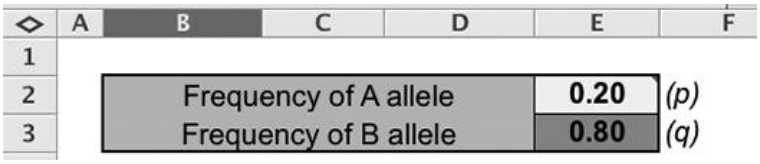
The spreadsheet for modeling evolution has four sheets: Small Population, Large Population, Selection, and Multiple Generations. This investigation starts on the "Small Population" sheet, which shows the initial gene frequencies in the population of 500 individuals. The alleles are represented by "A" and "B."

The Small Population model makes several assumptions, including:

- No mutations
- No selection
- No migration
- Sexual reproduction and alleles of each gamete are randomly selected
- Two-allele, simple-dominance pattern of inheritance
- All gametes form a viable zygote that lives to become an adult
- Population size remains constant

1. Open the mathematical model spreadsheet file. Save the file locally with a different filename. Select the Small Population sheet (click on the tab).

2. Enter some different frequencies for the A allele in cell E2.



   a. When the frequency of the A allele changes, what happens in cell E3? How does the spreadsheet determine the value for E3?

      NOTE: To see the formula for any part of the spreadsheet, simply select the cell of interest and look in the formula bar (f_x).

      The spreadsheet automatically calculates the frequency of *q* once *p* is entered. The value of *q* is calculated using the equation $q = 1 - p$.

   b. If a number greater than 1.00 is entered in cell E2, the message box labeled "Allele Frequency Invalid" appears. Use the Hardy–Weinberg equation to explain why the model requires a value less than or equal to 1.00 entered in cell E2.

      Because 100% of the alleles must be accounted for, the sum of the allele frequencies must equal 1.00 (where the frequency 1.00 designates 100%).

3. In the model population, a gamete can contain either an A allele or a B allele. Which of the two alleles is in any given gamete is random, so columns B and C have been set up using the spreadsheet's RANDOM function; this function generates a random number between 0 and 1.

   Observe how the RANDOM function works by entering the formula "=RAND()" into cell G2. Press the "*F9*" key on a Windows® computer or the "*command"* and "=" keys simultaneously on a Mac® computer to randomly generate a new number in this cell. Repeat this command three or more times.

4. Notice that the command not only generated a new value in G2, but data in other parts of the spreadsheet also changed. This is because some cells contain formulas that involve the RAND() function. For example, this function is part of the formula the spreadsheet uses to randomly determine if an A or a B allele is in a gamete. In this case, the function is not dependent on the *F9* or "*command =*" commands, though. Change the frequency of the A allele in cell E2 and press the *enter (*or *return)* key; the gamete columns change, as does the summary chart, and a new value appears in G2.

   NOTE: Delete the RANDOM function formula from cell G2 before continuing.

5. A formula using the RAND() function has been set up in cells B6 to B55 and C6 to C55 as follows:

   =IF(RAND()<=E$2,"A", "B")

   Click on cell B6 and look for the equation in the f_x window. This equation directs the spreadsheet program to first generate a random number between 0 and 1. Each time a random number is generated, the IF statement checks to see if it is less than or equal to the frequency of the A allele. If so, an "A" gamete is produced, whereas a random number that is greater than the frequency of the allele causes a "B" gamete to be produced.

   In the 50 rows of the table, gametes are randomly selected to be "A" or "B" and 50 zygotes are generated from the combination of these gametes.

5	Gametes		Zygote	AA	AB	BB
6	A	A	BB	0	0	1
7	B	B	BB	0	0	1
8	A	B	AB	0	1	0

6. If the frequency of the A allele is 0.50 and the RAND() function generates a value of 0.49, the gamete will be assigned an A allele. Why does the model use the current allele frequency to determine which allele is assigned to a gamete?

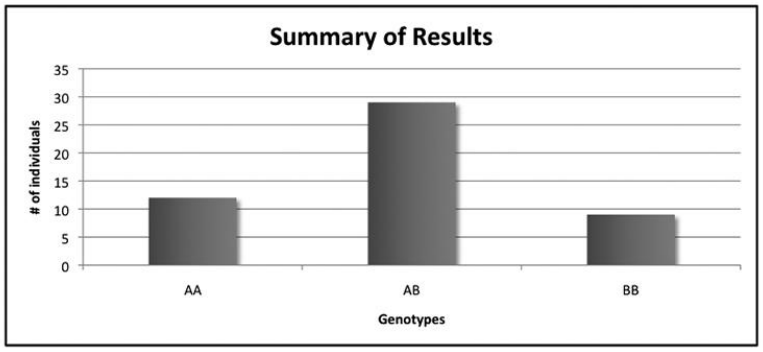
   The model uses the current allele frequency because the outcome of a random pairing within a population is subject to the allele frequencies of that population.

7. Is the spreadsheet model an accurate representation of reproduction in real populations? In other words, does the random selection of alleles and random combining of gametes occur in real populations? Explain your answer.

   The spreadsheet model is not an accurate representation. The model is a simplified version of a real population. The gametes may randomly combine but the individuals they are selected from are likely the product of non-random mating. On the spreadsheet, they are the product of random mating.

Another part of the spreadsheet is the results summary—a summary of the population's gene pool. Formulas in cells N5, O5, and P5 determine the number of zygotes of each genotype and this information is used to determine the number and frequency of A and B alleles in the new generation. The bar graph provides a visual summary of the genetic makeup of the population.

Totals for each genotype:	**AA**	**AB**	**BB**
	12	29	9
Next generation—number of alleles:	**A**		**B**
	53		47
Next generation—allele frequency:	***p***		***q***
	0.53		0.47



8. Click on cell N8.

   a. What formula is the spreadsheet program using to calculate the value for this cell?

   =N5*2+O5

   b. Explain what this formula is doing and explain the reason for using this formula to determine the number of A alleles.

   The formula is taking the number of individuals with the AA genotype and multiplying them by two (since two A alleles are present) then adding the number of individuals with the AB genotype to get the total number of A alleles in the population.

9. Set the starting A allele frequency in the model to 0.50. Create a data table to record the frequencies of the A and B alleles (*p* and *q*) for 5 generations, as instructed below.

   a. Record in the table the initial frequency of each allele and the next generation allele frequencies from the summary chart.

   NOTE: The next generation was automatically calculated when you pressed enter/return *after setting the initial* p *frequency to 0.50.*

b. Enter the value from N11 (the frequency of *p* in the next generation) into E2. Record the A allele frequency of the 2nd generation into the data table. Recalculate the model until you have created five generations. Do not forget to enter the new allele frequency in E2 *each time*.

Allele frequency changes in a small population

Generation	1st Trial	
	(p)	(q)
Initial	**0.50**	**0.50**
1	0.38	0.62
2	0.38	0.62
3	0.25	0.75
4	0.20	0.80
5	0.15	0.85

10. How did the allele frequencies change in the population over five generations? Are changes in allele frequency evidence that the population is evolving? Explain your answer.

The allele frequencies changed in the model: in the sample data the frequency of *p* decreased by 0.35, and *q* increased by the same amount. The change in allele frequency indicates that the population is evolving but not as a result of natural selection. This model demonstrates the potential for random changes, which provide the variability natural selection requires.

11. Repeat the process to produce four more data sets showing the change in allele frequency over five generations. For each data set, start with initial frequencies of 0.50 as you did before. (Create additional data tables for the new data sets.)

Comparison of five trials of allele frequency changes for a small population

Generation	2nd Trial		3rd Trial		4th Trial		5th Trial	
	(p)	(q)	(p)	(q)	(p)	(q)	(p)	(q)
Initial	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**
1	0.58	0.42	0.50	0.50	0.51	0.49	0.41	0.59
2	0.59	0.41	0.50	0.50	0.50	0.50	0.44	0.56
3	0.71	0.29	0.45	0.55	0.47	0.53	0.43	0.57
4	0.72	0.28	0.42	0.58	0.44	0.56	0.44	0.56
5	0.67	0.33	0.41	0.59	0.53	0.47	0.45	0.55

12. Are the results the same in all five data sets? If yes, why do you think this is the case? If no, what might be a reason for the differences? Do you detect any trends?

The direction and magnitude of the change was different in each trial. This is the result of the random selection of gametes. Once an allele drifts toward a higher or lower frequency, that trend will be more likely to continue, given the small sample size.

13. Repeat the above test showing the change of allele frequency, but set the initial p frequency to 0.90.

Change in allele frequency in a small population, beginning with $p = 0.90$ and $q = 0.10$

Generation	1st Trial		2nd Trial		3rd Trial		4th Trial		5th Trial	
	(p)	(q)	(p)	(q)	(p)	(q)	(p)	(q)	(p)	(q)
Initial	**0.90**	**0.10**	**0.90**	**0.10**	**0.90**	**0.10**	**0.90**	**0.10**	**0.90**	**0.10**
1	0.88	0.12	0.91	0.09	0.90	0.10	0.90	0.10	0.90	0.10
2	0.85	0.15	0.93	0.07	0.88	0.12	0.90	0.10	0.87	0.13
3	0.92	0.08	0.93	0.07	0.87	0.13	0.89	0.11	0.83	0.17
4	0.95	0.05	0.97	0.03	0.85	0.15	0.88	0.12	0.79	0.21
5	0.95	0.05	0.97	0.03	0.89	0.11	0.90	0.10	0.85	0.15

14. After reviewing your results from each scenario, what can you conclude about the effect of the initial allele frequency on the change in frequency over five generations?

The allele is more likely to go to fixation the closer the starting frequencies are to zero and one. In two trials of the sample data, the final frequency of the A allele was 0.95 or greater.

15. Do the changes in frequencies generation-to-generation progress in one direction? Why might a high A allele frequency in a small population be detrimental to the survival of that population?

The changes in allele frequencies in the population are random but due to sampling error. In a smaller population there is a greater risk that an allele will go to fixation and reduce the genetic variability of the population.

16. Move to the next tab in the spreadsheet, Large Population. The Large Population sheet is identical to the Small Population sheet except the population size has been increased to 5,000 individuals.

Set the starting frequency of the A allele to 0.90 and create three data sets of five generations each, as you did before. Create data tables to record the results.

Change in allele frequency in a large population, beginning with $p = 0.90$ and $q = 0.10$

Generation	1st Trial		2nd Trial		3rd Trial	
	(p)	(q)	(p)	(q)	(p)	(q)
Initial	**0.90**	**0.10**	**0.90**	**0.10**	**0.90**	**0.10**
1	0.904	0.096	0.897	0.103	0.901	0.099
2	0.906	0.094	0.897	0.103	0.904	0.096
3	0.903	0.097	0.901	0.099	0.906	0.094
4	0.903	0.097	0.903	0.097	0.904	0.096
5	0.903	0.097	0.901	0.099	0.906	0.094

17. Did the allele frequencies change similarly in the small and large populations over five generations? How do you explain the difference (if any) between the populations?

In a larger population, the allele frequencies change but to a much smaller degree. In the sample data, the largest change in frequency was 0.006. In a large population, the sampling error of the population is greatly reduced.

18. Move to the Selection sheet in the spreadsheet. On this sheet, the survival rate of each genotype can be manipulated and the gene frequencies are shown for a population of 500 individuals. Set the starting frequency to 0.50 for the A allele and set the survival rate of the BB genotype to 0%. The survival rate of AA and AB should be set at 100%.

    Run the model for five generations, again setting the allele frequency in E2 after each run. Record the new allele frequency of each generation in a table.

    Change in allele frequency with 0% survival rate of the BB genotype

Generation	Trial	
	(p)	(q)
Initial	**0.50**	**0.50**
1	0.66	0.33
2	0.76	0.24
3	0.78	0.22
4	0.81	0.19
5	0.83	0.17

19. In some populations there is a *heterozygote advantage*. Change the spreadsheet in a way you think models heterozygote advantage. Describe what you change and record the results in a table for five generations. Record both the allele frequencies for each generation and the number of individuals with each genotype.

    To model heterozygote advantage, the starting frequencies were set to 0.50 and 0.50, the survival percentage for the homozygotes was set to 75%, and the survival percentage for the heterozygotes was set to 100%.

    Change in allele frequency with heterozygote advantage

Generation	Trial		AA Individuals	AB Individuals	BB Individuals
	(p)	(q)			
Initial	**0.50**	**0.50**	**-**	**-**	**-**
1	0.48	0.52	85	245	107
2	0.47	0.53	83	247	107
3	0.48	0.52	83	256	101
4	0.46	0.54	82	241	113
5	0.46	0.54	77	251	110

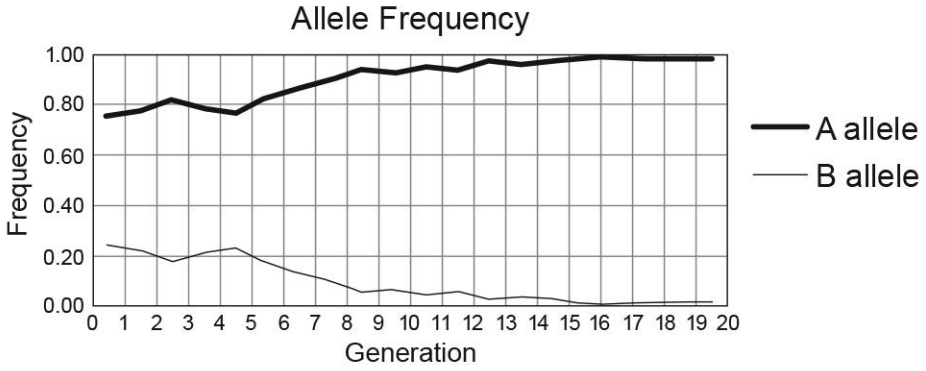
20. How did the selection against the BB genotype affect the allele frequencies over five generations? How do these results compare to those of the model reflecting heterozygote advantage?

    With selection against the BB genotype, the A allele frequency increased and the B allele frequency decreased. The allele frequencies did not change as much when there was a heterozygote advantage; in the sample data, the final frequency was 0.54 for the A allele. For the selection against the BB genotype, the final frequency was 0.83 for the A allele.

21. Which of the four situations, if any, represented a population in Hardy–Weinberg equilibrium? Justify your answer with evidence. For any population that was not in equilibrium, identify the factor or factors that disrupted the equilibrium.

    The large population most closely reflected a population in Hardy–Weinberg equilibrium. The other populations were either too small or were subjected to selective pressures. The Hardy–Weinberg equilibrium is a null test for a population that is not evolving. To determine if the model populations are in Hardy–Weinberg equilibrium or not, the data would need to undergo chi-square analysis.

22. Move to the final sheet in the spreadsheet, Multiple Generations, which models a population of 500 organisms for 20 generations. In this sheet, the allele frequencies are shown in the table and on the graph. Set the initial frequency to 0.80 for the A allele and observe the population changes for 20 generations. Instead of creating a data table, graph your results.



23. Compare your results to those of several other classmates or groups. How do they differ? Do differences in the results indicate a flaw in the model? Explain your reasoning.

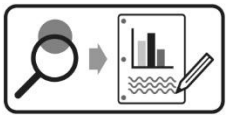
The results for each group were different. Some groups' model predicted the allele would go to fixation, others had different frequencies but both alleles were still represented after 20 generations. The differences can be explained by the random function and the relatively small sample size. The results do not indicate a flaw but an idealized population that is subject only to chance; no conditions of the Hardy–Weinberg model have been violated.

24. Computer modeling is a powerful tool for biologists but, as with all models, it has limitations. For the spreadsheet models you used to investigate population genetics and evolution, identify two useful aspects of computer modeling and two limitations.

The model saves time and allows biologists to run many simulations quickly and tabulate the results instantly. The limitations of this model include the inability to investigate multiple alleles, vary population by generation, and introduce non-random mating.

Design and Conduct an Experiment

The Hardy–Weinberg equilibrium model states that both allele and genotype frequencies in a population remain constant from generation to generation unless specific disturbing influences are introduced into the population. The mathematical model(s) used in this experiment simulate a population over multiple generations and can be used to simulate a population where a disturbance is introduced into the population. Students may use the provided model, create their own, or use other population genetics simulations.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If students are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- How does a homozygote recessive (bb) advantage affect the time to allele fixation?*
- Does a heterozygote advantage preserve alleles within a population?
- Does a homozygote advantage decrease the time to allele fixation?
- How does a population bottleneck affect the allele frequency of a population?

Design and Conduct an Experiment: Data Analysis

As part of their experiment design, students should explain how they will analyze their results.

1. Based on your model and data, describe how the independent variable in the models affected allele frequencies in the population.

   To create a homozygous recessive advantage in the population, the basic selection model was used and the homozygous dominant and heterozygous genotypes were given lower survival rates of 75% each with a starting allele frequency of 0.5 for both alleles. This model was run for 15 generations and the final allele frequencies were compared to a control where each genotype had 100% survival. The final allele frequencies did not show much change with 0.46 (p) and 0.54 (q) compared to 0.51 (p) and 0.49 (q) for the control. This makes sense since the heterozygote survival will maintain an equal frequency of alleles and the homozygotes both experience the same negative selection.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   The mathematical model is based on the randomness of the alleles assigned to a gamete, but the model needs to be run at least 5 times to ensure the validity of the results through a large enough sample size. The model produces data that is reliable enough to see the effects of single variables. Since real populations are usually subject to multiple variables at a time, a more complex model would need to be developed and tested before being used for predictive or management purposes.

Synthesis Questions

1. A great deal of genetic variation is found within healthy populations. This goes against the logic that natural selection works toward genetic uniformity in a population—the most fit genotypes produce the most offspring, thereby increasing the frequency of some alleles. Describe factors that could cause this genetic variation.

   The factors that could lead to genetic variation in a population are mutations, natural selection, genetic drift, gene flow, and non-random mating.

   Mutations that occur to the genetic code of an organism can lead to increased genetic variation and even cause different phenotypes to arise within a population.

   Natural selection, the concept of survival of the fittest, results in alleles being passed to the next generation in proportions different from their relative frequencies in the present and past generations.

   Genetic drift causes variation due to the unpredictable fluctuation in allele frequencies that results from a change in population size (such as the founder effect or bottleneck effect).

   Gene flow results when a population gains or loses alleles by genetic additions (immigration) or subtractions (emigration).

   Non-random mating causes allelic frequency to fluctuate due to mating patterns based on physical and behavior patterns.

2. The assumptions of the Hardy–Weinberg equilibrium are stringent and are seldom observed or never completely met in real populations, so why do genotype frequencies of many populations not deviate significantly from Hardy–Weinberg expectations?

   Genotype frequencies of many populations follow Hardy–Weinberg expectations because there is little or no selective pressure to favor one allele in the population.

3. Changes in allele frequencies lead to an evolving population. In a population of 200 peppered moths where white coloration is dominant over dark coloration, there are 32 dark peppered moths.

   a. What is the genotype breakdown of the population for the peppered moths?

      With the frequency of recessive allele q equaling 0.4 (using $p^2 + 2pq + q^2 = 1.0$, $q^2 = 32/200 = 0.16$, so $q = 0.4$) and the frequency of dominant allele p equaling 0.6, the population breakdown for the peppered moth is as follows:

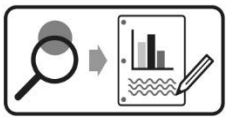
      Homozygous dominant: 72 peppered moths; Heterozygous: 96; Homozygous recessive: 32.

   b. Environments by nature are consistently changing. Identify a particular change and describe how it would affect the allele frequency of the peppered moth. Explain how the Hardy–Weinberg principle would be affected.

      The variety of coloration of peppered moths allows them to blend into their environments, providing camouflage and protection from predators. If their environment, such as a tree, were to change color, the likelihood of survival of peppered moths whose coloration provides greater camouflage would increase while other peppered moths would be exposed to predators as the changed coloration of their environment decreases their camouflage. Thus the population with the increased camouflage will see an increase in their allele frequency while the population with a decreased level of camouflage will see a decrease in their allele frequency. This natural selection occurring within the peppered moth population causes the population to evolve; it is not in Hardy–Weinberg Equilibrium.

Design and Conduct an Experiment Key

The Hardy–Weinberg equilibrium model states that both allele and genotype frequencies in a population remain constant from generation to generation unless specific disturbing influences are introduced into the population. The mathematical model used in this experiment simulates a population over multiple generations and can be used to simulate a population where a disturbance is introduced into the population.



Develop and conduct your experiment using the following guide.

1. For a population in Hardy–Weinberg equilibrium, what five factors or conditions must be taking place in order to maintain the equilibrium of the population?

   The five factors or conditions needed in the population to maintain the Hardy–Weinberg equilibrium are: 1) no mutations, 2) a large population size, 3) no natural selection, 4) no genetic drift, and 5) random mating.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   How does a homozygote recessive (BB) advantage affect the time to allele fixation?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   If a population is undergoing selection, one allele could be favored over the other. If the homozygous individuals are more fit, then that allele will become fixed in the population faster than if it were favored or if there was no selection.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   Selection toward the homozygote is the independent variable. In the model, the survival rate of heterozygous (AB) and homozygous dominant (AA) genotypes will be lowered to 75%, while the survival rate for the homozygous recessive (BB) genotype will be at 100%. This experimental group will be compared to a control group where the survival of each genotype is 100%.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The dependent variable is the allele frequency, which is determined using the Selection sheet of the mathematical modeling simulation spreadsheet.

6. Write a testable hypothesis (If…then…).

   If the homozygotes have a greater fitness in a population, the alleles will go to fixation sooner than if there was no selection.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   The starting allele frequency (p = 0.50 and q = 0.50) in each trial group and the number of generations (5) tested will be held constant, randomness will be assured, and no mutations will occur.

8. How many trials will be run for each experimental group? Justify your choice.

   Three of each group trials will be run, using the mathematical model, to ensure the validity of the data.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   The number of generations to fixation will be obtained using the model run with no advantage for the homozygotes and will be compared with the results of the model run with a homozygous recessive advantage. We expect the average time to fixation for the population with homozygous advantage will be shorter.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

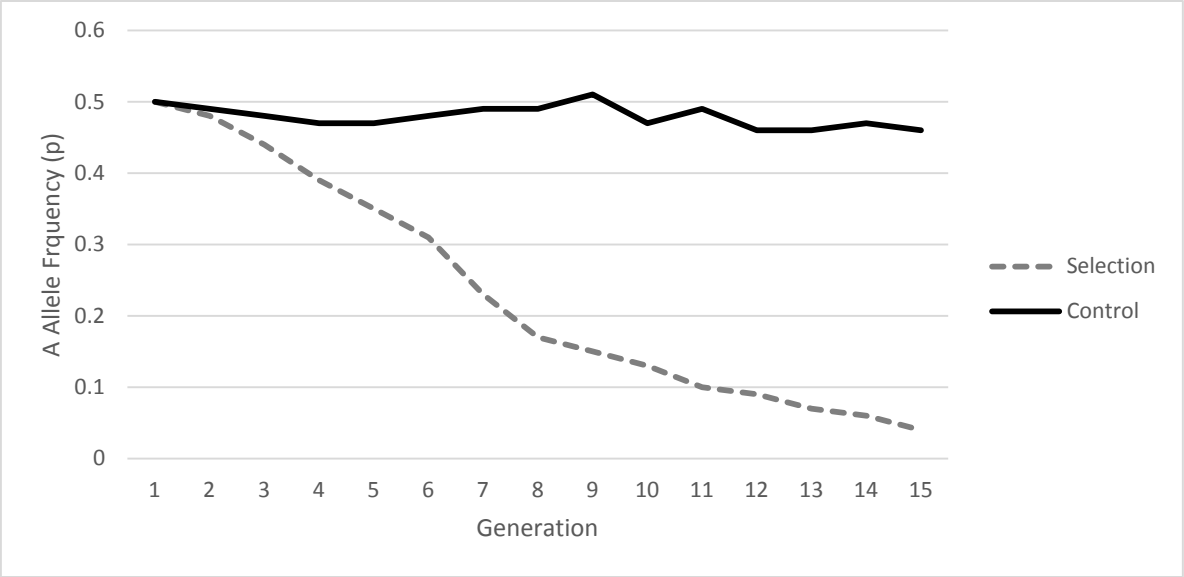
    Possible sources of errors for the model are the inputting of directions into the spreadsheet that eliminate the randomness of the model, sampling error from small populations or limited number of generations, or the misinterpretation of the data.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to replicate the results of this procedure.)
    a. Start the mathematical model with 500 individuals in the generation.
    b. The initial allele frequencies will be p = 0.50 and q = 0.50, with no selection taking place.
    c. Recalculate the model for five generations and record the final allele frequency obtained.
    d. Repeat the trials with the survival rate set to 75% for both heterozygous and homozygous dominant genotypes in successive tests. Homozygous recessive genotype will remain at a 100% survival rate.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

Frequency of the A allele with and without selection advantage for homozygous recessive BB



The results above were obtained using the selection model with 500 individuals. The control data had 100% survival rates for all genotypes while the selection data reduced the AA and AB genotype survival rate to 75%.

22. ANIMAL BEHAVIOR

Lab Overview

Students test the response of fruit flies to different stimuli and determine if there is a significant change in their behavior. They do this by constructing a choice chamber from drinking straws and cotton swabs and exposing the flies in the chamber to two environments, one on each end of the straw. Students then conduct a chi-square test to determine if the flies display taxis and are indeed favoring one environment over another.

Pacing and Length of the Lab

Initial Investigation	
Teacher Preparation Time	10 min
Initial Investigation	60 min

Student-Designed Experiment	
Experiment Design	15 min
Experiment	45 min
Data Analysis	15 min

AP Connections*

The concepts covered in this lab align to the "AP Biology Curriculum Framework."

Essential Knowledge	2.D.1, 2.E.3, 4.A.6, 4.B.4
Science Practices	1.3, 2.2, 3.2, 4.2, 5.1, 6.1, 7.2
Learning Objectives	2.22, 2.23, 2.24, 2.38, 2.39, 2.40, 4.14, 4.15, 4.16, 4.19

Materials and Equipment

For Each Student Station

- Clear drinking straw[1]
- Droppers (2)
- Cotton swabs (10)
- Timer
- Sheet of white paper
- Wingless fruit flies (10), or similar small organism[1]
- Mashed ripe banana, 10 mL[1]
- Mashed unripe banana, 10 mL[1]
- Distilled water, 10 mL

[1]For detailed information, refer to the Lab Preparation section.

Additional equipment recommended for the student-designed experiment:

- Cold and warm packs
- Aluminum foil
- Light source
- Condiments (such as ketchup and mustard)
- Solution with low pH (HCl)
- Solution with high pH (NaOH)
- Ammonia
- Soil or sand

*AP is a registered trademark of the College Board, which was not involved in the production of, and does not endorse, this product.

Prerequisites

Students should be familiar with the following concepts:

- Learned and innate behavior
- The role of phototaxis, chemotaxis, thermotaxis, and geotaxis in animal behavior.
- Genetic basis of behavior and the role of a taxis
- The impact of abiotic and biotic factors on the taxis of animal behavior
- Chi-square analysis and its application
- Null hypothesis
- Natural selection

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles when working with chemicals.
- Students should not eat any food items in the lab.
- Treat all living organisms with care.

Lab Preparation

These are the materials and equipment to set up prior to the lab:

1. Wingless fruit flies

   Wingless fruit flies can be purchased at any local pet store that sells reptiles or food for reptiles. They are also available from most science suppliers.

2. Drinking straws

   Obtain straws that are clear. Avoid purchasing straws with bendable ends to prevent adding an uncontrolled variable to the experiment.

   To indicate different sides of the choice chamber, divide each straw evenly into two sections using a permanent marker.

3. Bananas

   Ripe and unripe (green) bananas are needed to complete the initial investigation. Each type needs to be mashed so the banana can be transferred to a dry cotton swab.

Teacher Tips

Tip 1 – Small organisms

Wingless fruit flies are ideal for use inside the drinking straws. However, other small organisms, such as crickets or pill bugs, can be used. A choice chamber can be constructed from clear PVC or acrylic pipe 0.5–2 inches in diameter, plastic drinking bottles, or Petri dishes, to use with other organisms.

Tip 2 – Digital cameras

Students may have trouble capturing a correct count of fruit flies every minute in the choice chamber. Have them use a digital camera to take a snapshot at the correct interval for analysis. This can also expedite the lab, as students can run multiple trials simultaneously if they are organized.

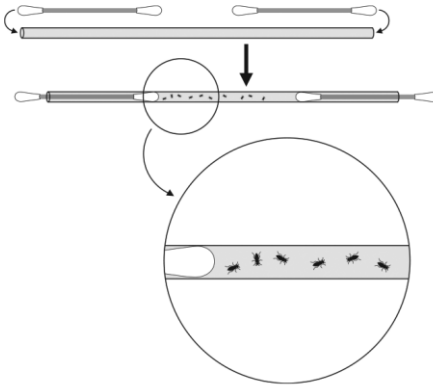
Tip 3 – No-choice zone for choice chamber

The drinking straws can be divided into thirds using a permanent marker. The middle third would indicate a "no-choice" zone when counting the organisms every minute to account for the flies not moving toward either end of the choice chamber. Use 9 or 12 flies instead of 10 so expected values are whole numbers.

Initial Investigation

The Initial Investigation is designed to familiarize students with the materials and techniques necessary to design their own experiment.

From the student handout:



Choice chamber setup

1. Place 5–10 drops of distilled water onto the end of a cotton swab and place the swab into one end of a clear drinking straw.

2. Using a paper funnel, place 10 wingless fruit flies into the drinking straw.

3. Place 5–10 drops of distilled water onto a second dry cotton swab and place it into the other end of the drinking straw.

4. Lay the choice chamber with the fruit flies onto a white sheet of paper. Label one end of the straw as side A and one as side B. Allow the fruit flies 5 minutes, with no disturbances, to acclimate to the choice chamber.

5. What is the purpose of collecting data for a situation in which fruit flies are given the same choice on either side of the choice chamber?

   This configuration is used as a control to ensure that no unknown variables are affecting the outcome of the experiment. If the flies don't exhibit a statistically significant preference for one side of the chamber, then additional variables can be introduced.

6. After the 5 minute acclimation period, start a 5 minute observation period. Count the number of flies on side A and the number of flies on side B every minute for 5 minutes. Record your observations in a data table arranged like Table 2.

7. For the following situations using the choice chamber, predict whether you expect fruit flies to exhibit a preference or whether you predict the null hypothesis to be supported (that the flies will have no preference).

   (a) ripe bananas vs distilled water

   Fruit flies should have a positive taxis toward the bananas when given a choice between distilled water and bananas, since bananas are a food source for them.

   (b) ripe bananas vs unripe bananas

   When given a choice between ripe and unripe bananas, the fruit flies are expected to have a positive taxis towards the ripe banana because the fermentation process that the banana is undergoing produces alcohol, which attracts the flies. The ripening fruit also provides a place to lay eggs that hatch into larvae in hours.

8. Using the same choice chamber and fruit flies, repeat the above procedures to expose the flies to the two combinations of substances specified in the previous step. Record the data in your lab notebook for each one.

9. Calculate the average number of flies on each side of the chamber for each situation.

10. Complete a chi-square analysis of the results to determine if the flies' distribution in the choice chambers is significant. (Table 1 is provided for reference.)

Use this null hypothesis for all experiments: The fruit flies do not have a preference for either substance in the choice chamber.

Table 1: Chi-square distribution

	Fail to reject				Reject	
Degrees of Freedom	**Probability *p* Value**					
	0.75	0.50	0.25	0.10	0.05	0.01
1	0.10	0.46	1.32	2.71	3.84	6.64
2	0.58	1.30	2.77	4.60	5.99	9.21
3	1.21	2.37	4.11	6.25	7.82	11.34
4	1.92	3.36	5.39	7.78	9.49	13.28

Table 2: Determination of significant trends

Time (minute)	**Number of Fruit Flies**					
	Water vs Water		**Water vs Ripe Banana**		**Ripe vs Unripe Banana**	
	A	**B**	**A**	**B**	**A**	**B**
1	2	8	4	6	7	3
2	5	5	2	8	8	2
3	6	4	1	9	9	1
4	7	3	0	10	10	0
5	4	6	0	10	10	0
Total (Observed *o*)	24	26	7	43	44	6
Average(Observed *o*)	4.8	5.2	1.4	8.6	8.8	1.2
Expected *e*	5	5	5	5	5	5
$(o - e)^2/e$	0.008	0.008	2.592	2.592	2.888	2.888
$\chi^2 = \sum[(o - e)^2/e]$	0.016		5.18		5.86	
Null Hypothesis	Fails to be rejected		Rejected		Rejected	

Table 2 shows sample data and the chi-square values for each trial, and indicates if the null hypothesis was rejected or not.

In the first test with water on both ends of the choice chamber, the null hypothesis failed to be rejected; the chi-square test yields a p-value >0.05. (According to the chi-square distribution table, there is more than a 75% probability that the behavior of the flies in the water vs water chamber was due to chance). In other words, the chi-square value was less than 3.84, the critical value for $p = 0.5$ and one degree of freedom. This result indicates that the difference in the number of flies on each side of the chamber was due to chance rather than preference.

In the remaining trials, the p-value for the calculated chi-square value was less than 0.05 (the chi-square value is equal to or greater than 3.84), indicating there is strong evidence against the null hypothesis, so the null hypothesis is rejected. In both trials using ripe bananas the location of the flies in the straw was due to preference and not to chance. As was expected, the fruit flies were attracted to ripe bananas.

Example calculation for the Water vs Water test:

Side A: $(\text{Observed} - \text{Expected})^2/\text{Expected} = (4.8 - 5)^2/5 = 0.04/5 = 0.008$

Side B: $(\text{Observed} - \text{Expected})^2/\text{Expected} = (5.2 - 5)^2/5 = 0.04/5 = 0.008$

$\chi^2 = 0.008 + 0.008 = 0.016$

The degrees of freedom (the number of parameters which may be independently varied) is $N - 1$, where N is the number of groups being compared (or in this case, the number of choices provided to the flies). With two groups, the degrees of freedom is one. By convention, if the chi-square value located in the table (in the row with the appropriate degrees of freedom for the experiment) results in a probability greater than 5% (that is, 0.05), the null hypothesis fails to be rejected; any difference observed between the two groups is the result of chance.

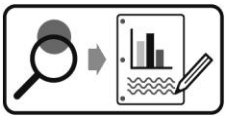
Since $\chi^2 = 0.016$ corresponds to a p value greater than 0.05, the null hypothesis fails to be rejected, which is the expected outcome when flies are given the same choice (water) on either side of the chamber.

11. After reviewing the data and completing a chi-square test, what is your conclusion? Did the fruit flies demonstrate a chemotaxis to any of the substances that were tested? Was it a positive or negative taxis?

    The fruit flies demonstrated a chemotaxis, specifically a positive taxis, to the ripe bananas, a decaying fruit that is a natural food source for fruit flies.

Design and Conduct an Experiment

Organisms exhibit a variety of behaviors that can be classified as taxis or kinesis behavior. Using a choice chamber and small organism, select an environmental factor and conduct an experiment to determine if that factor produces a taxis or kinesis behavior in the organism.



Students can design their experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NOTE: A key for the Design and Conduct an Experiment Worksheet follows the synthesis questions. The key describes a sample inquiry question and experimental protocol for which there are sample answers in the Data Analysis section.

Suggested Inquiry Questions

The Design and Conduct the Experiment section, along with students' background knowledge, guide students to generate a testable question. If students are not ready to create their own testable questions, students can use one of the following.

NOTE: Questions ending with an asterisk (*) have sample data shown at the end of the lab.

- Do fruit flies exhibit taxis behavior for light versus dark environments?*
- Do fruit flies exhibit a preference to certain odors?*
- Do fruit flies exhibit taxis behavior for warm versus cool environments?*
- Do fruit flies exhibit a preference to unripe banana versus water?*
- Do male fruit flies' taxis behavior differ from that of females?
- Do taxis behaviors in fruit flies change at different stages of development?
- Do fruit flies with different phenotypes (such as eye color or winglessness) exhibit different taxis behaviors?
- Is one type of taxis, such as chemotaxis, dominant over another type of taxis, such as geotaxis?

Design and Conduct an Experiment: Data Analysis

The answers in this section are sample answers that apply to the suggested inquiry question: "Do fruit flies exhibit taxis behavior for light versus dark environments?" Results of this experiment are shown in the Sample Data section.

As part of their experiment design, students should explain how they will analyze their results. Many analysis tools can be found in PASCO's data logging software. For some activities, students may want to export their data to other software, such as Microsoft Excel or the Google Docs™ program. Instructions for using analysis tools and exporting data can be found in the software help and user guide.

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected the behavior of the wingless fruit flies. Does chi-square analysis of your data indicate the null hypothesis can be rejected? Justify your claim with evidence from your experiment.

      According to the data collected, fruit flies show no preference for a light or dark environment; in other words they do not exhibit phototaxis.

      All trials resulted in a chi-square value less than 3.84 (the critical value for p = 0.5, degrees of freedom = 1), so the null hypothesis fails to be rejected. (The chi-square values ranged from 0.004 to 2.704.) The chi-square values correspond to, a *p* value greater than 0.05, so the alternate hypothesis that the fruit flies preferred a light environment is not supported.

      NOTE: the chi-square analysis for this inquiry is shown in the Sample Data section.

   b. Based on the evidence you collected, explain why the results occurred.

      In the other trials where the flies did exhibit a taxis, they were drawn to food and odors that emanated from food sources. The lack of attraction to light or dark environments suggests a phototaxis may not provide an evolutionary advantage, at least compared to other behaviors. Some research suggests that phototaxis is highly variable and not a heritable trait in most species of *Drosophila*.[24] Additionally, research suggests that phototaxis in fruit flies may be dependent on flies being startled by a stimulus, which was not the situation the flies were exposed to in this experimental design.

2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

   There is no evidence of error or uncontrolled variables in this experiment. The data is reliable and the alternate hypothesis is not supported by these results.

3. Identify any new questions that have arisen as a result of your research.

   Answers will vary based on the student's knowledge, experience, and results.

[24] Kain J. S.; Stokes, C; Bivort, B.; Phototactic personality in fruit flies and its suppression by serotonin and *white*. *Proceedings of the National Academy of Sciences*. Retrieved 3/2014 from http://www.pnas.org/content/early/2012/11/13/1211988109.full.pdf

Synthesis Questions

1. Animal adaptive behaviors are crucial for survival. Two types of adaptive behaviors are *innate behaviors* and *learned behaviors*. Explain how these two behaviors can impact an individual organism.

   Innate behavior is a behavior that is the same for the entire population of a species despite internal and environmental differences during development or during life experiences. This behavior is hereditary and the organisms do not need to be taught these behaviors. They will behave instinctively when the time or condition is right to trigger that behavior. For example, a young rattlesnake instinctively strikes when threatened or to capture food. This innate behavior helps the rattlesnake survive. Another example is migration behavior. Monarch butterflies do not "learn" to migrate; rather they are born with migration as an innate behavior.

   Learned behavior is the opposite of innate behavior in the sense that a behavior is modified based on specific experiences. An example of learned behavior is the learning of a specific call in songbirds. While vocalizing ("singing") is an innate behavior, birds learn a song with practice and listening to other birds of the same species nearby. The learning of a song is often important for reproductive success for an individual.

2. Animals exhibit behaviors that are classified as either a *taxis* or *kinesis*. What is the difference between taxis and kinesis in relation to animal behavior? In addition to chemotaxis, what other types of taxis responses exist?

   A kinesis refers to a change in activity level in response to a stimulus within an environment. A taxis is an automatic stimulus-caused movement toward or away from an environment. The main difference between the two is that in kinesis, any movement within the population is non-directional with regards to the location of the stimulus. The organism moves in a random way until it is in a more preferable location. In contrast, a taxis is a direct movement either toward or away from a specific stimuli or condition.

   Different types of taxis behavior include geotaxis, phototaxis, and thermotaxis.

3. An experiment was performed to investigate aggressive behavior in olive fruit flies (*B. oleae*).[25] Aggressive behavior was observed in swarms of flies around olive trees—males fighting to occupy leaves to perform courtship displays and females fighting for sites for laying eggs. Additionally, both sexes could gain access to food sources by occupying leaves or fruits on the tree. Investigators recorded fly behavior with high-speed video cameras and determined three behaviors to categorize as aggressive: wing waving, fast running toward the opponent, and pouncing and boxing on the head and thorax of the foe. One of the driving questions of the investigation was: Do resident flies win more combats than non-resident flies?

   NOTE: A resident fly is a fly placed into the chamber first, allowing it to establish a territory ("residence") before other flies are added to the testing chamber. A "win" is awarded to a fly if it remains on an olive leaf for at least 30 seconds after an aggressive interaction that displaces another fly.

Table 3: Observations of aggressive interactions in olive fruit flies

Sex	Initiator of an Aggressive Interaction		Winner of an Aggressive Interaction	
	Resident	Non-resident	Resident	Non-resident
Males	16	14	21	9
Females	19	11	22	8

   a. Write a null hypothesis for the driving question of the experiment and use chi-square analysis to determine if the null hypothesis should be accepted or rejected. Are the results different for different sexes?

      Null hypothesis: There will be no difference in the frequency of wins for resident flies compared to non-resident flies.

Male–Male

Parameters	Resident Is the Winner	Non-resident Is the Winner
Observed (o)	21	9
Expected (e)	15	15
$(o - e)^2/e$	2.40	2.40
$\chi^2 = \sum[(o - e)^2/e]$	4.80	
Null Hypothesis	Rejected	

Female–Female

Parameters	Resident Is the Winner	Non-resident Is the winner
Observed (o)	22	8
Expected (e)	15	15
$(o - e)^2/e$	3.27	3.27
$\chi^2 = \sum[(o - e)^2/e]$	6.54	
Null Hypothesis	Rejected	

For both sexes, 70% of the time the resident fly was the winner of the aggressive interaction. The null hypothesis is rejected because the chi-square values of 4.80 and 6.54 are greater than 3.84, the critical value for p = 0.5 and one degree of freedom. There is a 95% probability that the difference in wins for resident flies versus non-resident flies is not due to chance; the data support the idea that residency affects a fly's ability to remain on a leaf when challenged.

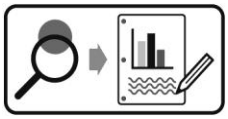
[25] Benelli, G. Aggressive Behavior and Territoriality in the Olive Fruit Fly, Bactrocera oleae (Rossi) (Diptera: Tephritidae): Role of Residence and Time of Day. *Journal of Insect Behavior* (2014) 27:145–161. doi 10.1007/s10905-013-9411-7.

b. Aggressive behavior has a genetic basis and has been conserved in insect evolution. In other words, the behavior is common in many insect taxa. Explain the relationship between natural selection and behavior in organisms.

If a behavior has a genetic basis, then it can be acted upon by natural selection. Genes that confer an insect's ability to defend a food source or increase its probability to mate are more likely to be passed on to future generations than genes that confer less aggressive behavior. If aggressive behavior makes an organism more fit in its environment, the trait is selected for by natural selection and becomes more common in a population.

Design and Conduct an Experiment Key

Organisms exhibit a variety of behaviors that can be classified as taxis or kinesis behavior. Using a choice chamber and a small organism, select an environmental factor and conduct an experiment to determine if that factor produces a taxis or kinesis behavior in the organism.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of animal behavior, what environmental factors (abiotic or biotic) could affect an organism's behavior?

   Factors that could affect behavior include light, odor, moisture, texture, temperature, and pH.

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

   Do fruit flies exhibit taxis behavior for light versus dark environments?

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

   As one of the most ubiquitous model organisms, fruit flies are kept in labs around the world. A better understanding of their behavior could help scientists provide a more suitable artificial environment. Additionally, if a taxis is observed, further research into the adaptation or evolutionary significance can be conducted.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

   The independent variable for the experiment is the amount of light in the environment. The light will be manipulated in the experiment by placing one end of the choice chamber in natural light and the other end in complete darkness (covering one end of the straw with aluminum foil).

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

   The number of wingless fruit flies on each side of the chamber is the dependent variable and will be counted every minute for 10 minutes.

6. Write a testable hypothesis (If…then…). Is this hypothesis the null hypothesis or alternate hypothesis for the chi-square analysis?

   If the wingless fruit flies are placed in a choice chamber that provides the options of light and dark, the fruit flies will be found more frequently on the side with light. This is the alternate hypothesis. The null hypothesis is that there will be no difference in the number of flies observed on the light and dark sides.

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

   The conditions to be held consistent are the type of straw used for the choice chamber, the intervals and total length of time measurements are made, the ambient environmental conditions, and the type of organism (wingless fruit fly).

8. How many trials will be run for each experimental group? Justify your choice.

   Three trials will be performed. This should ensure that the movement of the fruit flies in the choice chamber is due to the environmental condition and not to the random movement of the flies.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

   Data will be collected by counting the number of flies in each side of the chamber every minute for 10 minutes. After the data is collected, it will be analyzed for preference for light or dark using a chi-square analysis to determine if the distribution of the flies was significant.

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

    The most significant source of error in this experiment is the physiological differences of the fruit flies. Age, gender, and differences in the flies' eyes and light perception could all affect their behavior. Further, since the flies will be subject to testing in groups of ten, flies in the group could influence an individual's behavior, potentially masking or amplifying a taxis response.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to replicate the results of this procedure.)

    1. Construct a choice chamber with half of the chamber covered in aluminum foil or a similarly opaque material.
    2. Place 10 fruit flies into the choice chamber and set it in an area with plenty of ambient light to illuminate the other half of the chamber.
    3. Allow the fruit flies to become acclimated to the choice chamber for two minutes.
    4. Count the number of fruit flies in each half of the choice chamber every minute for ten minutes and record the data.
    5. Average the data for each side of the chamber and run a chi-square analysis with the null hypothesis that the flies have no preference and will be evenly distributed.
    6. Determine if the flies have a significant preference for one side of the chamber and if the hypothesis is supported.

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.

Sample Data

Null hypothesis: The fruit flies do not have a preference for either substance, or condition, in the choice chamber.

Animal behavior in a choice chamber with varying light*

Time (minute)	Number of Fruit Flies									
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
1	4	6	3	7	4	6	5	5	6	4
2	3	7	4	6	3	7	6	4	2	8
3	5	5	3	7	3	7	3	7	2	8
4	6	4	3	7	4	6	3	7	4	6
5	4	6	4	6	2	8	2	8	6	4
6	3	7	2	8	2	8	2	8	8	2
7	2	8	6	4	3	7	1	9	5	5
8	1	9	3	7	1	9	6	4	5	5
9	5	5	6	4	1	9	4	6	7	3
10	6	4	5	5	1	9	4	6	6	4
Total (o)	39	61	39	61	24	76	36	64	51	49
Average (o)	3.9	6.1	3.9	6.1	2.4	7.6	3.6	6.4	5.1	4.9
Expected (e)	5	5	5	5	5	5	5	5	5	5
$(o - e)^2/e$	0.242	0.242	0.242	0.242	1.352	1.352	0.392	0.392	0.002	0.002
$\chi^2 = \sum[(o - e)^2/e]$	0.484		0.484		2.704		0.784		0.004	
Null Hypothesis	Fails to be rejected									

*The light used was ambient light. The dark half of the choice chamber was kept in total darkness.

Animal response in a choice chamber to certain odors*

Time (minute)	Number of Fruit Flies									
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Non-odor	Odor	Non-odor	Odor	Non-odor	Odor	Non-odor	Odor	Non-odor	Odor
1	4	6	3	7	3	7	4	5	6	4
2	3	7	4	6	2	8	3	7	3	7
3	2	8	4	6	1	9	2	8	2	8
4	2	8	2	8	3	7	3	7	1	9
5	1	9	1	9	2	8	1	9	1	9
6	2	8	2	8	1	9	1	9	0	10
7	1	9	2	8	1	9	0	10	0	10
8	2	10	1	9	0	10	1	9	1	9
9	1	9	0	10	0	10	1	9	2	8
10	1	9	0	10	1	9	0	10	1	9
Total (o)	19	83	19	81	14	86	16	83	17	83
Average (o)	1.9	8.3	1.9	8.1	1.4	8.6	1.6	8.3	1.7	8.3
Expected (e)	5	5	5	5	5	5	5	5	5	5
$(o-e)^2/e$	1.922	2.178	1.922	1.922	2.592	2.592	2.312	2.312	2.178	2.178
$\chi^2 = \sum[(o-e)^2/e]$	4.100		3.844		5.184		4.624		4.356	
Null Hypothesis	Rejected									

*The conditions consisted of distilled water in one chamber (non-odor) and vinegar in the other (odor).

Animal response within a choice chamber to temperature options*

Time (minute)	Number of Fruit Flies									
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold
1	4	6	5	5	4	6	7	3	6	4
2	6	4	5	5	3	7	7	3	7	3
3	5	5	6	4	4	6	5	5	6	4
4	7	3	3	7	4	6	6	4	5	5
5	5	5	3	7	5	5	4	6	3	7
6	3	7	4	6	6	4	3	7	6	4
7	4	6	2	8	2	8	4	6	8	2
8	3	7	2	8	3	7	7	3	7	3
9	2	8	4	6	2	8	8	2	6	4
10	4	6	1	7	3	9	5	5	5	5
Total (o)	43	57	35	63	36	64	56	44	59	41
Average (o)	4.3	5.7	3.5	6.5	3.6	6.4	5.6	4.4	5.9	4.1
Expected (e)	5	5	5	5	5	5	5	5	5	5
$(o-e)^2/e$	0.098	0.098	0.450	0.450	0.392	0.392	0.072	0.072	0.162	0.162
$\chi^2 = \sum[(o-e)^2/e]$	0.196		1.125		0.784		0.144		0.324	
Null Hypothesis	Fails to be rejected									

*Fruit flies were given a choice between a hot and cold environment. The hot environment was obtained using a heating pad, with an average temperature of 33 °C. The cold environment was obtained using an ice pack or bag of ice and was maintained at an average temperature of 15 °C.

Animal response within a choice chamber to certain foods*

Parameters	Number of Fruit Flies	
	Water vs Unripe Banana	
	A	B
Total (o)	8	37
Average (o)	1.6	7.4
Expected (e)	5	5
$(o-e)^2/e$	2.312	1.152
$\chi^2 = \sum[(o-e)^2/e]$	3.46	
Null Hypothesis	Fails to be rejected	

*Fruit flies were given a choice between water and an unripe banana.