



Bio- logy

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College Biology for AP[®] Courses

Lab Manual

Teacher Version

**OpenStax**

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To the Student:

Congratulations on being accepted into, and having the courage to take, an Advanced Placement biology class! You are about to delve deep into some very detailed biology concepts. This lab manual aims to help you better understand these concepts through hands-on experiences in the laboratory. In addition, it will challenge you to critically think about biology concepts, scientific methods, and experimental design as part of its inquiry-based framework.

Inquiry-based learning involves challenging yourself to learn through self-discovery. Instead of simply presenting you with facts to memorize, this manual encourages you to ask questions about the material that you will then answer through your own exploration. By creating your own hypotheses and then planning and carrying out your own experiments on a variety of topics in the lab manual, you will hopefully learn biology by satisfying your own curiosity.

In this AP lab manual, the inquiry-based structure includes the following components:

1. **Pre-assessment section.** This section contains a list of questions that you should answer before starting each activity. These are meant to get you thinking about the main concepts of each lab. The pre-assessment questions are designed to connect the concepts in each lab to your experiences in daily life. Whether you realize it or not, you observe biology constantly in the world around you. Therefore, you are likely familiar with more biology topics than you realize! The pre-assessment questions are meant to tap into the biology knowledge you already have and apply it to what you will learn in each lab. As a result, your answers to these questions may not be graded and you will benefit greatly by discussing your answers as a class. This also allows your teacher to measure how familiar you and your classmates are with the material.
2. **Structured Inquiry.** In this section, you will be introduced to an experimental system by doing a well-laid out experiment with detailed steps. This section is meant to guide you in using the equipment in a “safer” activity before planning and performing an entire experiment. However, you will still be posing questions, predictions, and hypotheses in the structured inquiry. You will also critically think about how to achieve the most accurate and reliable results during the structured inquiry in preparation for creating your own experiments in the guided inquiry.

3. **Guided Inquiry.** In the guided inquiry, you will use the familiarity you gained during the structured inquiry to perform your own self-investigation. The experimental setup of the guided inquiries is often identical to that used in the structured inquiry. Therefore, you will be working with equipment and methods that you have already tried in the structured inquiry. This time, you will pick a variable to study, create a hypothesis, and fully design an experiment to test your hypothesis. You will determine which equipment and methods you should use to collect accurate and precise data.

Once you have planned your experiment, be sure to have your plan approved by your teacher, who will also ensure that your plan is safe and appropriate for the equipment available.

Finally, you will analyze your own data and make conclusions based on your experimental evidence. If time allows, you will then refine and re-run your experiments or test additional hypotheses that you find interesting. In many ways, the guided inquiry step is meant to engage you in the same processes that scientists have used to discover information about our world and universe!

Components of Structured and Guided Inquiry Sections

To ensure that an inquiry-based approach is implemented in each activity, both the structured and guided inquiries also contain each of the following steps at least once:

Hypothesize/Predict: This is where you will be creating hypotheses, which are questions or predictions about what will happen during an experiment. Be sure that your hypotheses are clear, specific, and t

estable.

Good hypothesis: The volume of water in a container will be higher when a 2-gram mass is added compared to when a 1-gram mass is added.

Poor hypothesis: The volume of water in this experiment will increase as larger objects are added.

Good hypothesis: The speed of a vehicle traveling down the 30° ramp will be lower than the speed of the vehicle traveling down the 60° ramp.

Poor hypothesis: The vehicle will travel fast down the ramp with the greater amount of slant.

Student-led Planning: Each inquiry contains at least one step where you and your lab partners will plan how to properly conduct your experiment. During the Structured Inquiry, you will generally plan proper techniques for getting the best results possible using the available equipment and described methods. As with many things in life, two or more heads are often better than one, and you and your group members should come to a consensus on a plan

before proceeding. This will lay the groundwork for the Guided Inquiry; you and your group will need to plan an entire experiment in this step.

Critical Analysis: This step typically occurs near the end of each inquiry. Here you will critically analyze your results, judge their validity, and explain why your hypotheses were supported or not supported by your results. You will also suggest ways that your experimental methods could have been improved to get more accurate or precise data as well as determine new questions to ask related to your results.

A Note About Your Notebook

As part of the challenge of taking an AP course, this lab manual does not contain data tables where you record your findings. Therefore, you will be required to design your own tables, answer assessments, and do any other note-taking in a separate notebook. You should use the same notebook for biology lab throughout the year. This will allow you to easily refer back to previous labs when you need to reference earlier content. Do not put non-biology content in your biology notebook, as your teacher may collect and grade your notebook throughout the year.

Components of a Lab

Main introduction:

Each lab contains an introductory section under the title. This introduces the “big picture” concepts of the lab as well as how they connect to everyday life. They will also introduce the pioneering physicists and experiments that led to our current knowledge of each lab topic. Relevant equations that you will use in the labs are also introduced here, including definitions of their variables. Many of the labs involve measuring the value of these variables so that you can later perform your own calculations. Please read the lab and activity introductions carefully before your lab period. Then, before the lab starts, ask your teacher about any concepts of which you are unsure.

In this lab you will learn:

This section presents learning objectives for the lab. These are the “take away points” that you should be able to explain or perform after doing the activities. It is helpful to read these objectives before each lab to prime yourself for what you will learn. It is then helpful to reread these at the end of each lab to ensure that you have achieved all of the learning objectives.

Activities:

Each lab is divided into 2-3 activities. Please note that your teacher may or may not have you perform all activities in a given lab, so pay close attention to your teacher’s instructions throughout the lab.

Safety precautions:

These bullet points list important safety issues that will prevent injury to yourself or your classmates during the lab activities. Each activity has its own safety precautions section. **Please read and understand all safety precautions before beginning each activity!**

For this activity you will need section:

This section lists all of the materials needed for each activity. Before you start the lab, make sure that you can identify all items on this list. Also, pay close attention to your teacher's instructions, as you may be using different equipment for these labs than those on this list.

Activity introduction:

These are short introductions relevant to specific activities. As with the main introduction, the activity introductions may contain formulas, equations, or other background information needed to successfully carry out and understand the activities. As with the main introduction, please read these introductions carefully before your lab period. Then, before the lab starts, ask your teacher about any concepts of which you are unsure.

Process steps:

These are the steps you will perform to carry out the activities. Please read through **all** of the process steps and setup diagrams **before** starting Step 1. Ask your teacher if there are any steps you don't understand prior to starting. This will help you perform the activities correctly the first time, preventing the need to redo activities or having to leave your laboratory period with unusable data.

Assessments:

The assessment sections provide questions that test your knowledge of the lab material. Your teacher will instruct you on how to submit answers to the assessments for grading.

College Board® (CB) Standard Alignment:

College Board® standards are summarized in a table format at the beginning of each lab. The College Board's® AP Biology Course and Exam Description was used to provide this information. In addition, standards tags are found on the assessments, allowing you to quickly identify which standard is addressed by each question.

Lab 1: Lab Safety, Scientific Method, and Measurements

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED.

SCIENCE PRACTICES

Science Practice	Skills
SP 2 Visual Representations <i>Analyze visual representations of biological concepts and processes</i>	2.D Represent relationships within biology molecules, including mathematical models, diagrams, and flow charts.
SP 3 Questions and Methods <i>Determine scientific questions and methods.</i>	3.A Identify or pose a testable question based on an observation, data, or model. 3.B State the null and alternative hypotheses, or predict the result of an experiment. 3.C Identify experimental procedures that are aligned to the questions, including variables and controls. 3.D Make observations, or collect data from representations of laboratory setups or results. 3.E. Propose a new/next investigation.
SP 5 Statistical Tests and Data Analysis <i>Perform statistical tests and mathematical calculations to analyze and interpret data.</i>	5.A Perform mathematical calculations.

Many dangers are associated with working in the lab, so you must be aware of lab safety. **Lab safety** is the knowledge of how to safely work in the lab by following safety procedures. You need to know the location of various kinds of safety equipment, the hazards of the laboratory, and what to do when you encounter hazards. The **Occupational Safety and Health Administration (OSHA)** is a federal agency whose mission is to enforce safety and health laws, including the handling of chemicals in the lab. Hazards and handling information can be found on the **material safety data sheets** for any materials used in a lab. You should also be aware of good laboratory practices, such as how to properly maintain your lab notebook, which will help make it easier to write your final report. Maintaining the equipment properly will help it last longer and remain available to others who will use the equipment after you.

People are naturally curious about the world around them. The **scientific method** is a method of inquiry—a way of investigating the world around us. It allows us to consider a problem or a situation, make observations, ask questions about those observations, and generate a statement that can be tested about what we believe to be true (a **testable hypothesis**). Then, we test the hypothesis using an experiment. The results of the experiment, if it is well-designed, will either reject or support the hypothesis. Scientists often avoid saying that a hypothesis or theory is ever *proven*, as it implies that there is no need for further study. Therefore, we always refer to hypotheses in this lab manual as being *supported* by data, not *proven* by data.

To help us understand the world around us, we have developed standards and tools of measurement to determine quantities such as mass and volume. Although we use the **British System** in the United States, scientists use the International System (SI), or metric system. There are standards in weight (gram), volume (liter), and length (meter). To convert one measure to another, there are simple conversion factors, due to the metric system being based on multiples of ten. An easy way to remember the order of units to determine the conversion factor is to use this mnemonic (memory) device: King Henry died unusually, drinking chocolate milk. King = Kilo, Henry = Hecto, Died = Deca, Unusually = Unit, Drinking = Deci, Chocolate = Centi, and Milk = Milli (see Table 1.1). For example, let's say a problem asked you to measure the length of a pencil using a metric ruler, and you found it to be 7 cm. The ruler allows you to measure in centimeters (cm), but the question needs you to give the answer in meters (m). To convert from centimeters to meters, you need a conversion factor, such as that shown in Table 1.1. There are 100 cms in a meter. Therefore, the calculation would be

$$7 \text{ cm} \times \frac{1 \text{ m}}{100 \text{ cm}} = 0.07 \text{ m}$$

Table 1.1: Conversion Table

King	Henry	Died	Unusually	Drinking	Chocolate	Milk
Kilo 10x10x10x LARGER than a unit 1 kilo = 1,000 units	Hecto 10x10x LARGER than a unit 1 hecto = 100 units	Deca 10x LARGER than a unit 1 deca = 10 units	Unit Meter (length) Liter (liquid volume) Gram (mass/weight) 1 unit	Deci 10x SMALLER than a unit 10 deci = 1 unit	Centi 10x10x SMALLER than a unit 100 centi = 1 unit	Milli 10x10x10x SMALLER than a unit 1,000 milli = 1 unit
km = kilometer kL = kiloliter kg = kilogram	hm = hectometer hL = hectoliter hg = hectogram	dam = decameter daL = decaliter da = decagram	m = meter L = liter g = gram	dm = decimeter dL = deciliter dg = decigram	cm = centimeter cL = centiliter cg = centigram	mm = millimeter mL = milliliter mg = milligram

You will perform unit conversions during this lab.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- If your students have not had a lab class in a long time, or if this is their first time being in a lab, it may be helpful to begin the first lab by discussing the importance of lab safety prior to showing the lab safety video referenced in the cited link.
- Examples of points to emphasize include common lab safety mistakes that lead to injury, such as failure to use personal protection equipment including goggles, lab coats, and gloves while conducting experiments. It would be useful to underscore the importance of advance preparation by reading the lab material ahead of time to ensure that students know what to expect for a given class period.
- Additional safety points to consider include the location of items such as fire extinguishers, fire blankets, fire safety valves, and first-aid kits.
- Upon finishing with the discussion of lab safety, introduce the major content of the lab for the day by discussing the scientific method and its importance in conducting sound science. Outline the steps and ask, after each step, why students believe that particular step is important. This will help you grasp their understanding of the topic before proceeding.
- A simple diagram such as a flow chart, drawn on the board listing each of the steps of the scientific method, may also help students visualize how each step of the method leads to the next.
- Because some students may have issues performing the conversion calculations, a diagram of a chart outlining the metric system units of measurement and their corresponding conversion factors should serve as a reference point. This may be provided to the students separately as a handout. It would be beneficial to perform a few conversion problems with the students, prior to letting them conduct the lab, to underscore the importance of this skill in analyzing data and writing lab reports.

In this lab, you will learn

- How to safely work in the lab,
- How to use the scientific method, and
- How to measure weight and volume.

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook.** Name one piece of lab safety equipment. What is it used for?
2. **Answer the following question in your notebook.** What is one hazard of the lab, and why is it hazardous?
3. **Discuss the answers to questions 1 and 2 with your class.**

Activity 1: Lab Safety

Safety Precautions

- Take care as you move around the lab.
- Wear all appropriate safety equipment.

For this activity, you will need the following:

- Access to the internet or a downloaded version of the safety video
- Lab safety equipment
- Lab safety agreement

Activity 1: Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Review the tenets of lab safety with your students. Consider having students handle this activity individually or in pairs.
- Assist students in locating lab safety items. This may be the first time students have been in your lab room, so covering the location of safety items is important. You should also demonstrate the proper usage of each item including the first-aid kit, emergency exits, shower, fire extinguisher, eyewash, waste containers (broken glass waste), safety glasses bin, gloves, and any other safety equipment and supplies in the lab.
- Demonstrate for the students how to wear and remove the personal protection equipment (e.g., goggles, lab coats, gloves). Students should also know where to properly dispose of lab waste.
- Remind students of the importance of abiding by all safety rules of the lab during each class period.

- Lead the students in a group discussion on lab safety after the activity to grasp their understanding of the topic. It may be useful to give a small quiz on the topic to grasp their understanding of the topic before proceeding.
- Collect the signed lab safety agreements after completion of this activity.

Step 1: Locate the following safety equipment and supplies in the lab: first-aid kit, emergency exits, shower, fire extinguisher, eye-wash, waste containers (broken-glass waste), safety glasses bin, gloves, and any other safety equipment and supplies in the lab.

Step 2: Draw a diagram of the lab showing the location of the safety equipment and supplies.

Step 3: Watch the lab safety video, either the one linked [here](#) or another of your choosing.

Step 4: Review the personal lab safety equipment shown in Figure 1.1.

Step 5: Record and sign the lab safety agreement, which states: “I have been trained in lab safety, and I agree to follow the rules of the lab.”

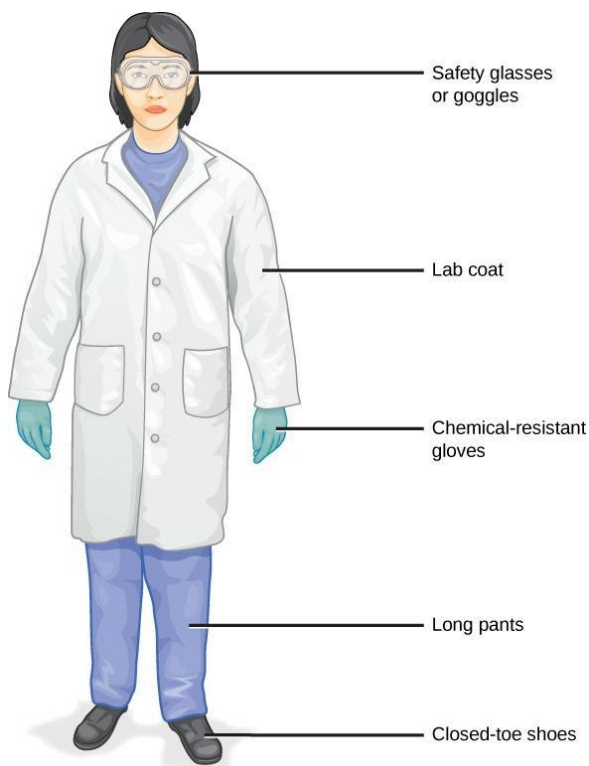


Figure 1.1: Basic personal lab safety equipment.

Assessments

1. Describe what you would do in each of the following situations. [SP 1, Concept Explanation; SP 3, Questions and Methods]
 - a. There is a chemical spill in the lab.
 - b. A fire starts in the lab.
 - c. A beaker breaks in the lab.
2. List personal safety equipment needed to work in the lab. [SP 3, Questions and Methods]
3. Name one rule that you learned from the video. [SP 3, Questions and Methods]

[Answer Key]

1.
 - a. Alert the teacher to the type of spill.
 - b. Alert the teacher and use the fire extinguisher to put out the fire.
 - c. Alert the teacher, clean up the glass with a broom and dustpan, and dispose of the glass into a broken-glass box.
2. Goggles, gloves, lab coat, long pants, and closed-toe shoes. Other answers can include long sleeves, hair ties, etc.
3. Answers will vary but could include not eating or drinking in the lab and washing your hands before leaving the lab.

Activity 2: Pre-Assessment

1. **Answer the following question in your notebook.** When doing an experiment, why is it beneficial to alter only one experimental condition at a time?
2. **Answer the following question in your notebook.** How can you ensure that your experiment results aren't influenced by any outside conditions?
3. **Discuss the answers to questions 1 and 2 with your class.**

Activity 2: The Scientific Method

A simple experiment will contain two types of variables: **independent** and **dependent**. The *independent* variable is the variable being tested and manipulated by the experimenter. The *dependent* variable is the variable being analyzed (counted or measured) for how it responds to changes in the independent variable. Experiments also need **controls**, which are additional setups that have predictable results. If the controls show the predicted response, this indicates that the experiment was set up and run properly and that there are no outside factors influencing the experiment. A **positive control** confirms that the experiment can detect a change in the dependent variable when the independent variable changes. Conversely, a **negative control** confirms that the experiment does *not* detect a change in the dependent variable when the independent variable *does not* change. For example, consider a test for the presence of sugar. The test yields a blue color when sugar is present but does not change color when sugar is absent. A positive control for this test would be to test several sugars and ensure that they always produce a blue color. A negative control would be to test several non-sugars, (e.g. water or proteins) and ensure that they never produce a blue color. In this way, positive and negative controls ensure that the experiment will generate reliable results. However, all experiments should be done multiple times (replicated) to reduce the influence of human error when performing them.

Safety Precautions

- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Water
- Sugar or salt
- Beakers
- Ice
- Hot plate
- Thermometer

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 2:

- Remind students of the importance of lab safety and that they should wear appropriate clothing and shoes to work in the lab. Additionally, because a hot plate is being used for this activity, it is important to underscore the importance of the proper use of this piece of equipment to avoid burn injuries and possible fire hazards.
- Provide students with instructions on the proper handling of mercury thermometers (if being used in this activity), as well as what to do in the event that one breaks during lab.
- Have extra materials available while prepping for this experiment, as students working in pairs may require a bucket or other carrying container for their materials (e.g., ice).
- The first part of this activity requires students to examine materials available in the lab for which they are to design a test for a hypothesis, so have a variety of materials for the students to use. If any potential safety hazards are associated with the materials, discuss these hazards with the students prior to letting them conduct the experiments and/or handle the materials.
- Discussing the proper way to state or format a hypothesis is important to this exercise. This information could also be reviewed in your initial discussion for the lab. Remind students of the steps of the scientific method and encourage them to follow these steps when performing this exercise. Also, remind students that they should have controls for their experiment and that they should also identify their independent and dependent variables for their experiments.

The following are recommendations for the Structured Inquiry and for the Guided Inquiry for Activity 2:

- Students can remain in groups of two while doing the Guided Inquiry project, but you may wish to have each student write his or her own redesign of the experiment. This is to ensure that each student contributes equally to each group.
- Check the students' hypothesis and initial experimental design during the Structured Inquiry before allowing them to proceed with performing their experiment. This will help to ensure that they are performing an experiment that can actually be conducted in your lab, given the available equipment and time you have allotted for this activity.
- Offer students advice on how to determine what controls to use for their experiment as well as how to identify their independent and dependent variables. Remember that students may not otherwise include all vital components of an experiment due to their inexperience.

- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.
- In Step 4, clarify to students how to analyze their results. Often students don't understand the importance of why they must perform experiments multiple times or have multiple variables and controls. Remind them of the importance of the reproducibility of data when trying to analyze results for scientific significance. Instruct students on how to determine if any human error could have been introduced into the experiment that could have skewed the results. Remind them that they should come up with a completely new hypothesis based on the results of their first study, if needed.
- Provide a grading rubric for this activity with criteria describing each of the mandatory parts of this activity to help guide students to include the proper components in their experiment.

Structured Inquiry

Step 1: Hypothesize/Predict. Examine the materials available in the lab. Think of a question you could examine about the available materials (e.g., does sugar dissolve faster in warm water than in ice-cold water?). Formulate a clear, specific hypothesis that you could test during the lab period. Record your hypothesis in the data table.

Step 2: Student-Led Planning. Design an experiment to test your hypothesis. Set up a data table in your notebook to record your data. Write out the steps of your experiment, and devise positive and negative controls. Discuss your plan with your teacher before proceeding.

Step 3: After your teacher approves, implement your experiment and record the data in your data table.

Step 4: Critical Analysis. Does your data support your hypothesis? Why or why not? What methods could you use to improve your results? Discuss with your partner, and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict. Redesign the experiment you previously performed in the Structured Inquiry. Create a more specific hypothesis, or create a follow-up hypothesis that you can test based on the results of the Structured Inquiry.

Step 2: Student-Led Planning. Create a plan for your experiment, including controls and data tables. As before, your teacher should approve your plan before you start your experiment.

Step 3: After your teacher approves, execute your experiment and record the data in your data table.

Step 4: Critical Analysis. Does your data support your hypothesis? Why or why not? How did you improve your experiment over what you did in the Structured Inquiry? How could you further improve your results? Discuss with your partner, and then write your answers in your notebook.

Assessments

1. What is the purpose of the scientific method? [SP 3, Questions and Methods]
2. You observe that plants sitting in one location in your house grow faster than plants sitting in another location. Create a hypothesis to test why this occurs. Then, describe an experiment that would test your hypothesis that includes controls. [SP 3, Questions and Methods]

[Answer Key]

1. The purpose of the scientific method is to understand the world around us using a systematic method of inquiry.
2. Answers will vary. Sample answer: The plants in the first location grow better because they have more available light than the plants in the second location. To test this hypothesis, half of the plants could be switched to the opposite location while the other plants should remain in their locations to serve as controls during the experiment.

Activity 3: Pre-Assessment

1. **Answer the following questions in your notebook.** What are the conversions for the following measurements?
 - a. 50 ml = ____ L
 - b. 20 g = ____ mg
 - c. 700 μ l = ____ ml
 - d. 1 gal = ____ pt
 - e. 2000 ml = ____ L
2. **Answer the following questions in your notebook.** Visit the weighing station. What is the weight of a weighing boat? Some balances have a method of subtracting it by pressing the “zero” or “tare” button. Weigh three items. Record the results in your notebook.
3. **Discuss the answers to questions 1 and 2 with your class.**

Activity 3: Measurement

In this lab, you will need to use instruments to make your measurements. There are instruments to use for each type of substance (liquid or solid). Some of the instruments to measure volume are graduated cylinders (shown in Figure 1.4) and pipettes (shown in Figure 1.2 and Figure 1.3). Some instruments to measure solids are balances and scales (a scale is shown in Figure 1.5). You may also need to adjust the instrument or container you are using based on the amount of substance you need to measure.

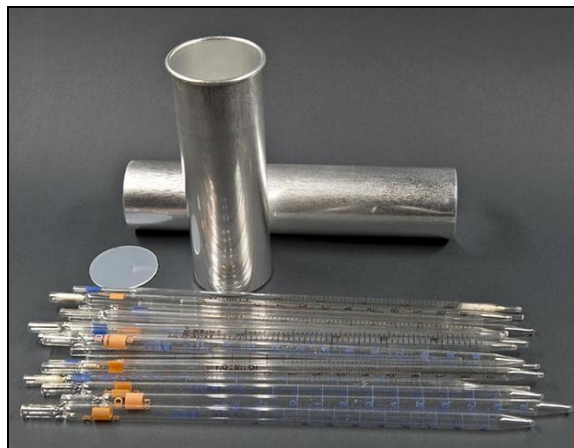


Figure 1.2: Serological pipettes are used to measure volumes of 1 to 50 ml.



Figure 1.3: Micropipettes are used for measuring micro quantities of liquid from 1 to 1,000 μl .



Figure 1.4: Graduated cylinders are used to measure volumes from 10 ml to 1 L.



Figure 1.5: A scientific scale is used to find the mass of solids.

Also, when recording your measurements in a data table, it is important to be aware of significant figures. The number of **significant figures** is the number of digits needed to express the accuracy of a calculation. The rules for significant figures are presented in Figure 1.5.

To use these rules, let's look at an example. An experiment requires you to measure the weight of 10 marbles. The scale shows a weight of 32.46547 g. All the digits in this measurement are significant figures. However, in the number 32.465470, how do we know if the last 0 is significant or not? There are three rules on how to determine significant figures:

- Non-zero digits are always significant.
- Any zeros between two significant digits are significant.
- A final zero or trailing zeros in the decimal portion only are significant.

So, based on this rule, the final 0 in 32.465470 is significant, as is the first zero in 32.046570. However, the 0 in 0.324657 would *not* be significant because it is simply a placeholder.

Safety Precautions

- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- A balance
- A weighing boat
- A graduated cylinder
- Weighable objects

For this activity, you will work *in pairs*.

Activity 3 Instructor Preparation and Teaching Notes

The following are recommendations for the Structured Inquiry for Activity 3:

- Because the first part of this activity requires students to predict the mass of various objects, have a variety of materials for the students to use. If any potential safety hazards are associated with the materials, discuss these hazards with the students prior to letting them conduct the experiments and/or handle the materials.
- Demonstrate the proper technique for using the balance/scale by using a weighing boat as well as a graduated cylinder and then zeroing the scale prior to filling your chosen items with a substance. Instruct the students on how to record the data from the scale in their lab notebooks. This demonstration will help students who have not previously used the balance/scale.

- Demonstrate how to use all other required materials, including the micropipette and pipettes, as well as how to properly read the meniscus of liquid in a graduated cylinder when performing measurements.
- Perform a few sample calculations using the provided equations in this lab activity. This will help clarify any inquiries regarding calculations.
- Clarify what significant figures are by providing students with an overview on this topic as well, prior to letting them conduct their exercises and record their data.

The following are recommendations for the Structured Inquiry and for the Guided Inquiry for Activity 3:

- Continue to have students work in pairs while doing the Guided Inquiry project. You may wish to have each student write his or her own hypotheses and calculations separately.
- Check the students' devised plan before they proceed with determining how to calculate the density of the objects they are testing.
- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.
- In Step 5, clarify to students how to analyze their results. Often students don't understand the importance of why they must perform experiments multiple times or have multiple variables or controls. Remind them of the importance of the reproducibility of data when trying to analyze results for scientific significance. Instruct them on how to determine if any human error could have been introduced into the experiment that could have skewed the results. Remind students that they should come up with a completely new hypothesis based on the results of their first study, if needed.
- Provide a grading rubric for this activity with criteria describing each of its mandatory parts to help guide students to include the proper components in their experiment.

Structured Inquiry

Step 1: Create a data table for your measurements, and show your calculations in your notebook.

Step 2: Hypothesize/Predict. Examine one of the objects. Predict the object's mass, and write the predicted mass in your notebook.

Step 3: Student-Led Planning. Listen to your teacher's instructions on how to use the balance. Discuss with your partner how you should determine the weight of the object and how many significant figures your measurement should have. Write your ideas in your notebook.

Step 4: Determine the weight of the object using the scale. Repeat the measurement twice more, and record all results in your notebook.

Step 5: Critical Analysis. How much did your measurements vary among the three trials? Were you able to accurately measure the number of significant figures stated in Step 3? Why or why not? Could you have improved your methods to get more accurate measurements or obtain a greater number of significant figures? Why or why not? Discuss with your partner, and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Volume is a measure of how much space matter occupies, and it is used to calculate density (d),

$$d = \frac{m}{v} ,$$

where m is the mass of the object and v is the object's volume. Pick another object available in your lab and predict whether it has a higher or lower density than the object you used during the Structured Inquiry. Write your prediction in your notebook.

Step 2: Student-Led Planning: How can you calculate the density of your two objects? Devise a plan for finding the density of both objects and present it to your teacher for approval. Create the data tables you need and decide how many times you should measure each object as well as how many significant figures you will use.

Step 3: After your teacher approves, gather the measurements you need to calculate the density of both objects. Write your final densities in your notebook.

Step 4: Critical Analysis: How did the object's size influence its mass and its volume? What would you have to do to increase the mass, volume, and density of an object? How could you increase the accuracy of your measurements? Discuss with your partner and then write your answers in your notebook.

Assessments

1. Why does the weight of the weighing boat need to be subtracted when measuring the weight of an object sitting in the weighing boat? [SP 5, Statistical Tests and Data Analysis]
2. What is the most likely instrument used to measure the following volumes? [SP 5, Statistical Tests and Data Analysis]
 - a. 200 ml
 - b. 50 μ l
 - c. 1 L

3. Calculate the density of an object that is 3 g and that displaces water in a graduated cylinder by 3 ml. [SP 5, Statistical Tests and Data Analysis]

[Answer Key]

1. The weighing boat is not part of the object and therefore should be excluded from the weight of the object.
2.
 - a. graduated cylinder
 - b. 100 μ l pipette
 - c. 1 L graduated cylinder
3. 1 g/ml

Lab 2: Introduction to Microscopy

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED.

REQUIRED COURSE CONTENT

Topic	Enduring Understandings	Learning Objective	Essential Knowledge
2.1 Cell Structure: Subcellular Components	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.D Describe the structure and/or function of subcellular components and organelles.	SYI-1.D.1.Ribosomes synthesize protein. SYI-1.D.2 Ribosomes are found in all cell types, reflecting common ancestry of all known life. SYI-1.D.3 Endoplasmic reticulum occurs in two forms, smooth and rough. SYI-1.D.4 Chemical modification of newly synthesized proteins occurs in the Golgi, a series of membrane-bound sacs. Other membrane-bound organelles include mitochondria, chloroplasts, lysosomes, and vacuoles.
2.2 Cell Structure and Function	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.E Explain how subcellular components and organelles work together to contribute to the function of the cell.	SYI-1.E.1 Organelles, including the ER, mitochondria, lysosomes, and vacuoles interact to support cellular function.
2.3 Cell Size	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.B. Explain the effect of surface area-to-volume ratios on the exchange of materials between cells or organisms and the environment.	ENE 1.B.1 Surface area-to-volume ratios affect the ability of cells to exchange chemicals and energy with the environment.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation <i>Explain biological concepts, processes, and models presented in written format.</i>	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations <i>Analyze visual representations of biological concepts and processes.</i>	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams.
SP 3 Questions and Methods <i>Determine scientific questions and methods.</i>	3.D Make observations, or collect data from representations of laboratory setups or results. 3.E Propose a new/next investigation based on evidence from an experiment or an evaluation or the methods.
SP 6 Argumentation <i>Develop and justify scientific arguments using evidence.</i>	6.B Support a claim with evidence from biological principles, concepts, processes, and/or data.

Arguably, the invention of the microscope was a defining moment of biology in the 17th and 18th centuries. Before its invention, scientists did not have a tool with which to see objects and structures below the resolution limit of the human eye. The ability to magnify objects allowed scientists to see the cellular and subcellular structure of animals and plants. Because of the microscope, scientists were able to develop many foundational theories of biology, including the **cell theory** (where all living things are made up of cells) and the **germ theory** (where infectious diseases are caused by microscopic organisms).

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Explain with the simple diagram of a magnifying lens how images are magnified. Show students how to trace rays. Introduce the terms real object, the specimen, and its virtual image, lens, focus, and light-bending effect of glass. Emphasize that this is a simple explanation and that a microscope is more complicated because of the number of lenses in the optical path.
- Lead a discussion on handling the microscope with care stressing that this is an expensive and delicate instrument. Review that rough handling of the microscope may result in the misalignment of the optical path and loss of the quality of the images.

- Review the cell theory. Remind students that, with few notable exceptions, cells' dimensions fall within a narrow range although living organisms vary widely in sizes. Ask students, what are the constraints on cell size?
- Ask students for current applications of light microscopy. Students should mention basic biological research and diagnosis. Remind them that they will use their skills in microscopy in almost all future biology classes.
- Students may not be aware that microscopy has many applications in fields other than biology such as material science or inspection of computer chips. Ask students to suggest other applications. Students will realize that microscopy is a widely used current technique and not a relic from the past.
- Review the three main factors in microscopy: magnification, contrast, and resolution.
- Explain the concept of total (final) magnification and remind students that all their drawings should have a title and a final magnification. Don't allow students to limit themselves to taking pictures. They may take pictures if they wish to do so, but it is no substitute for the exercise of sketching one's own observations.

In this lab, you will learn

- to identify the parts of a microscope
- to effectively use a microscope to conduct observations

Activity 1: Pre-Assessment

Answer the following questions in your notebook.

1. A microscope has two sets of lenses. What do you think is the purpose of each lens?
2. Why do you think scientists use microscopes? Think of useful functions for the microscope and discuss your ideas with your partner.

Activity 1: Parts of a Microscope

A microscope magnifies the image of an object through a series of lenses. The **condenser lens** focuses the light from the microscope's lamp onto the specimen. The light then passes through the object and is refracted by the objective lens. The **objective lens** is the more powerful lens of a microscope and is closest to the object. The light then travels to the **ocular lens**, which focuses the image onto the user's eye. Usually, the power of the ocular lens is fixed for a given microscope.

Different microscopes can magnify objects hundreds, thousands, or even millions of times. **Magnification** refers to how much larger the image is compared to the original object. As magnification increases, the image becomes larger. The total magnification is the power of the objective multiplied by the power of the ocular lenses. **Resolution** refers to the ability of a microscope to distinguish two points on the image. As resolution increases, objects that are closer together appear as separate points. **Contrast** refers to the ability of a microscope to distinguish an object from its background. The higher the contrast, the greater the difference in intensity between an object and the background.

Safety Precautions

- Handle microscopes and lenses with care.
- Do not drop or crush slides. Alert your teacher immediately to any broken glass so it can be properly disposed of.

For this activity, you will need the following:

- Compound microscope
- Slide with the letter *e*; if you do not have premade slides, cuttings from a newspaper can be taped to a slide

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Review good practices in handling a microscope. Students may have received vague instructions in previous classes and can be reluctant to abandon bad habits.
- Remind students to clear working space for the microscope and the supplies which will be necessary to perform observations. The microscope should be positioned on a flat surface with no notebook or other items underneath it.
- Tell students that using a microscope correctly will allow them to work better and faster in the lab and learn more from their observations. They should not be satisfied with viewing blurry images with poor contrast.
- Demonstrate proper focusing of the microscope and ask students to write down the steps as you perform them:
 1. Once the slide with the specimen is secured with clamps or stage clips, raise the stage to its uppermost position. While looking through the ocular, bring the specimen into focus. Make the point that one should always start at the highest or lowest position of the stage, but not in a middle position. This approach avoids the hit-or-miss focusing technique. It is preferable to start with the slide close to the objective and focus down to avoid hitting the slide. This is especially the case when working with a higher magnification objective, X400.

2. Explain that a microscope with parfocal lenses will allow them to focus at higher magnification with little adjustment. The microscope available to them may not necessarily have parfocal lenses.
 3. Tell students that they can move to higher magnification after they are comfortable working with the low objective lens.
- Stress that the coarse adjustment knob is used with the lower magnification objectives, typically X4 and X10. Only the fine adjustment knob should be used with high power, X40. Use of the coarse adjustment knob with a high magnification objective easily results in smashing the objective on the slide. It will scratch the objective lens and spread glass shards on the stage of the microscope.
 - Stress the importance of bringing down the stage before removing a slide to avoid bumping into the objective lens.
 - Emphasize that only lens paper is to be used. Paper towels or tissues will scratch the lenses.

Tips for Instructor

- Check students individually to make sure that each can focus a microscope. When students work in pairs, frequently one student actually does all the focusing.
- Lens tissue and lens cleaner should be readily available. The lens tissue should be lightly moistened with cleaning fluid. Do not wipe an optical surface with a dry lens tissue as particles can scratch the lens.
- Focusing the microscope by looking on the side and lowering the objective lens towards the slide is considered poor practice and should be avoided because it does not teach students how to bring the specimen in sharp focus.
- There is no need to clean lenses routinely. Clean lenses only if the specimen appears cloudy or sample contaminates the lens. For example, students may use too much liquid in a wet mount and cloud the objective lens.

The following are recommendations for the Guided Inquiry for Activity 1:

- Demonstrate refraction to students by asking them to observe a drinking straw in a glass of water.
- Explain that refraction refers to the bending of a light ray as it crosses a boundary between two different media. Explain that the magnification of a microscope is due to the bending of light by the magnifying lenses in the light path. Students may also be familiar with Snell's law from physics class, but it is not required to understand the concept of refraction.
- Ask students: how many boundaries does light typically cross when a wet mount specimen is observed? They should answer glass, water, and air.
- Ask students why the letter C or the letter A would not have been a good choice to observe the specimen's change in orientation under the microscope.

Tips

- Have ready diagrams of the light paths through a compound microscope and an inverted microscope as references.
- Review the paths of light and the changes in the light beam when moving the parts of the microscope before the lab session.

Misconception of Students

- Students often will notice that a specimen appears upside-down, but not that it is also flipped left-to-right.

Structured Inquiry

Step 1: When moving a microscope, grasp the neck firmly with one hand and place your other hand under the microscope's base. Do not bang the microscope on the desk. Once you have set down your microscope, turn the revolving nosepiece so that the lowest power objective is pointed at the stage.

Step 2: Throughout this lab, handle slides by their edges and do not touch the specimen area to avoid smudges. Place the slide with the letter *e* on the stage. Make sure it is oriented so that you can read the letter *e* correctly with the naked eye while standing at the microscope.

Step 3: You will now observe the slide containing the letter *e*. Center the specimen on the stage and look through the ocular lens. Adjust the coarse and fine focus knobs until the letter comes into focus. Be careful while focusing so that the slide does not touch the lenses.

Step 4: Hypothesize/Predict. From your observations of the letter *e*, what do you think is the function of each part of the microscope? Write the function of each in your notebook.

Step 5: Student-Led Planning. Using your textbook and other resources, research each part of the microscope. Identify each part of the microscope in Figure 2.1. Label each part of the microscope in Table 2.1. Write the function of each part in Table 2.1.

Step 6: Critical Analysis. You place a sample on the stage of a microscope and you look through the ocular lens. All you see is black in the field of view. What would you adjust to fix this problem?

Step 7: Microscope Care and Maintenance. When you are finished using a microscope, make sure the lowest power objective is in place and take the slide off the stage. Use only lens paper to wipe the lenses if they are dirty. Support the microscope by the base when carrying it as described in Step 1. If you are continuing with the other activities in this lab, keep the microscope out.

Guided Inquiry

Step 1: Find the light source on the microscope. Turn on the light source and trace the path of light from the source to the ocular lens.

Step 2: Hypothesize/Predict. Knowing the function of each part of the microscope, draw how the light path of the microscope bends and changes as it travels from the light source to the observer on Figure 2.1.

Step 3: Student-Led Planning. Using your textbook and other resources, research how light travels through the microscope. Use a piece of paper to block the light path through each component of the microscope to confirm that it is part of the light path.

Step 4: Critical Analysis. Sometimes, scientists want to study living cells that are kept in dishes. The dishes are very deep and filled with media. The cells are on the bottom of the plate. In order to see these cells on the microscope, the microscope has to be inverted so that the objective lens is below the sample instead of above it. Draw a diagram of how you would design the light path of an inverted microscope.

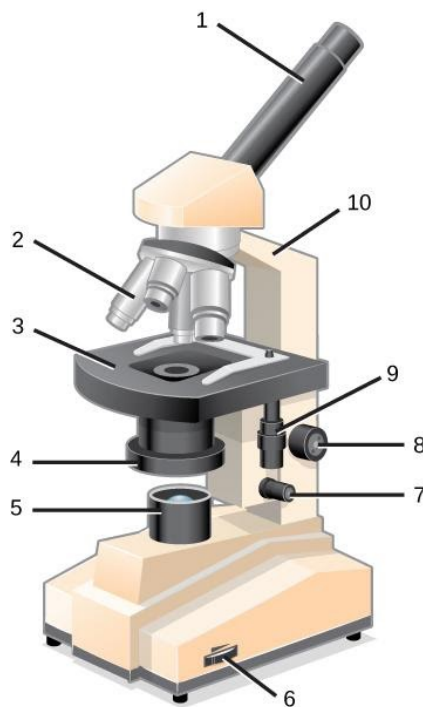
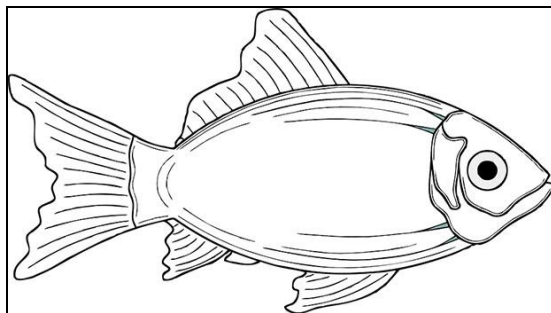


Figure 2.1: Can you identify all of the parts of the microscope?

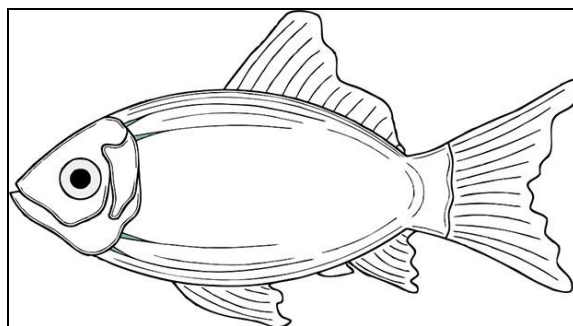
Assessments

1. What are three precautions you must take to prevent damage to the microscope? [SP 3, Questions and Methods]
2. A scientist uses a 40x objective to observe a specimen using a 10x ocular lens. What is the total magnification of the object? [SP 3, Questions and Methods]
3. A magnifying glass is also used to magnify objects. How is a compound microscope different from a magnifying glass? [SP 3, Questions and Methods; SP 1, Concept Explanation]
4. Draw how Figure 2.2 (below) would appear under a compound microscope. [SP 3, Questions and Methods; SP 2, Visual Representations]



[Answer Key]

1. Always carry the microscope by the base. Always place the slide on the stage with the lowest objective in place. Always use lens paper to clean the lenses.
2. 400X
3. A compound microscope uses two sets of lenses to magnify the object.
4. Under a typical compound microscope, the fish will appear backwards and upside-down.



Activity 2: Pre-Assessment

1. **Answer the following question in your notebook.** So far, you have only viewed a slide at the lowest power of the microscope. How do you think the field of view will change as you move to higher powers on the microscope?
2. **Answer the following question in your notebook.** The microscope makes small organisms appear larger so that scientists can more carefully observe them. How can you measure the size of small organisms on the slide of a microscope?

Activity 2: The Field of View

When you first place a specimen on the microscope's stage, the image you see in the ocular lens will likely appear blurry. This is because light rays from the object are not reaching your eye at the same time. **Focusing** is used to improve the image. Focusing moves the stage up and down, which changes the point at which the light rays from the object converge. When the stage is at a position so that the light rays exactly converge on the retina of your eye, the image will appear sharp and clear.

When focusing the specimen, you must be careful! The distance between the objective and the specimen is very small, and it is possible to crush the specimen against the lens if you move the stage too far. The proper distance between the specimen and the object is called the **working distance**.

When you look at a specimen through the microscope's lenses, you will likely not see the entire specimen at one time. Because the microscope enlarges the image of the object, only a small portion of the object will be visible at any given time. The **field of view** refers to the portion of the object that is seen through the ocular lenses.

Safety Precautions

- Handle microscopes and lenses with care.
- Do not drop or crush slides.

For this activity, you will need the following:

- Compound microscope
- Slide with the letter *e*; if you do not have premade slides, cuttings from a newspaper can be taped to a slide
- Slide with a stage micrometer or a clear millimeter ruler taped across the center of the slide

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Notes

The following are recommendations for the Structured Inquiry for Activity 2:

- Explain that a microscope must have good resolution for observation. Compare it to the familiar notion of poor resolution images or videos on the internet. Optimal resolution depends both on the quality of the optical lenses and on careful focusing.
- Ask the students to observe how the amount of light entering the objective lens varies with the magnification of the lens.
- Remind students to lower light intensity when they switch from high magnification to low magnification because a low magnification lens allows more light in and it will have a blinding effect.

The following are recommendations for the Guided Inquiry for Activity 2:

- Measure the number of *e*'s that fit in the field of view at each magnification before class to have a ballpark figure to compare to students' results. Allow for some margin of error in the students' measurements.
- Ask students to show you and explain all their calculations when comparing the size of the field of view at different magnifications. Make sure that they understand the relation between field of view and magnification and don't just copy another group's calculation.
- Be prepared to go over the calculation on the board after students have completed their own calculations.
- Ask students which magnifications are suitable to observe swimming living organisms. Students should understand that lower magnifications are more suitable because organisms would rapidly disappear from the field of view.

Structured Inquiry

Step 1: Turn the revolving nosepiece of the microscope so that the lowest power objective is pointed at the stage.

Step 2: Place the slide with the letter *e* on the stage. Make sure it is oriented so that you can read the letter *e* correctly with the naked eye while standing at the microscope.

Step 3: Hypothesize/Predict. How do you think the letter *e* will appear under different magnifications? Draw your predictions in your lab notebook.

Step 4: Student-Led Planning. Change the magnification by turning the revolving nosepiece. Draw your observations in your lab notebook.

Step 5: Critical Analysis. How do your observations compare to your predictions? Why do you suppose the letter *e* looks different under the microscope and at different magnifications?

Guided Inquiry

Step 1: Hypothesize/Predict. From your observations of the letter *e*, hypothesize how large the field of view is under the low power objective. To do this, measure the letter *e*, in millimeters, and then calculate how many letter *e*'s would fit in the field of view. From that hypothesize the size of the field of view, in mm. Will the size of the field of view change when you change magnifications? Based on the change in magnification, hypothesize the size of the field of view under the high-power objective.

Step 2: Student-Led Planning. With your partner, formulate a plan to measure the field of view using either the stage micrometer or the slide with the attached ruler. Observe the micrometer or ruler under the microscope to understand how it works before measuring. Then take three measurements of the field of view under low power. Repeat under high power. Write your results in your notebook.

Step 3: Critical Analysis. How did your estimated size of the field of views from Step 1 compare to the measurements you made using the micrometer or ruler? How does the size of the field of view change as the magnification changes?

Assessments

1. When you take a picture on your phone, does the field of view get larger or smaller as you increase the magnification? How does this compare to the microscope? Explain. [SP 3, Questions and Methods; SP 1, Concept Explanation]
2. As you increased in power on the microscope, the image became darker. Why do you suppose this is so? [SP 3, Questions and Methods; SP 2, Concept Explanation]

[Answer Key]

1. The field of view gets smaller in both the microscope and the camera as you increase the magnification. This is because less of the image can fit on the screen when the image is magnified.
2. Because you are looking at a smaller part of the field, less total light enters the lens at higher powers.

Activity 3: Pre-Assessment

1. **Answer the following questions in your notebook.** When you focused the slides in the previous activities, what part of the microscope was moving? Why do you think this changes the focus?
2. **Answer the following questions in your notebook.** Review the definition of life. What are characteristics found in all living things?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 3: Depth of Field and Wet Mount of Specimens

As you work with a microscope, you will notice that even when an object is in focus, you can continue to adjust the focus and see new things in the lenses. This is because most specimens are very thick. As you move up and down with the stage, different planes of the object come into focus. The **depth of field** refers to how thick each plane is. It tells you how much of a specimen is in focus at any given time. Although you may think that a higher depth of field is more useful because it allows you to see more objects, most scientists try to make the depth of field as narrow as possible. This prevents light from other focal planes from interfering with the observations.

The specimens you use on your microscope can either be wet-mounted or dry-mounted. A **wet-mount** refers to living tissues that are placed on a slide with an aqueous solution to keep them wet. Usually, a coverslip is placed on top of the specimen to flatten the specimen onto the slide. A **dry-mount** refers to preserved tissue that has been fixed and stained on a slide. This technique allows you to preserve specimens for a long time, and it also allows you to add chemicals to increase the contrast of a specimen from its background.

Safety Precautions

- Handle glass slides with care.
- Dispose of specimens as necessary.
- Dispose of coverslips in a broken-glass container.

For this activity, you will need the following:

- Compound microscope
- Slides with crossed colored fibers or crossed strands of hair
- Clean slides
- Coverslips
- *Elodea*, onion skin, pond water, or other samples

For this activity, you will work *in pairs*.

Activity 3 Instructor Preparation and Teaching Notes

The following are recommendations for the Structured Inquiry for Activity 3:

- Remind students to compare depth of field at different magnifications. Students should notice that the depth of field decreases at higher magnification.
- Ask students how changing focus can add to visualization of specimens because students may not be aware that many structures lie in different planes.

- Mention cells in plant vascular tissue and the striations of a skeletal muscle as examples of structures that do not lie on the same plane and are best observed by changing focus. If possible, have slides that demonstrate this point.

The following are recommendations the Guided Inquiry for Activity 3:

- Demonstrate the preparation of a wet mount because students may not be able to visualize the procedure from the written explanation.
- Emphasize the need to avoid the formation of air bubbles. Tell students to ignore a few small air bubbles on the slide. What they need to avoid are large bubbles which interfere with observation.
- Demonstrate how to dab excess mounting fluid – water or stain – with a tissue to avoid contaminating the objective lens.

Tips

- Tell students that they may use the edge of the coverslip or even the contours of an air bubble to focus the microscope.
- Show students how gently tapping the coverslip with the eraser side of a pencil can chase air bubbles to the edges of the coverslip in a wet mount.

Misconceptions of Students

- Students may think that the highest amount of light is always best for observation.
- Lowering light intensity improves viewing of living organisms in several ways.
 - It increases contrast while viewing unstained specimens and allows observation of internal structures.
 - Some organisms exhibit negative phototaxis and will swim away from the light.
 - The heat from the focused light beam often kills organisms and no movement will be observed.

Structured Inquiry

Step 1: Hypothesize/Predict. Look at the slide with crossed fibers or hair. How do you think you can use the microscope to determine which fiber is closest to the top of the slide and which fiber is closest to the bottom?

Step 2: Place the slide with crossed fibers or hair on the stage. Center the slide and focus it.

Step 3: Student-Led Planning. Turn the fine focus knob up and down. With your partner, decide how to determine which fiber is on top and which fiber is on the bottom of the slide. Record your results in your notebook.

Step 4: Critical Analysis. Take a look at the slide again without the microscope. With your naked eye, do you think the results of your analysis make sense?

Guided Inquiry

Step 1: Hypothesize/Predict. Based on the microscopy you have performed so far, what would be the challenges of viewing living organisms under the microscope?

Step 2: Student-Led Planning. You will now observe pond water or samples of living organisms provided by your teacher. Place a small drop of water from near the bottom of the sample jar on a slide. Place a cover slip at an angle so that one edge touches the drop. Slowly lower the coverslip onto the specimen. Avoid trapping air bubbles under the coverslip. Record drawings of your observations in your lab notebook. Make sure to record the magnification for each drawing.

Step 3: Critical Analysis: What were some of the differences you observed between your specimens? What were some of the challenges in viewing living organisms that move under the microscope? Be prepared to present your findings to the class.

Assessments

1. A microscope can focus on many fields of different depths. However, thick samples have to be sliced into very thin slices in order to be viewed under the microscope. Why do you suppose this is the case? [SP 3, Questions and Methods; SP 1, Concept Explanation]
2. *Elodea* plants appear as solid green objects to the naked eye, but appear mostly clear under a microscope. Explain this phenomenon. [SP 1, Concept Explanation]
3. A scientist takes a sample from a pond and examines it under their microscope. He thoroughly examines the wet mount and finds no living thing on the slide. He concludes that the pond is unable to support life. Is this a valid conclusion? Why or why not? [SP 1, Concept Explanation; SP 6, Argumentation]
4. Before the cell theory was developed, two previous theories were prevalent at different historical theories:
 - a. The Miasma Theory, where diseases were thought to be spread by miasma, a poisonous, foul-smelling vapor that can travel through the air.
 - b. The Theory of Spontaneous Generation, where living things could be generated from non-living matter, such as how maggots seem to appear spontaneously on meat left out in the open.

Explain how the invention of the microscope would lead to the replacement of both of these theories with the cell theory. [SP 1, Concept Explanation]

[Answer Key]

1. A thick slice lets less light through. A very thick slice will appear completely dark under a microscope.
2. The resolution of the naked eye is not high enough to distinguish between two subcellular structures, such as chloroplasts, which are the source of the green pigment. Under the microscope, we see that the green pigment is enclosed in the chloroplasts while the rest of the cell is colorless cytoplasm.
3. This is not a valid conclusion. The sample may not be representative of life in the rest of the pond. Also, there may be organisms that are too small to be resolved with the scientist's microscope.
4. The microscope would allow scientists to observe cells from patients affected by a disease and the changes in the cell's appearance when the cause of the disease is no longer present.
 - a. With a microscope, scientists would be able to analyze infection sites, mucus, etc. and see that it contains microscopic disease-causing organisms. They could also analyze putrid air and would find that it also contains these organisms, showing that it is not the air, itself, that causes disease.
 - b. With a microscope, scientists could examine a piece of meat left out in the open and would see fly eggs that hatch into maggots, showing that all organisms can only appear from other organisms.

Lab 3: Macromolecules in Common Food

This investigation addresses the following required course content and science practices from the AP CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
1.2 Elements of Life	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.A Describe the composition of macromolecules required by living organisms.	ENE-1.A.1. Organisms exchange matter with the environment to grow, reproduce, and maintain organization. ENE-1.A.2. Atoms and molecules from the environment, including carbon and nitrogen, are necessary to build new molecules.
1.3 Introduction to Biological Molecules	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.B Describe the properties of monomers and the types of bonds that connect monomers in biological macromolecules.	SYI-1.B.1. Hydrolysis and dehydration synthesis cleave and form covalent bonds between monomers.
1.4 Properties of Biological Molecules	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-B. Describe the properties of monomers and the types of bonds that connect monomers in biological macromolecules.	SYI-1.B.2 Structure and function of polymers, including proteins, carbohydrates, and lipids, are derived from the way their monomers are assembled.
1.5 Structure and Function of Biological Macromolecules	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.C Explain how a change in the subunits of a polymer may lead to changes in structure and function of the macromolecule.	SYI-1.C.1 Directionality of the subcomponents influences structure and function of polymers.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation <i>Explain biological concepts, processes, and models presented in written format.</i>	1.A Describe biological concepts and/or processes. 1.B Explain biological concepts and/or processes. 1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations <i>Analyze visual representations of biological concepts and processes.</i>	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B. Explain relationships between different characteristics of biological concepts, processes, or models represented visually in both theoretical and applied contexts. 2.C Explain how biological concepts or processes represented visually relate to larger biological principles, concepts, processes, or theories. 2.D Represent relationships within biological molecules, including diagrams.
SP 3 Questions and Methods <i>Determine scientific questions and methods.</i>	3.A Identify or pose a testable question. 3.B Predict the results of an experiment. 3.C Identify experimental procedures that are aligned to the investigative question. 3.D Make observations or collect data from representations or laboratory setups or results. 3.E Propose a new investigation.
SP 4 Representing and Describing Data <i>Represent and describe data.</i>	4.B Describe data from a table or graph.
SP 5 Statistical Tests and Data Analysis <i>Perform statistical tests and mathematical calculations to analyze and interpret data.</i>	5.D Use data to evaluate predictions.
SP 6 Argumentation <i>Develop and justify scientific arguments using evidence.</i>	6.A Make a scientific claim. 6.B Support a claim evidence from biological principles, concepts, processes, and/or data 6.D Explain the relationship between experimental results and larger biological concepts, processes, or theories. 6.E Predict the causes or effects of a change in, or disruption to, components in a biological system.

Macromolecules are important components in all living things, ranging from the smallest virus to the largest tree. Biologists typically define **macromolecules** as large, organic molecules that include carbohydrates, proteins, lipids and nucleic acids. Plants can produce all of these macromolecules using energy that has been converted from sunlight. The raw materials to build the building blocks of macromolecules come from simple molecules in the atmosphere and minerals from the soil. Bacteria, fungi, protists, and animals are not typically capable of obtaining the building blocks of macromolecules in this way. They must resort to eating other organisms for food and then rebuild the macromolecules they need within their cells.

Food labels are one way of understanding the types of macromolecules found in foods. The information on the labels is gathered by performing chemical tests to determine the presence and quantity of macromolecules in the food. Food processing companies and food agencies that monitor food use sophisticated equipment to test for macromolecules. Fortunately, there are a variety of simple macromolecule tests that were developed many years ago, before the advent of the instruments used today, that you can access. In this lab, you will use these simple chemical tests to investigate the macromolecular composition of different foods. Three of these tests are called colorimetric tests because they use a characteristic change in color to identify the molecules. A fourth test we will use depends on the solubility properties of a molecule to determine its presence.

So, how do these tests indicate the presence of the molecules you will be looking for? The Benedict's solution used to detect glucose contains an inorganic compound called copper citrate, which appears blue in solution due to the copper ion being in the Cu^{2+} state. Glucose is one of many sugars that contain an aldehyde group as part of its chemical structure. The aldehyde groups in carbohydrates are called **reducing agents** because they tend to cause chemical reduction by losing electrons and becoming oxidized. In the presence of copper citrate, glucose's aldehyde group reduces Cu^{2+} into Cu^+ . Cu^+ in the Benedict's solution then combines with oxygen to form a brick-red precipitate, as shown in Figure 3.1. No change in color indicates a negative result (–) and the absence of glucose while the change to brick red indicates a positive (+) result and the presence of glucose.

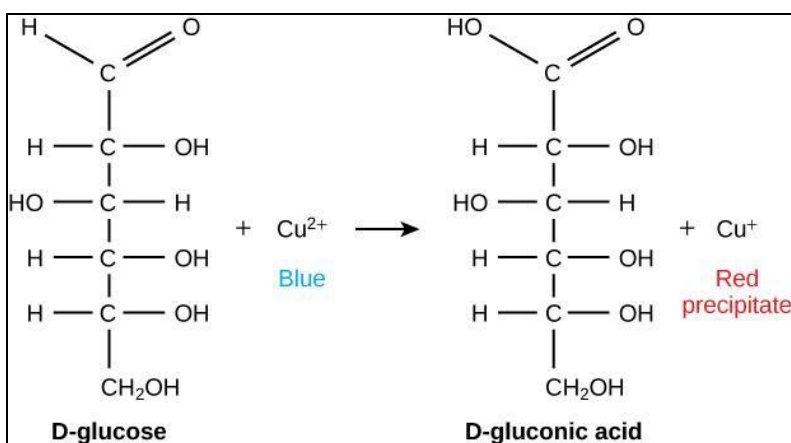


Figure 3.1: Reduction of copper by glucose.

The test of polysaccharides is highly specific for starch. It distinguishes starch from other types of polysaccharides as well as other types of molecules. Much of the starch found in our foods is a form called amylose, which has what are called alpha 1,4 bonds between the glucose subunits, as shown in Figure 3.2. In the presence of a potassium iodide solution, hydrogen bonds in the starch cause the starch to wrap around the electrically charged potassium iodide, as shown in Figure 3.3. In response to this reaction, the potassium iodide solution changes from an amber color, which indicates a negative result (–), to a dark blue or black color, which indicates a positive (+) result.

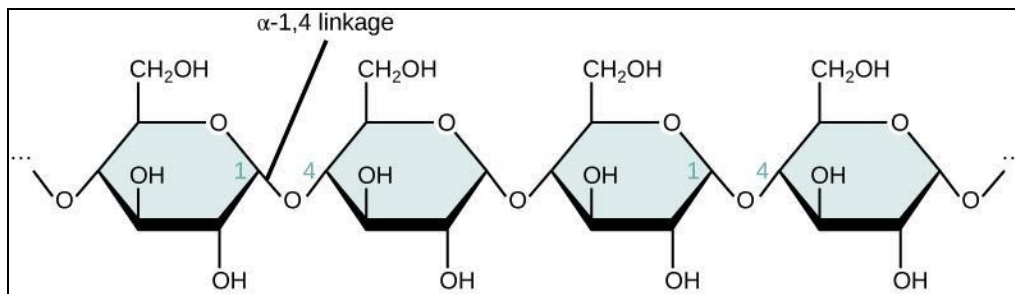


Figure 3.2: Starch alpha 1, 4 linkages.

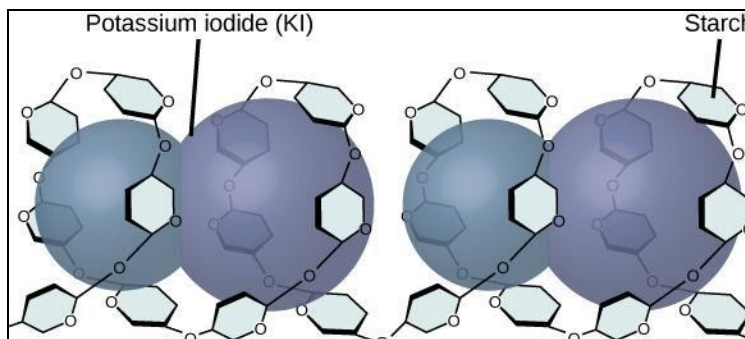


Figure 3.3: Starch wrapping around the potassium iodide molecules.

The **Biuret test** for detecting proteins relies on the ability of Cu^{2+} to react with the nitrogen in the peptide bonds of proteins, as shown in Figure 3.4. This can only occur under alkaline conditions. Biuret solution starts out a light blue and becomes purple in the presence of protein, meaning that purple indicates a positive test. You will work with two solutions of protein in order to be able to distinguish between dilute and concentrated levels of protein.

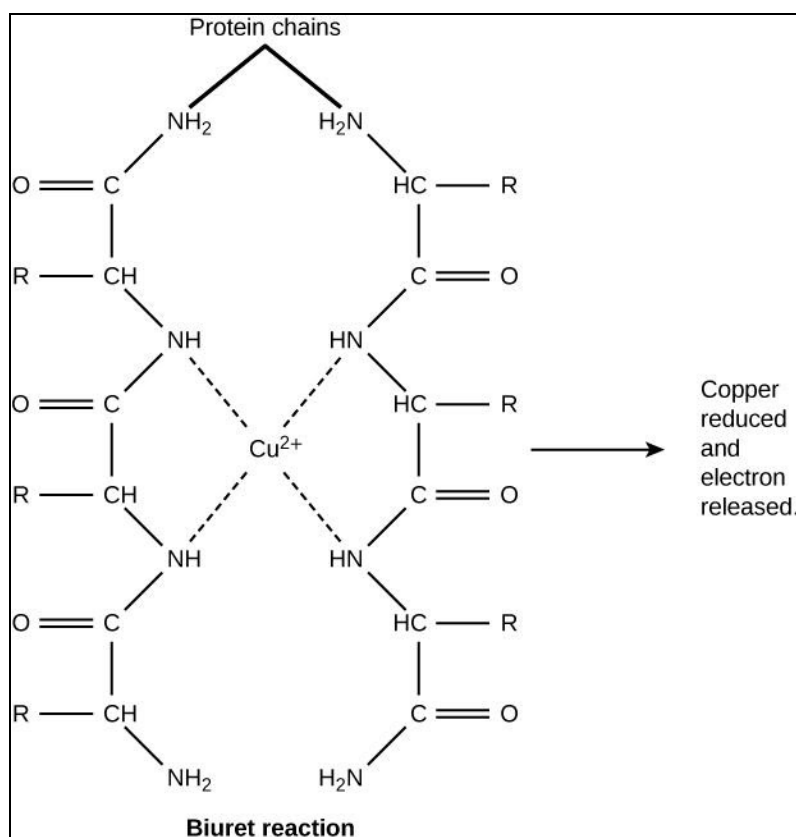


Figure 3.4: Chemistry of Biuret test.

There are several simple tests for detecting lipids. Unfortunately, the more accurate colorimetric tests use potentially harmful dyes that dissolve into the lipids as a way of indicating the presence of the lipids. In this test, therefore, you will use a classical food chemistry test that looks at the ability of lipids to make certain types of paper translucent. The best explanation of this is that when paper is dry, it reflects and scatters most of the light that falls upon it. Very little of the light passes through the paper, making it opaque. When lipids come in contact with paper, they adhere to the cellulose fibers and also fill in the spaces between the fibers. The lipids then act like a lens that lets most of the light pass through the paper rather than being reflected and scattered. Water and other liquids can produce a similar effect. However, it is not as intense and long-lasting as the translucent effect created by lipids. In this experiment you will look for the presence of lipids in pure vegetable oil and in a mixture of vegetable oil and water, which will serve as the positive control standards.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Review the structures and functions of the four classes of macromolecules.
- Ask students for examples of simple sugars. Most will mention hexoses such as glucose and fructose, but not other simple sugars such as glycerol, deoxyribose and ribose. Remind students that trioses and pentoses are also simple sugars.
- Ask students for examples of polysaccharides. Starch may be the obvious answer. Remind students that cellulose is one of the most abundant macromolecules in nature. Explain the role of the chitin in fungi and arthropods.
- Write on the board (or display taped on a slide) a table which lists the macromolecules and their building blocks.
- Be prepared to review the numbering of the carbon atoms in a simple sugar molecule because students will be confused by the mention of the alpha 1,4 bond linkage. Explain what an alpha carbon is.
- Review the formation of the peptide bond between amino acids.
- Explain why the fourth class, the nucleic acids, is not included in this exercise, because students will be puzzled by its absence. Here is a sample:
 - The main examples of nucleic acids are DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). Single nucleotides serve as important energy carriers in the cell, the most common one being ATP. Nucleic acids are not large contributors of nutrients in food. The Dische's diphenylamine test can be used to detect DNA in a mixture. In this test, a blue complex forms when diphenylamine binds to the sugar deoxyribose under acidic conditions. RNA does not yield a colored complex. The acidic Dische phenylamine solution is toxic and should be handled with extreme care.
- Be prepared to discuss the misconceptions that students have about the nutritional role of carbohydrates and fats which are considered “food villains” depending on the fad diet of choice. Remind students about the role of carbohydrates and fats in living organisms.
- Remind students that edibles used in the lab are not to be eaten no matter how tempting.

In this lab, you will learn

- to detect the presence of macromolecules based on their chemical structures;
- how the macromolecules found in a particular food provide evidence about the structure of the food;
- how changing the structure of macromolecules affects their functionality.

Activity 1: Pre-Assessment

Answer the following question in your notebook:

1. A chemical indicator called Benedict's solution turns from blue to brick red in the presence of certain monosaccharides. How would Benedict's test react in the presence of polysaccharides?
2. A chemical mixture containing copper sulfate undergoes a color change when it binds to the nitrogen atoms in certain macromolecules. What types of macromolecules can be detected using this solution?
3. Discuss the answers to questions 1 and 2 with your class.

Activity 1: Detecting Reducing Sugars, Starch, Proteins, and Lipids

The three major macromolecules that make up a bulk of our foods are carbohydrates, lipids, and proteins. Carbohydrates and proteins often consist of many smaller structural units, called **monomers**, linked together into long chains, called **polymers**. Before investigating the presence of these macromolecules in food, it is important to first perform what is called a positive control and negative control standards test for each macromolecule. Your positive control samples will contain the macromolecules glucose, starch, egg white protein, and vegetable oil. These samples will provide you with a reference for identifying each of the representative macromolecules in your food samples. Your negative control will be distilled water, which should provide results for the absence of the macromolecule in your food sample.

To begin your investigation, you will conduct a sequence of four tests for investigating carbohydrates. In test 1, you will use a procedure called the **Benedict's test** to confirm the presence of glucose, which is a monosaccharide found in many types of food. For test 2, you will use a solution of iodine to determine whether the polysaccharide starch is present in a solution. For test 3, you will use the Biuret test to identify if a solution contains protein. Then, in test 4, lipids will be detected using a very simple test called the **paper spot test**.

For this activity, you will work *in pairs*.

To expedite the lab activity, one team member should carry out the carbohydrate activities while the other team member does the tests on the lipids and the proteins.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Ask students to prepare a flow of work of the lab before coming to class in order to use their lab time efficiently.
- Review safety precautions stressing that goggles must be worn at all times, chemicals must be disposed of in the manner prescribed by the lab guide.
- Remind students that they are supposed to check their procedure with the instructor, who will circulate in the classroom to check each group.

- Demonstrate how to mark and read a tube at eye level to avoid parallax errors.
- For Benedict's test, remind students to label the upper part of the tube that is not immersed in water; otherwise, the writing will come off in the water bath.
- Point out that whenever the instructions mention *add*, the reagent should be added drop by drop along the side of the test tube to control the amount of solution added and to avoid splashes.
- Have students start their experiment by heating water for the Benedict's test and periodically measure the temperature. Students should record the temperature at which Benedict's test is performed.
- Remind students to 1) use a test tube holder when removing test tubes from the hot water bath, and 2) allow hot plates to cool down after use before moving or touching them. Hot plates should be turned off when not in use.
- If more than one group shares the beaker of hot water, remind students to number and add one lab partner's initials to the test tubes they will add to the water bath.
- Remind students that the Benedict's test is the only test that requires heat. Students may be confused and heat all the samples.
- Tell students to handle 10 percent NaOH with extreme care making sure that the Biuret test reagents are added in the correct order. In case of a spill, they should extensively wash their hands and alert the instructor. Any spill should be wiped carefully.
- If enough test tubes are available, save the tubes from Activity 1 as positive controls for Activity 2.

The following are recommendations for the Guided Inquiry for Activity 1:

- Have students write individual lab reports (papers) or detailed notes and conclusions in their lab notebooks. This is to ensure that each student contributes equally to the project, especially if students continued with the Guided Inquiry project in pairs. It will also encourage good lab practices while performing the actual experimental work.
- Remind students that they should submit their choices of food and procedure for approval before they proceed with research to ensure that they are testable given the available equipment and time. Some foods contain pigments that may give misleading results. For example, green pigment may yield a false positive in Benedict's reaction.
- Students often will not include all vital components of a research paper or presentation due to their inexperience. Provide a grading rubric for papers or presentations to help guide students to include the proper analyses and critical thinking in their papers/presentation.
- Provide an experimental set up to extract macromolecules from the foods analyzed. A pestle and mortar works well to mash most foods. Make sure that students choose food items that can be easily crushed.
- Ask students about which solvent they will use to extract macromolecules. Distilled water may be the obvious choice. It will be effective for proteins and carbohydrates.
- Ask students if distilled water will extract lipids. Students will have to crush food samples directly onto the paper.

- Remind students that no experiment is valid without a positive and negative control. Ask them what they will use as controls. The answer should be the tests from the structured inquiry.
- Ask students to reflect and discuss the qualitative versus quantitative aspect of their experiments. They should discuss the limitation of their experimental method. Discuss with the class the possibility of false positive and false negative results. For example, the green pigment in peas may give a false positive. An incomplete extraction may give a false negative.
- Discuss with students which components of a balanced diet were not included in their experiments. Students should mention vitamins, fibers, and minerals.

Safety Precautions

- Safety goggles must be used throughout the duration of the laboratory activity.
- It is highly recommended that you wear rubber gloves and a lab apron when handling any of the chemicals.
- Immediately inform your teacher of spills containing the test solutions. The acids and bases in the solutions can harm your skin.
- Immediately inform your teacher of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent other people from slipping.
- Immediately wash your hands if they directly come in contact with the test solutions. It is important to wash your hands after the laboratory activity to avoid any possible contamination of other surfaces with chemical residues that might be on your hands.

For this activity, you will need the following:

- Eight test tubes (one test tube per sample being tested)
- 10 ml graduated cylinder
- Grease pencil or washable marker
- One test tube rack
- One test tube holder
- Eight sample droppers or pipettes (do not mix pipettes from one solution to another)
- One thermometer
- Hot plate or microwave
- 250 ml beaker
- Brown paper bag
- Scissors
- Small ruler
- Hair dryer or lamp

Chemicals

- Distilled water
- Benedict's solution
- 1 percent copper sulfate solution or Biuret reagent
- 10 percent sodium hydroxide
- Lugol's solution or iodine-potassium iodide solution
- 5 percent glucose solution
- 2 percent starch solution
- 1 percent albumin solution or raw liquid egg white
- Vegetable oil

Structured Inquiry

Calibrating the test tubes:

Before beginning this activity, it is important to calibrate your test tubes to ensure accurate and consistent test results. To do this, you will need the test tube rack, seven test tubes, the 10 ml graduated cylinder, and a marker or grease pencil. For this laboratory activity, it is best to calibrate your test tubes in 1 ml increments. The maximum volume you will measure is 5 ml.

1. Collect eight test tubes, a test tube rack, the grease pencil or marker, and a 10 ml graduated cylinder.
2. Write out a procedure for calibrating your eight test tubes.
3. Get your procedure approved by your teacher before you continue with the calibration.
4. Use the grease pencil or marker provided by your teacher to draw the calibration lines.
5. Explain how estimating the quantities of a solution in an uncalibrated test tube could affect the results of an experiment. Explain if your study would have been more accurate if you calibrated your test tube using microliter units to mark your test tube. Record your answers in your laboratory notebook.

Carbohydrates:

As mentioned earlier, you will test for glucose and starch. In this investigation, explain which samples are the negative controls and which are the positive controls.

Use the following steps to test for the presence of glucose and starch.

Step 1: Place four clean test tubes in the test tube rack and use the grease pencil or marker to number each test tube as shown here.

Table 3.1

Test Tube Number	1	2	3	4
Test solution	Distilled water	Glucose test solution	Distilled water	Starch test solution

Step 2: Hypothesis/Prediction. Predict which of the tubes will show a positive result for the Benedict's test and which will show a negative result. Write your prediction in a table for recording the data from the tests.

Step 3: Which tubes represent the positive and negative controls for this experiment? Explain why in your notebook.

Step 4: Add approximately 150 ml of water into the 250 ml beaker. Use the hot plate or microwave to heat the water to about 80°C.

Step 5: Add 1 ml of distilled water to test tube 1. Then add 1 ml of the 5 percent glucose solution to test tube 2.

Step 6: Observe the appearance of each solution and record your observations in your table.

Step 7: Add 2 ml of the Benedict's solution to test tube 1 and to test tube 2. Observe the color of each solution and record your observations in your table.

Step 8: Place test tubes 1 and 2 in the beaker of heated water for 5 minutes. If using a hot plate, turn off the heat after removing the test tubes.

Step 9: Use a test tube holder to remove test tubes 1 and 2 and allow them to cool in the test tube rack. Observe the color of each solution and record your observations in your data table.

Step 10: Add 1 ml of distilled water to test tube 3. This will be your negative control standard for the starch test.

Step 11: Add 1 ml of the 2 percent starch solution to test tube 4. This will be your positive control standard.

Step 12: Observe the appearance of each solution and record your observations in your data table.

Step 13: Add 8 drops of the Lugol's solution or potassium iodide solution to test tube 3 and to test tube 4. Observe the color of each solution and record your observations in your data table. It may be useful to take photographs of your results.

Step 14: Cleanup:

- Empty test tubes 1 and 2 into a container designated for copper wastes or hazardous wastes.
- Empty test tubes 3 and 4 into a container designated for iodine wastes or hazardous wastes.
- Rinse out all of the test tubes with soap and water. Make sure to rinse out all of the soap from the test tubes.
- Dispose of the water from the 250 ml beaker into a sink only after it has cooled.
- Place the test tubes upside down in a test tube rack to dry.

Step 15: Critical Analysis: Discuss with your partner if your results match the expected results shown in Figure 3.5 and Figure 3.6. What could be some reasons for your results not matching the expected results in the figures? Explain the role of starch for plants and for the organisms that consume plants. Why can starchy foods be a problem for people with diabetes?

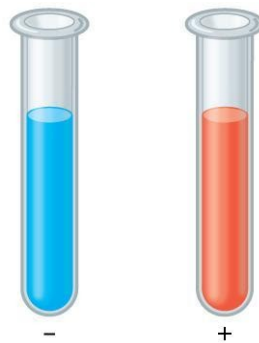


Figure 3.5: Benedict's test results.

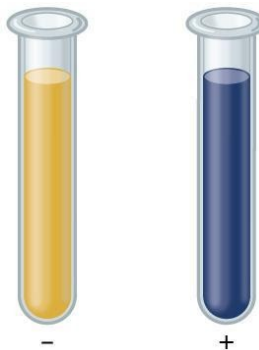


Figure 3.6: Iodine test results.

Proteins:

Step 1: Place three clean test tubes in the test tube rack and use the grease pencil or marker to number each test tube as shown here.

Table 3.2

Test Tube Number	1	2	3
Test solution	Distilled water	Dilute protein solution	Concentrated protein solution

Step 2: Hypothesis/Predict: Predict which of the tubes will show a positive result for the Biuret test and which will show a negative result. Write your prediction in a table for recording the data from the tests. Which tubes represent the positive and negative controls for this experiment? Explain why in your notebook.

Step 3: Add 2 ml of distilled water to test tube 1. Then add 1 ml of distilled water and 1 ml of the 1 percent albumin solution or raw egg white to test tube 2.

Step 4: Add 2 ml of 1 percent albumin solution or raw egg white to test tube 3.

Step 5: Observe the appearance of each solution and record your observations in your data table.

Step 6: Add 2 ml of the 10 percent sodium hydroxide to each test tube and carefully swirl the tubes to mix the solution.

Step 7: Add five drops of the 1 percent copper sulfate solution or Biuret reagent to each test tube. Carefully swirl the tubes to mix the solution.

Step 8: Observe the color of each solution and record your observations in your data table. It may be useful to take photographs of your results.

Step 9: Cleanup:

- Empty all test tubes into a container designated for copper wastes or hazardous wastes.
- Rinse out all of the test tubes with soap and water. Make sure to rinse out all of the soap from the test tubes.
- Place the test tubes upside down in a test tube rack to dry.

Step 10: Discuss with your partner whether your results match the expected results shown in Figure 3.7. What could be some reasons for your results not matching the expected results? Explain why the concentration of protein in a food is important in understanding a food's nutritional value for animals. Record your answers in your laboratory notebook.

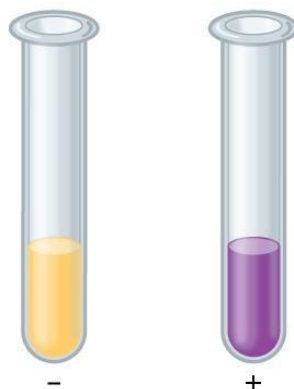


Figure 3.7: Biuret results.

Lipids:

As discussed in the introduction, you will use a strip of brown paper to indicate the presence of lipids in a solution. In this investigation, explain which samples are the negative controls and which are the positive controls. Why is it important to perform a negative control and a positive control before using these tests to analyze foods for lipids? Explain why it is important to test a lipid sample that is mixed with water. Write your answers in your laboratory notebook.

Use the following steps to test for the presence of lipids.

Step 1: Design a table for recording the data from the lipid test. You should show your table to the teacher before proceeding with the activity.

Step 2: Place three clean test tubes in the test tube rack and use the grease pencil or marker to number each test tube as shown here.

Table 3.3

Test Tube Number	1	2	3
Test solution	Distilled water	Water mixed with vegetable oil	Vegetable oil

Step 3: Use the scissors to cut a piece of the brown paper bag into a 7 cm × 15 cm rectangle. At each 5 cm interval along the long side of this rectangle, draw a line using the grease pencil so that you have three equal boxes. Label the cut sections of the bag according to Figure 3.9.

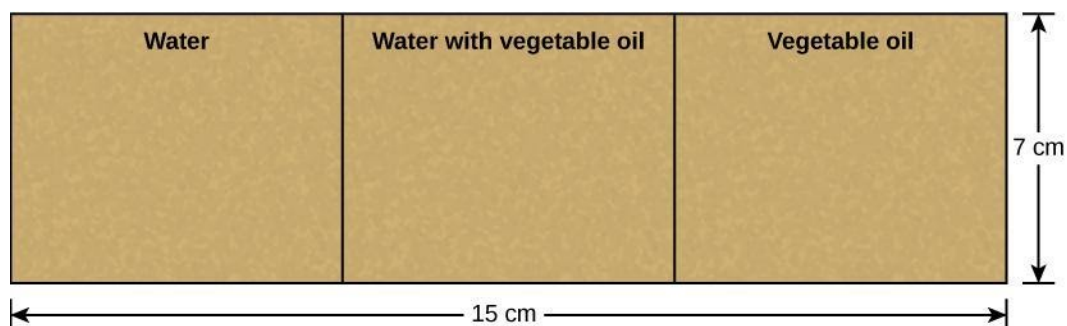


Figure 3.9: Labeled section of brown paper bag for lipid test.

Step 4: Add 1 ml of distilled water to test tube 1.

Step 5: Add 1 ml of distilled water and 1 ml of vegetable oil to test tube 2.

Step 6: Add 1 ml of vegetable oil to test tube 3.

Step 7: Use a pipette to collect the distilled water from test tube 1. Transfer the liquid to the brown paper bag. Add enough of the liquid to make a spot 1 cm in diameter on the section of the brown paper bag labeled *Water*.

Step 8: Gently swirl test tube number two until the solution is uniform. Quickly, use a pipette to collect the solution.

Step 9: Add enough of the liquid to make a spot 1 cm in diameter on the section of the brown paper bag labeled *Water mixed with vegetable oil*.

Step 10: Use a pipette to collect the vegetable oil from test tube 3. Transfer the liquid to the brown paper bag. Add enough of the liquid to make a spot 1 cm in diameter on the section of the brown paper bag labeled *Vegetable oil*.

Step 11: Record your results in your table. It may be useful to take photographs of your results.

Step 12: Critical Analysis: Discuss with your partner whether your results match the expected results shown below in Figure 3.10. What could be some reasons for your results not matching what was determined in the examples given in the figure? Explain the roles of fats in animals and plants. Why is knowing the lipid content of a food essential for human health? Record your answers in your laboratory notebook.

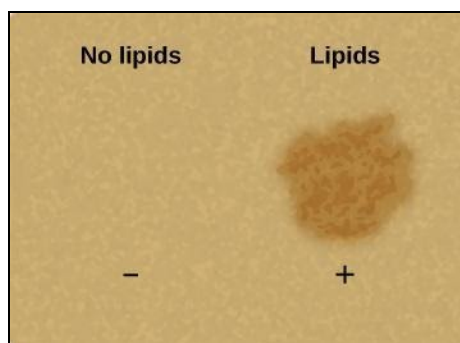


Figure 3.10: Paper spot test for lipids.

Step 13: Cleanup:

- Empty all of the test tubes into a laboratory sink.
- Rinse out all of the test tubes with soap and water. Make sure to rinse out all of the soap from the test tubes.
- Place the test tubes upside down in a test tube rack to dry.
- Dispose of the paper sheet in a trash can.

Guided Inquiry

Step 1: Hypothesize/Predict. Explain the types of nutrient macromolecules you would expect to find in food grouping 1, which includes potato chips, breakfast cereal, and popcorn. Do the same assessment for food grouping 2, which includes peanuts, beans, and peas. Make a hypothesis for which of these food groupings would be more appropriate for a balanced diet. Feel free to use a food pyramid and other resources to help with your assessment. Write your ideas in your laboratory notebook.

Step 2: Student-Led Planning. Write out the steps you will need to analyze the macromolecule content of the potato chips and the peanuts. Select only one food from each grouping. Remember to keep in mind how you will work safely in the lab and how you will dispose of the lab wastes. Submit your experimental procedure to your teacher. Once your teacher approves of your procedure, perform your experiment and record your data in an appropriate format. Write your results in your laboratory notebook.

Step 3: Critical Analysis. Which of the foods had the best balance of nutrients according to the United States Department of Agriculture? What about your procedure would you have to modify to test for the total amount of glucose available in both food groupings once these foods are processed by a person's digestive system?

Assessments

1. A student needs to calibrate an unmarked beaker in order to mix a food sample into 100 ml of distilled water and 50 ml of dilute acid. The student is provided with a 1 ml pipette, a 5 ml pipette, a 50 ml graduated cylinder, and a 100 ml flask. The student selects the 5 ml pipette to use in making markings at 50 ml increments on the beaker.
 - a. Did the student carry out the most accurate way for calibrating the beaker? Why or why not? [SP 3, Questions and Methods]
 - b. Which would be the correct apparatus for calibrating a test tube in two 1 ml increments? [SP 3, Questions and Methods]
2. Starch and glycogen are both composed of 1,4 bonded glucose polymers. Is it possible to use potassium iodide to detect the presence of glycogen in a food sample? Explain why or why not. [SP 1, Concept Explanation]
3. Glycoproteins are protein macromolecules that have carbohydrate groups attached to a polypeptide chain. Which of the tests that you used would likely be for detecting glycoproteins? Explain why. [SP 1, Concept Explanation; SP 3, Questions and Methods]
4. You need a definitive test to determine if an unknown food substance comes from an animal or a plant. Which of the four chemical tests would be the best test to distinguish animal from plant cells? Explain why. [SP 1, Concept Explanation; SP 3, Questions and Methods]
5. You are doing a study on crop nutrition. The study is investigating the effects of nitrogen fertilizer concentration on the protein content of plants. Design a simple experiment for carrying out this investigation. [SP 3, Questions and Methods]
6. Use the image of Benedict's test (Figure 3.11) to help you design an experiment to compare the glucose levels of different foods. [SP 3, Questions and Methods; SP 2, Visual Representations]

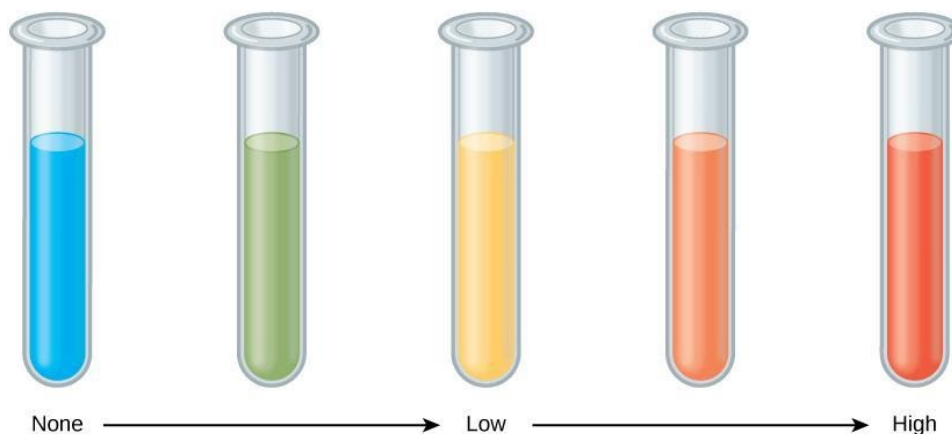


Figure 3.11: Benedict's test results for glucose concentration.

[Answer Key]

1.
 - a. No, the student should have selected the 50 ml graduated cylinder and marked off the beaker in 50 ml increments.
 - b. The student should select the 1 ml pipette to calibrate the test tube into 1 ml increments.
2. The specific arrangement of glucose molecules that characterizes starch causes the starch to wrap tightly around the potassium iodide. This tight wrapping causes the potassium iodide to turn blue or black. Glycogen may have enough similarity to starch that it may partly wrap around potassium iodide. In turn, glycogen may show a partially positive test.
3. It is more probable that the protein component of the glycoprotein has the same types of peptide bonds as other proteins. The carbohydrate component may or may not have the 1,4 bonds needed for the potassium iodide test to work. Therefore, is it more likely that the Biuret test would be the best test to use to detect the glycoproteins.
4. Unlike animals, plants contain starch, which is exclusively detectable with the potassium iodide test.
5. The Biuret test should be used to detect the protein in the plant samples, which were given different levels of nitrogen. In order to get accurate results, you would have to standardize the Biuret test so that low levels and high levels of protein appear in the test.
6. Students have to make sure the mass of each food is equal and the volumes of the reagents are each equivalent to ensure that the glucose per gram measurements of the foods are consistent.

Lab 4: Prokaryotic and Eukaryotic Cells

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED.

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
2.1 Cell Structure: Subcellular Components	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.D Describe the structure and/or function of subcellular components and organelles.	SYI-1.D.1 Ribosomes synthesize proteins according to mRNA sequence. SYI-1.D.2 Ribosomes are found in all cells, reflecting common ancestry of all known life. SYI-1.D.3 Rough endoplasmic reticulum compartmentalizes the cell and is associated with membrane-bound ribosomes in eukaryotic cells, whereas detoxification and lipid synthesis occurs in smooth ER SYI-1.D.4 Modification and packaging of newly synthesized proteins occur in the flattened membranous sacs of the Golgi. Other organelles include mitochondria, lysosomes, vacuoles, and chloroplasts.
2.2 Cell Structure and Function	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.E Explain how subcellular components and organelles contribute to the function of the cell. SYI-1.F Describe the structure features of a cell that allow organisms to capture, store, and use energy	SYI-1.E.1 Organelles that interact to support cell function include ER, mitochondria, lysosomes, and vacuoles. SYI-1.F.1 The folding of the inner membrane increases the surface area, which allows for more ATP to be synthesized. SYI-1.F.3 to SYI-1.F.9 The processes of photosynthesis and cellular respiration occur in the chloroplasts and mitochondria, respectively.

2.3 Cell Size	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.B Explain the effect of surface area-to-volume ratios on the exchange of materials between cells or organisms and the environment.	
2.11 Origins of Cell Compartmentalization	EVO-1 Evolution is characterized by a change in the genetic makeup of a population over time and is supported by multiple lines of evidence.	EVO-1.A Describe similarities and/or differences in compartmentalization between prokaryotic and eukaryotic cells.	EVO-1.A.1 Membrane-bound organelles evolved from once free-living prokaryotic cells via endosymbiosis. EVO-1.A.2 Prokaryotes generally lack internal membrane-bound organelles but have internal regions with specialized structures and functions. EVO-1.A.3 Eukaryotic cells maintain internal membranes that partition the cell into specialized regions.
7.6 Evidence of Evolution	EVO-2 Organisms are linked by lines of descent from common ancestry.	EVO-2.B Describe the fundamental molecular and cellular features shared across all domains of life, which provide evidence for common ancestry.	EVO2-2.B.1 Many fundamental molecular and cellular features and processes are conserved across organisms. EVO2-2.B.2 Structural and functional evidence supports the relatedness of organisms in all domains.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation <i>Explain biological concepts, processes, and models presented in written format.</i>	1.A Describe biological concepts and/or processes. 1.B Explain biological concepts and/or processes. 1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations <i>Analyze visual representations of biological concepts and processes.</i>	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B Explain relationship between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams.
SP 3 Questions and Methods <i>Determine scientific questions and methods.</i>	3.D Make observations, or collect data from representations of laboratory setups or results.
SP 5 Statistical Tests and Data Analysis <i>Perform statistical tests and mathematical calculations to analyze and interpret data.</i>	5.A Perform mathematical calculations, such as ratios.
SP 6 Argumentation <i>Develop and justify scientific arguments using evidence.</i>	6.A Make a scientific claim. 6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. 6.C Provide reasoning to justify a claim by connecting evidence to biological theories.

Cell theory states that all cells come from preexisting cells and that cells are the basic structure of living organisms. The subcellular structures within cells provide all the necessary machinery for life. An organism can be single-celled or multicellular. The human body contains trillions of cells. The latest research shows that you have as many bacteria in you as your own human cells. You have probably spent time in the past making lists of the parts of cells, but what will help you identify which type of cell you are looking at under a microscope?

All cells contain DNA, RNA, proteins, and a plasma membrane. There are two basic types of cells: prokaryotic and eukaryotic. Prokaryotic cells are simpler, do not contain internal membranes, and tend to be small (1 to 10 μm). Generally, prokaryotes are single-celled organisms, although recent studies suggest that prokaryotes can be multicellular (see References). There are two domains within prokaryotes: archaea and bacteria. Bacteria have

cell walls, which include the molecule peptidoglycan. Eukaryotic cells are more complex. DNA is enclosed in a membrane-bound structure called a nucleus. Eukaryotic cells also contain organelles (membrane-bound compartments), and tend to be larger (generally, 10 to 100 μm , with some egg cells large enough to be visible to the human eye). See Figure 4.1 for an illustration of the relative sizes of cells.

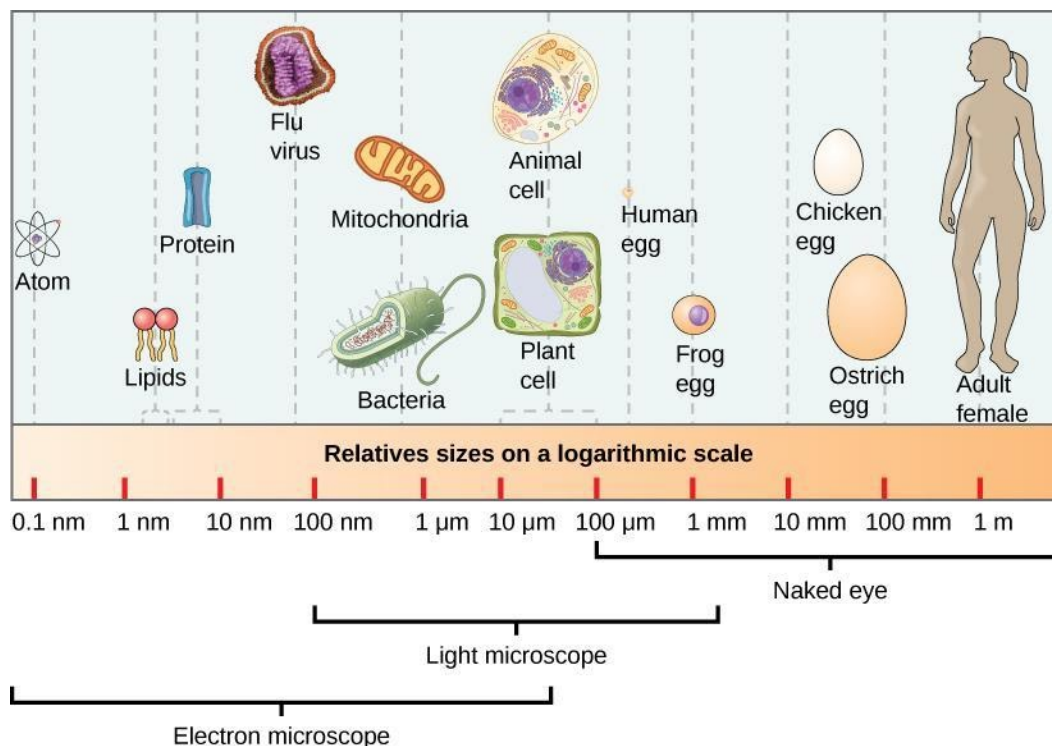


Figure 4.1: This figure shows relative sizes of microbes on a logarithmic scale. Recall that each unit of increase in the logarithmic scale represents a 10-fold increase in the quantity being measured.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Show diagrams of bacterial, plant, and animal cells so that students can compare and contrast each cell structure.
- Provide a scale diagram that compares the size of a typical bacterial cell to the size of a typical eukaryotic cell to help students visualize differences in cell size.

- Show micrographs of different cells so that students can see how cell structure varies. Bacterial micrographs could include coccus (spherical or round), bacillus (rod-shaped), and spiral (corkscrew-shaped) bacteria. A plant micrograph could be a cross section of a leaf. Point out that kidney-shaped guard cells, which differ in structure from other cells of the epidermis, allow air to travel in and out of the leaf. Animal cell micrographs could include nerve cells. Point out that the long extensions of the nerve cells allow communications to travel from one part of the body to another.
- Ask students to think about how they could determine if an unknown cell is a bacterial cell, plant cell, or animal cell. Then show the students one example of each cell type and ask them to identify what type it is.

In this lab, you will learn

- how to make wet mounts of bacteria, plant and animal cells and view them under the microscope
- how to observe and identify differences between cells and cell structures under low and high magnification and record observations
- how and why microscope stains are used when viewing cells under the microscope

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** What is the difference between the cells of a bacterium and the cells of your own body?
2. **Answer the following question in your notebook:** Compare and contrast the structures of prokaryotic and eukaryotic cells.
3. **Discuss the answers to questions 1 and 2 with a partner and then the class.**

Activity 1: Observation of *Anabaena* or *Nostoc*

Prokaryotes, unicellular organisms lacking a nucleus, include cyanobacteria (formerly blue-green algae; this name is now considered inaccurate because algae are eukaryotes). Cyanobacteria, like those shown in Figure 4.2, are **photoautotrophs**—organisms that carry out **photosynthesis** by using light energy, water, and carbon dioxide from the air and converting to sugars, and providing oxygen to the atmosphere as a waste product. Cyanobacteria contain pigments capable of capturing light energy but do not contain chloroplasts. Cyanobacteria are single-celled organisms, but some can form colonies with differentiated cell types. For example, some species can form specialized cells called **heterocysts**—structures containing enzymes which can take atmospheric nitrogen (**nitrogen fixation**) from the air and convert into usable molecules for DNA, RNA, and protein synthesis. Nitrogen fixation involves converting nitrogen gas (N_2) from the atmosphere to ammonia (NH_3). Ammonia is a form of nitrogen that can be used to build other molecules, including DNA, RNA, and proteins. Oxygen, a waste product of

photosynthesis, interferes with a key enzyme in nitrogen fixation. Thus, only a few cells in a colony (about 1 in 10) become heterocysts. Resources, such as ammonia and sugars from photosynthesis, are then shared between cells. These cells range from 1–40 micrometers in size. Not all bacteria can carry out photosynthesis; there are many species of heterotrophic bacteria living virtually everywhere on Earth. These cells are much smaller ranging from 0.5–8 micrometers. Eukaryotic cells have many more features and organelles and range in size from 10–500 micrometers.

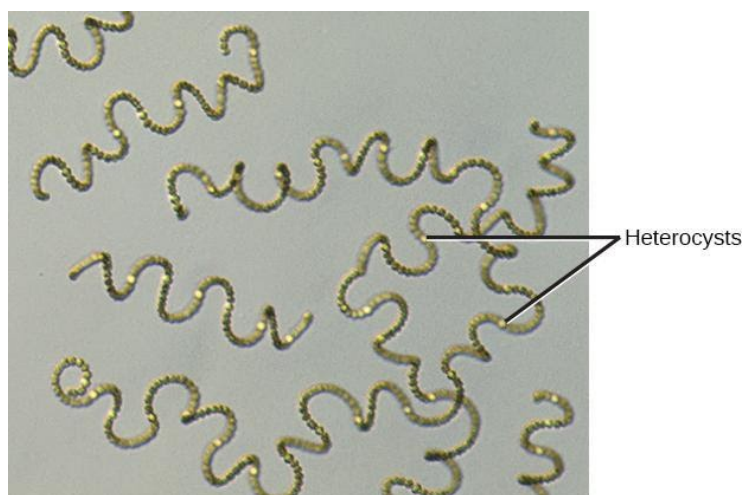


Figure 4.2: Cyanobacteria *Nostoc* with larger heterocysts.

Safety Precautions

- Be careful when handling glass slides, as the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Wash your hands with soap and water after handling live organisms.

For this activity, you will need the following:

- Light compound microscope (10x, 40x, 100x)
- Lens paper
- Prepared slide of *Anabaena* or *Nostoc* or images of *Anabaena* or *Nostoc*
- Special slide with micron ruler or clear millimeter ruler (you can photocopy ruler on overhead transparency, then cut and tape to microscope slide)

For this activity, students will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Explain that slides should be handled at the edge only so they do not get smudged with fingerprints. Remind students to be careful when handling glass slides and to inform you immediately if glass breaks. Be sure that students know how to dispose of broken glass.
- Explain that a microscope is delicate and must be handled with care. Tell students to use two hands to carry the microscope and to use only lens paper to clean the lenses.
- Identify the parts of the microscope, including the stage, coarse focus knob, fine focus knob, nosepiece, objective lenses and ocular lens. Show students how to adjust the amount of light that enters the microscope and how to change the objective lens. Tell students to watch carefully as they adjust the magnification so they do not strike the lens against the slide.
- Explain that magnification can be calculated by multiplying the magnification of the ocular lens by the magnification of the objective lens. Have students calculate all possible magnifications for the scopes they are using.
- Explain how to focus the microscope. Initially, adjust the focus using the coarse focus knob with the low power (10x) objective lens in place. Once the object is visible, use the fine adjustment knob to sharpen the focus. To increase magnification, switch to the medium power lens and use the fine focus knob to adjust the focus. Repeat this process to switch to the high-power lens. Make students aware that they must never use the coarse adjustment knob when focusing at medium or high magnification. If students cannot see the object they are trying to focus at high magnification, they will need to switch back to the low power lens and start over.
- Show students what an air bubble or a piece of lint looks like as students can mistake these for cells. Have students tell you when they think they have the cells in focus so you can confirm that they are actually looking at cells. Make sure students know how to calculate the magnification and to estimate the cell size.

Structured Inquiry

Step 1: Estimate the size of the field of view at all the magnifications of your microscope by placing a clear millimeter ruler on the stage of the microscope. This will allow you to estimate the sizes of cells. Convert your millimeter estimates to micrometers for this activity.

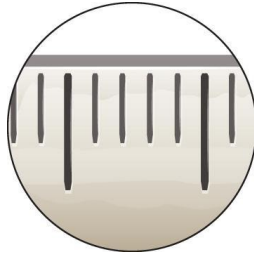


Figure 4.3: Example of a millimeter ruler taped to a microscope slide.

Step 2: Hypothesize/Predict. In your notebook, predict (draw) what you would expect to see through the microscope. How big do you predict the cells will be? What features do you expect to see? Do you expect to see organelles or a cell wall?

Step 3: In your notebook, create a detailed drawing, with a sharp pencil, of the structure of the cyanobacterium. An example of a detailed drawing is seen in Figure 4.4. Record the estimated size of the cells at the magnification used. Use color in your drawings if appropriate. Identify the colors used and label any obvious structures. Note the shapes or organization of the cells.

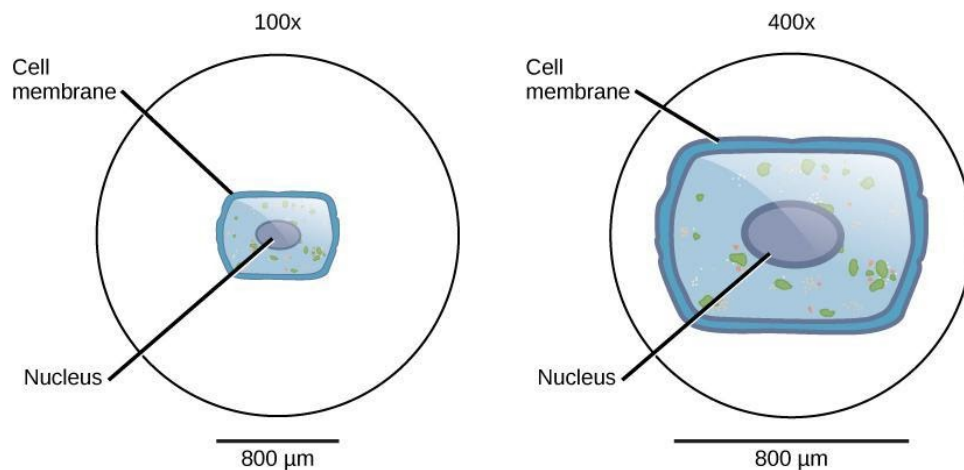


Figure 4.4: Example of a student drawing including labels and magnification.

Step 4: Critical Analysis: Think about the cell types you observed. Do your observations match your expectations? For example, given that the cyanobacteria is photosynthetic, was it the color you expected? Why? Did you expect the cells to have organelles? Did they? Did they have a cell wall? Did you find heterocysts? Discuss with your partner and write your answers in your notebook.

Assessments

1. Create a diagram of a general prokaryotic cell and a general eukaryotic cell. Label the cell structures that differ between the two cell types. [SP 2, Visual Representations]

2. How would internal membrane-bound structures, such as chloroplasts and mitochondria, allow chemical reactions to occur more efficiently in cells? [SP 1, Concept Explanation]

[Answer Key]

1. The main differences between the two diagrams should be that the eukaryotic cell should contain membrane-bound organelles, such as chloroplasts, mitochondria, etc., as well as a nucleus. The prokaryotic cell should not contain any of these organelles.
2. Internal membrane-bound structures can create isolated compartments for chemical reactions. Within these compartments, the cells can create ideal conditions for the chemical reactions, allowing them to proceed at a faster rate.

Activity 2: Pre-Assessment

1. **Answer the following question in your notebook:** What new structures would you observe in *Elodea* cells which are not present in a cyanobacterium cell?
2. **Answer the following question in your notebook:** Which of those structures would you expect to observe in an onion skin cell? Can you explain why some structures will be present in an *Elodea* cell but not in an onion epidermal cell?
3. Discuss the answers to questions 1 and 2 with a partner and with the class.

Activity 2: Comparing Plant Cells

Plant cells are eukaryotic; they have subcellular organelles. Like the bacteria, they have a **cell wall** to help keep the cell rigid—in plants, the cell wall is composed of a complex carbohydrate called *cellulose*. Plant cells, like that shown in Figure 4.5, also have a **nucleus** with DNA and a **large central vacuole** full of water and other important substances for maintaining life (such as carbohydrates, non-nutrients, and wastes) and cell pressure.

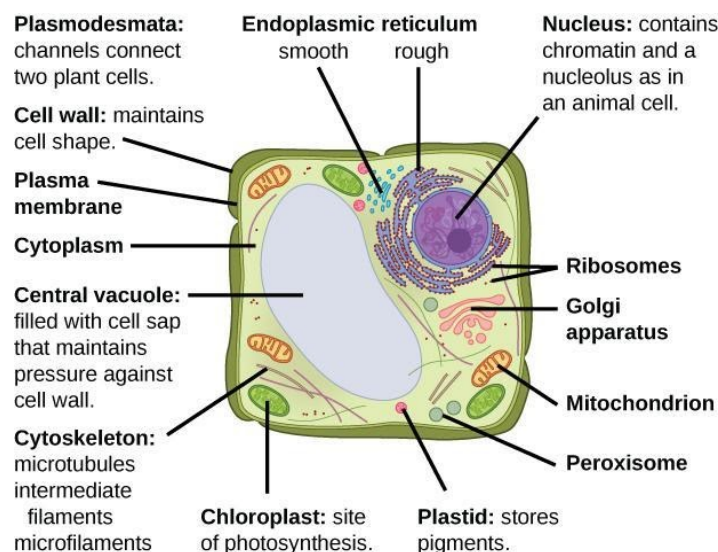


Figure 4.5: Structures found in a typical plant cell.

Safety Precautions

- Be careful when handling glass slides, the edges may be sharp.
- Dispose of used cover slips in a glass disposal box.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Wash your hands with soap and water after handling live organisms.

For this activity, you will need the following:

- *Elodea anacharis*, moss or *Spirogyra* green algae
- Light compound microscope
- Clean microscope slides and cover slips
- Lens paper

- Water and dropper
- Yellow onion
- Forceps
- Iodine solution (optional)

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Notes

The following are recommendations for the Guided Inquiry for Activity 2:

- Explain that leaf sections must be very thin or not enough light will pass through for students to see the cells. Explain that to prepare the slide, students should lay the leaf flat and add a drop of water. Then lay the cover slip over the liquid. Air bubbles can be removed by carefully pressing down on the edge of the cover slip. Explain that even in a very thinly sliced leaf, multiple cell layers will be present. The lens can be adjusted to focus on different layers.
- Ask students to note the color of the unstained cells and to think about why the color of *Elodea* leaves is different from that of onion leaves (*Elodea* leaves are photosynthetic and contain green pigment.)
- Explain that a drop of dye can be added at the edge of the cover slip while the slide is still on the stage. Ask students to think about why the dye is used, and to predict what structures will be visible in the absence and presence of dye.
- Students that have difficulty preparing a good slide can be directed to look at a better slide prepared by a different pair of students, or consult another group to determine how to re-prepare the slide.

Guided Inquiry

Step 1: Hypothesize/Predict. What features do you expect to see in the plant cell *Elodea* and the onion skin under low and high magnification? Draw and label your prediction in your notebook.

Step 2: Student-led Planning. Prepare wet mounts of *Elodea* and the onion skin. View with water only, and again in iodine solution (biological stain). Record your observations as drawings. Use color if present, label the magnification, and estimate the size of the cells in your notebook. Each partner is expected to prepare one sample. Each person should view, draw, state the size and magnification, and label each sample.

Step 3: Critical Analysis. What differences do you expect to see between *Elodea* and the onion? What similarities? If you used iodine as a stain, did that reveal any other differences in either

plant? Why would these organisms have anything in common based on your prediction in Step 1?

Assessments

1. Were the features you predicted to see in *Elodea* cells and the onion cell visible at low and high magnification? Which structures in Figure 4.5 can you identify in the *Elodea* cells? What about the onion cells? Why do you think this is the case? [SP 2, Visual Representations; SP 6, Argumentation]
2. What are the similarities and differences between cyanobacterium and a plant cell? [SP 1, Concept Explanation]

[Answer Key]

1. In the *Elodea*, you can clearly see the cell walls, and probably see cytoplasmic streaming of the chloroplasts around the central vacuole. The nucleus is not obvious. In the onion cell, the cell walls and central vacuole are visible, but there are no chloroplasts. This part of the onion is not exposed to sunlight and does not have active chloroplasts, so it is not green. Sometimes you can see the nucleus as a circular object. The nucleus is much more visible when an iodine solution is added to the onion.
2. Prokaryotic cells, like those of cyanobacteria, are smaller than eukaryotic cells, like those of plants. In *Elodea*, the obvious chloroplasts are membrane-bound organelles, which prokaryotic cells lack. In addition, the nucleus visible in the onion cells is not present in prokaryotic cells like the cyanobacteria.

Activity 3: Pre-Assessment

1. **Answer the following question in your notebook:** How do plant cells and animal cells differ? Why would these differences likely evolve in plant and animal cells?
2. **Answer the following question in your notebook:** What microscope techniques could help us see more structures within cells?
3. **Discuss the answers to questions 1 and 2 with a partner and then the class.**

Activity 3: Observe Animal Cells and Identify their Components

Animal cells are eukaryotic and possess subcellular components in common with the plant cells you observed in Activity 2. Organelles that plant and animal cells share in common include the nucleus, **Golgi apparatus**, **mitochondria**, **ribosomes**, and the **endoplasmic reticulum**. These are all participants in protein synthesis. An illustration of an animal cell is shown in Figure 4.6. There are some exceptions to these general components. For example, mature red blood cells (**RBC**) which have ejected their nuclei to have more room for **hemoglobin**, the protein that carries oxygen around the body. One of the easiest eukaryotic cells to obtain in the lab is the **squamous epithelial cell** (your cheek cells). These cells are arranged in a flat layer and are easy to remove and observe.

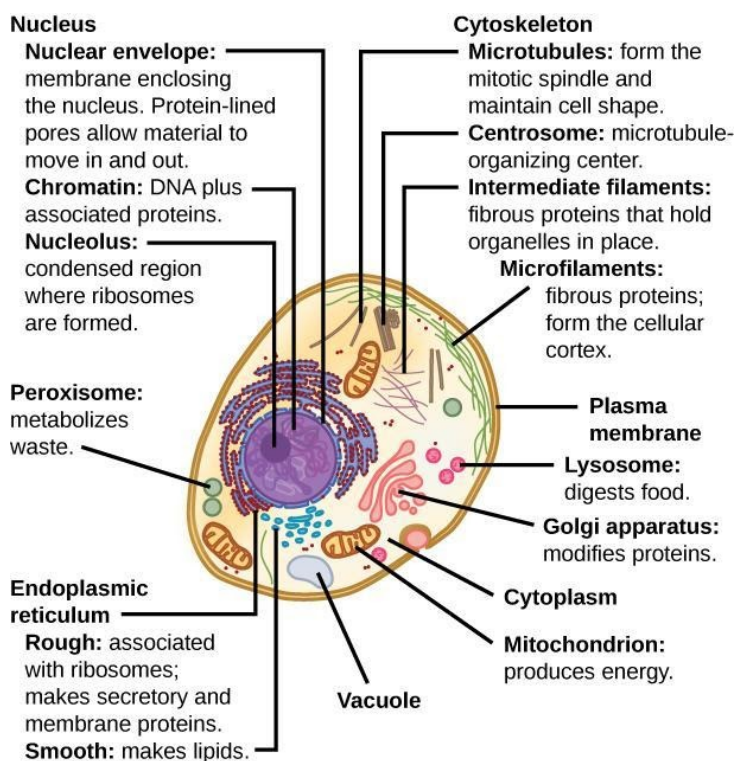


Figure 4.6: Diagram of the parts of an animal cell.

Safety Precautions

- Be careful when handling glass slides, as the edges may be sharp.
- Dispose of used cover slips in a glass disposal box.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.

- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Used cotton swabs are considered biohazard; dispose of swabs in the biohazard trash container as soon as you have used them.
- Methylene blue is a dye; be cautious not to ingest methylene blue.
- Wash your hands with soap and water after handling live organisms.

For this activity, you will need the following:

- Prepared slide of red blood cells
- Light compound microscope
- Clean microscope slide, cover slip
- Clean cotton swab
- 0.5–1 percent methylene blue solution
- Dropper or pipette
- Small squares of paper towels

For this activity, you will work *in pairs*.

Activity 3 Instructor Preparation and Teaching Notes

The following are recommendations for the Guided Inquiry for Activity 3:

- Remind students to handle the dye with care and to be careful with broken glass.
- Tell students to carefully wash their hands after preparing the cheek cell slides; otherwise they could spread germs.
- Ask students to think about how the color and shape of animal cells differs from that of bacterial cells and plant cells.
- Remind students that only lens paper can be used to clean the lenses and to use two hands to put the scope away.

Guided Inquiry

Step 1: Hypothesize/Predict. Predict the different features you expect to see in the animal cell versus the plant cell. Predict the differences you will see between animal cells and prokaryotic cells under low and high magnification. Include in your prediction the size differences between a cyanobacterium, plant cells, and animal cells. Create a table in your notebook to draw and label your predictions in your notebook.

Step 2: Student-led Planning. Observe the red blood cell prepared slide. Record your observations (draw and label any visible parts, use color if visible, include magnification and size of cells) in your notebook. Both partners should view, draw, state the size and magnification, and label each sample.

Step 3: Prepare your cheek cell slides as shown in Figure 4.7 and Figure 4.8 and outlined below.

- Take a clean cotton swab and gently scrape the inside of your mouth.
- Smear the cotton swab on the center of the microscope slide for 2 to 3 seconds.
- Add a drop of methylene blue solution (a dye) and place a coverslip on top.
- Remove any excess solution by allowing a paper towel to touch one side of the coverslip.
- View the slide at all magnifications.
- Record your observations as drawings. Use color if present, label the magnification, and estimate the size of the cells in your notebook. Record your observations (drawings, color if present, labels, magnification, and size of cell) in your notebook.

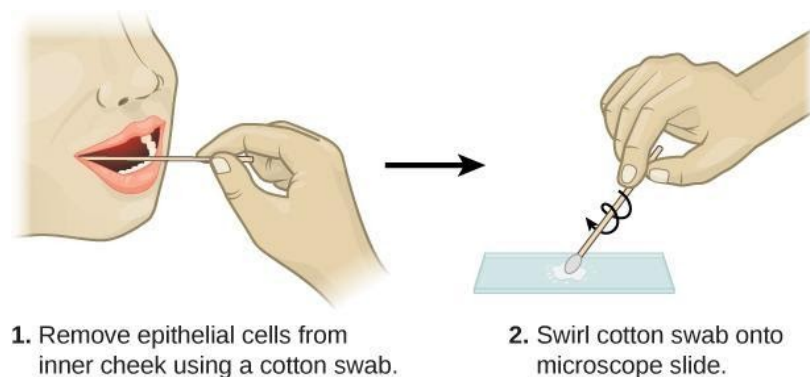


Figure 4.7: How to prepare a cheek swab.

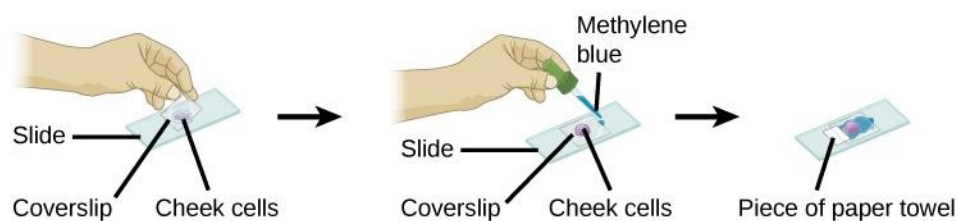


Figure 4.8: How to stain cheek cells with methylene blue dye.

Step 4: Critical Analysis. Were differences observed between the RBC and the cheek cell? What does the methylene blue stain reveal in the cheek cell? There should be small blue dots visible on the cheek cells much smaller than the nuclei. Hypothesize what those blue dots might be?

How does the animal cell compare to the plant cells in Activity 2 and the cyanobacteria in Activity 1? Record the answers to these questions in your notebook.

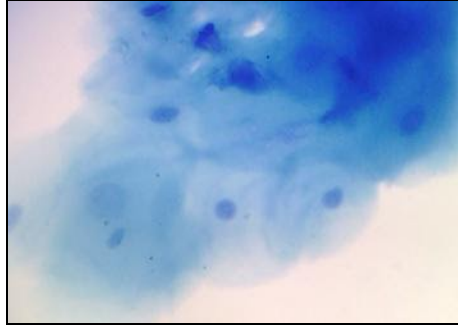
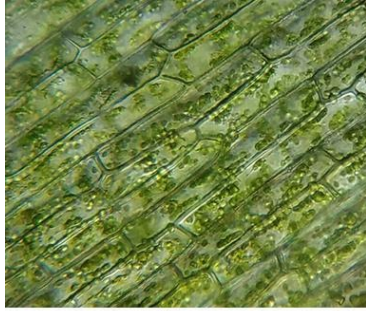


Figure 4.9: Cheek cells stained with methylene blue dye.

Assessments

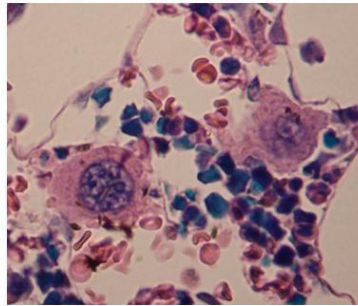
1. Based on the staining technique you performed in this activity, how could you distinguish stained prokaryotic cells from stained eukaryotic cells? [SP 1, Concept Explanation; SP 2, Visual Representation; SP 3, Questions and Methods]
2. What do all cells have in common, whether prokaryotic or eukaryotic? What major differences would you expect to find? [SP 1, Concept Explanation]
3. Identify whether the following images show an animal cell, a plant cell, or a prokaryote cell. Explain how you know the difference. [SP 1, Concept Explanation; SP 2, Visual Representations]



(a)



(b)



(c)

[Answer Key]

1. The stained prokaryotic cells should be much smaller than the eukaryotic cells and would likely be located on the outside of the eukaryotic cells. In addition, the eukaryotic cell would contain a stained nucleus while the prokaryotic cells would not contain the nucleus.
2. In common: Living cells must have nucleic acid (DNA) whether present in an organelle in eukaryotes or in the nucleoid in prokaryotes. They must have a boundary membrane to separate their metabolic processes from the environment. Differences: Eukaryotes would be larger and have a visible nucleus.
3. Cell (a): Plant cell, cell wall, geometric shape, visible chloroplasts, central vacuole
 Cell (b): Prokaryote, very small at high magnification, no obvious subcellular features.
 Cell (c): Animal cell, no cell wall, obvious nucleus, visible subcellular structures, no chloroplasts or large central vacuole.

Lab 5: Subcellular Structures

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT:

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
2.1 Cell Structure: Subcellular Components	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.D Describe the structure and/or function of subcellular components and organelles.	SYI-1.D.1 Ribosomes comprise rRNA and protein and are the sites of protein synthesis. SYI-1.D.2 Ribosomes are found in all cells, reflecting common ancestry of all known life. SYI-1.D.3 Endoplasmic reticulum occurs in two forms—smooth and rough. Rough ER is associated with membrane-bound ribosomes and also compartmentalizes the cell. Smooth ER functions include detoxification and lipid synthesis. SYI-1.D.4 Folding and chemical modification of newly synthesized proteins occur in the flattened sacs of the Golgi complex. Other organelles include mitochondria, chloroplasts, lysosomes, and vacuoles.
2.2 Cell Structure and Function	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.E Explain how subcellular components and organelles contribute to the function of the cell. SYI-1.F Describe the structural features of a cell that allow organisms to capture, store, and use energy.	SYI-1.E.1 Organelles and subcellular components—including mitochondria, ER, ribosomes, lysosomes, and vacuoles—interact to support cellular function. SYI-1.F.1 The folding of inner membranes increases surface area, which allows for more synthesis of ATP.

			SYI-1.F.2 to SYI-1.F.7 The light-dependent reactions of photosynthesis occur in the grana of the chloroplasts, whereas the carbon-fixing reactions occur in the stroma.
2.11 Origins of Cell Compartmentalization	EVO-1 Evolution is characterized by a change in the genetic makeup over time and is supported by multiple lines of evidence.	EVO-1.A Describe similarities and/or differences in compartmentalization between prokaryotic and eukaryotic cells. EVO-1.B Describe the relationship between the functions of endosymbiotic organelles and their free-living ancestral counterparts.	EVO-1.A.1 & EVO-1.B.1 Membrane-bound organelles evolved from once free-living prokaryotic cells via endosymbiosis. EVO-1.A.3 Eukaryotic cells maintain internal membranes that partition the cell into specialized regions.
3.5 Photosynthesis	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.I Describe the photosynthetic processes that allow organisms to capture and store energy. ENE-1.J Explain how cells capture energy from light and transfer it to biological molecules for storage and use.	ENE-1.I.1 Photosynthesis first evolved in prokaryotic organisms, resulting in an oxygenated atmosphere. These pathways were the foundation of eukaryotic photosynthesis. ENE-1.J.2 Photosystems I and II are embedded in the internal membranes of chloroplasts.
7.6 Evidence of Evolution	EVO-2 Organisms are linked by lines of descent from common ancestry.	EVO-2.B Describe the fundamental molecular and cellular features shared across all domains of life, which provide evidence of common ancestry.	EVO-2.B.1 Many fundamental molecular and cellular features and processes are conserved across organisms. EVO-2.B.2 Structural and functional evidence supports the relatedness of organisms in all domains.

SCIENCE PRACTICES:

Science Practice	Skill
SP 1 Concept Explanation <i>Explain biological concepts, processes, and models presented in written format.</i>	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations <i>Analyze visual representations of biological concepts and processes.</i>	2.A Describe characteristics of a biological concept, processes, or model represented visually. 2.B Explain relationships between characteristics of biological concepts, processes, or models represented visually in applied contexts.
SP 3 Questions and Methods <i>Determine scientific questions and methods.</i>	3.B State and null and/or alternative hypotheses, or predict the results of an experiment. 3.D. Make observations, or collect data from representations of laboratory setups or results.
SP 6 Argumentation <i>Develop and justify scientific arguments using evidence.</i>	6.A. Make a scientific claim. 6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. 6.E Predict the cause or effects of a change to one or more components in a biological system based on data.

Eukaryotic cells are recognizable by the presence of subcellular structures like organelles. Organelles like **mitochondria** for cellular respiration, lysosomes, endoplasmic reticulum, and **chloroplasts** that capture light energy and manufacture energy rich compounds. Visualizing these structures under the light microscope can be difficult since the organelles appear transparent without the use of biological stains. However, some organelles are much easier to visualize due to the presence of organic molecules such as chlorophyll and other pigments.

Plastids are double-membraned subcellular structures found in algae and plants. The theory of endosymbiosis, illustrated in Figure 5.1, is supported by the findings of Lynn Margulis at the University of Massachusetts in the 1960s. Evidence that a cyanobacterium was engulfed and took up residence inside a eukaryotic ancestor is strengthened by the presence of circular DNA and bacterial sized ribosomes inside the plastids. Plastid DNA was transferred to the host DNA, leaving only the partial circular genomes of their free-living cousins.

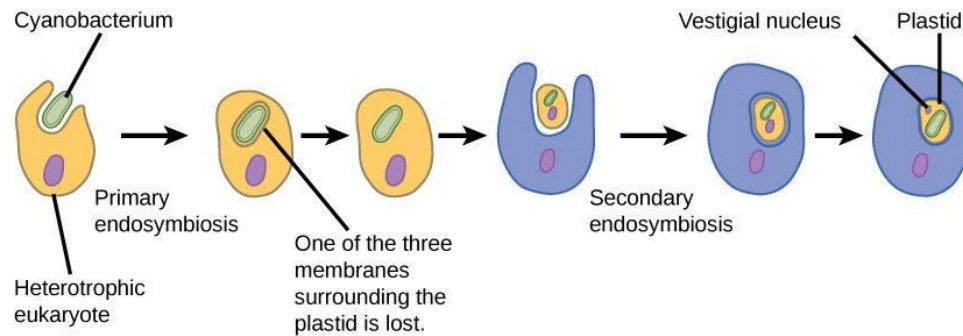


Figure 5.1: Endosymbiosis is the theory that eukaryotes gained membrane-bound organelles by evolving a symbiotic relationship with prokaryotic cells.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Make sure students understand the definition of symbiosis, and explain that a symbiotic relationship can be obligatory, meaning that one organism cannot survive without the other.
- Explain that mitochondria and chloroplasts are believed to have arisen through endosymbiosis, a process in which an ancestral eukaryotic cell engulfed a smaller cyanobacterium. The two cells formed an obligatory symbiotic relationship in which the cyanobacterium provides energy to the larger cell, and the larger cell protects the cyanobacterium.
- Show students diagrams of a chloroplast and mitochondrion. Ask them to identify evidence of endosymbiosis in these organelles, including the presence of inner and outer membranes, DNA, and ribosomes.
- Ask students to describe a common function of chloroplasts and mitochondria. Response should indicate that both of these organelles provide energy for the cell. Chloroplasts capture energy from sunlight, and mitochondria extract energy from food.
- Explain that a chloroplast is a type of plastid. Other types of plastids include chromoplasts, which contain colored pigments such as those found in flowers, and amyloplasts, which store starch.

In this lab, you will learn

- to prepare wet mounts of carrot slices and observe chromoplasts;
- to prepare wet mounts of potato slices and observe before and after staining with iodine;

- to make wet mounts of red onion skin;
- the effect of pH on red onion skin;
- to observe Nissl bodies in a neuron as an example of endoplasmic reticulum;
- to observe striated muscle as an example of cytoskeleton thin filaments;
- to visualize mitochondria using a biological stain.

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** Why would pigments make visualizing subcellular structures easier under the light microscope?
2. **Answer the following question in your notebook.** How could you tell the difference between a lipid-soluble pigment and a water-soluble pigment under a light microscope?
3. **Discuss the answers to questions 1 and 2 with a partner (think, pair, and share) and then the class.**

Activity 1: Chromoplasts and Amyloplasts

All plastids originate from proplastids that can differentiate into several mature types of plastids, as shown in Figure 5.2, similar to the idea of a stem cell. **Chloroplasts** (“green bodies”) are an example of a mature plastid that contain **pigments**, water insoluble lipid molecules that can be found in membranes and are capable of absorbing light energy. These start the process of photosynthesis, as shown in Figure 5.3. You will examine a few different types of plastids in this lab. The two plastids that are examined in this activity are the **chromoplasts** and the **amyloplasts**, shown in Figure 5.4 and Figure 5.5, respectively. Chromoplasts, or *colored bodies*, are mature plastids that are used primarily for the synthesis of pigments and their storage. Chromoplast pigments are found in many plant parts such as flowers, roots, and aging leaves. The purpose of these plastid pigments is to attract pollinators to the plant and to advertise to different animals that eat the fruit (such as mammals, birds, insects, and reptiles) and then disperse the seeds, generally with a bit of fertilizer. These pigments are also found in root vegetables such as carrots and yams. Amyloplasts, meaning *starch bodies*, are a type of leucoplast, or *white body*, that are colorless; these plastids synthesize glucose into starch, store starch, and help the plant detect gravity using statoliths.

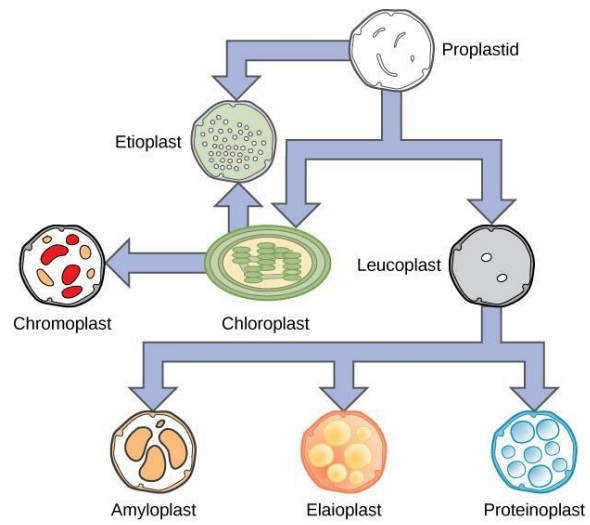


Figure 5.2: Protoplastids are the stem for several other plastids.

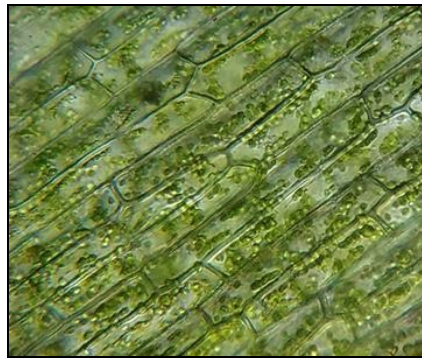


Figure 5.3: A micrograph of chloroplasts within plant cells.



Figure 5.4: A micrograph of chromoplasts within plant cells

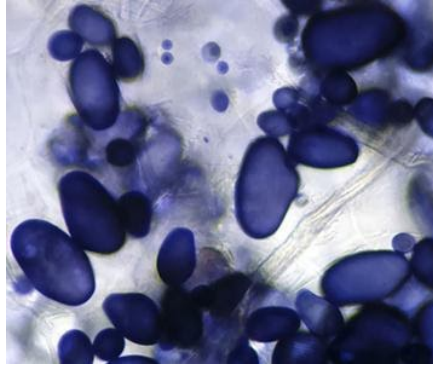


Figure 5.5: A micrograph of amyloplasts within plant cells.

Safety Precautions

- Be careful handling glass slides, the edges may be sharp.
- Cut away from your body. Sectioning of potato and carrots involves using a single-edged razor blade. Use care to avoid cuts.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Dispose of biological stains according to teacher instructions and local regulations.
- Wash your hands with soap and water after handling biological stains.

For this activity, you will need the following:

- Clean microscope slides and coverslips
- Single-edge razor blade (only for preparation purposes, **not** for students to use)
- Carrot and potato
- Iodine stain (or Lugol's stain) in dropper bottle
- Paper towels

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Let students know that slides should be handled at the edge only so they do not get smudged with fingerprints. This activity recommends you, as the instructor, use the razor blade or knife to pre-cut the carrot and potato slices. Remind students to be careful handling glass slides and to inform you immediately if glass breaks. Be sure students know how to dispose of broken glass.
- Explain that a microscope is delicate and must be handled with care. Tell students to use two hands to carry the microscope and to use only lens paper to clean the lenses.
- Identify the parts of the microscope, including the stage, coarse focus knob, fine focus knob, nosepiece, objective lenses, and ocular lens. Show students how to adjust the amount of light that enters the scope, and how to change the objective lens. Tell students to watch carefully as they adjust the magnification so they do not strike the lens against the slide.
- Explain that magnification can be calculated by multiplying the magnification of the ocular lens by the magnification of the objective lens. Have students calculate all possible magnifications for the scopes they are using.
- Explain how to focus the microscope. Initially, adjust the focus using the coarse focus knob with the low power (10x) objective lens in place. Once the object is visible, use the fine adjustment knob to sharpen the focus. To increase magnification, switch to the medium power lens and use the fine focus knob to adjust the focus. Repeat this process to switch to the high-power lens. Make students aware that they must never use the coarse adjustment knob when focusing at medium or high magnification. If students cannot see the object they are trying to focus at high magnification, they will need to switch back to the low power lens and start over.

Structured Inquiry

Step 1: Make extremely thin slices of the carrot. The slice should be as transparent as possible. Place the carrot slice on a clean microscope slide with a coverslip. If the coverslip does not lie flat, the slice is too thick. Observe under middle (100x) and high power (400x) and draw and label what you observe in your notebook, and record the magnification. Note: you do not need to add a drop of water before placing the coverslip.

Step 2: Hypothesize/Predict. In your notebook predict (draw) what you expect to see in the microscope with a potato slice. Will the addition of a biological stain (a dye that stains cellular structures) help visualize subcellular structures? Which structures do you think will be more obvious with the stain? Write your predictions in your notebook.

Step 3: Repeat Step 1 with the potato slice. Do not add iodine. Observe under high power (100x) and then draw and label what you observe in your notebook, recording the magnification.

Step 4: Add a drop of iodine stain to the edge of the coverslip. Grasp a small piece of paper towel and dab it to the opposite side of the coverslip to draw the iodine through the potato slice, as illustrated in Figure 5.6. Observe the slice under high power and draw and label what you observe in your notebook, recording the magnification.

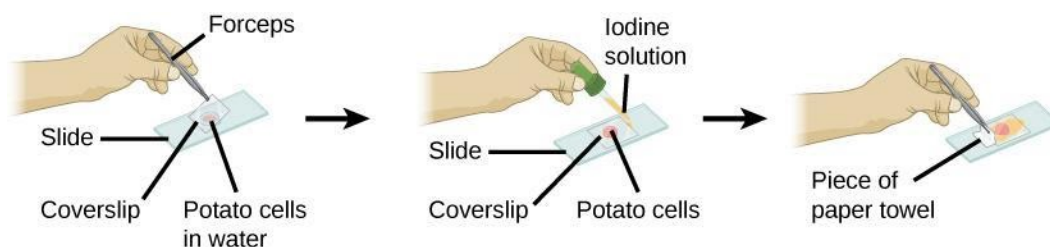


Figure 5.6: Process for making a wet mount cell of potato tissue.

Step 5: Critical Analysis: Are the predictions you made in Step 2 supported by your data and observations? Why or why not? Are there any methods you could use to improve your results? Discuss with your partner and then write your answers in your notebook.

Assessments

1. How do your drawings of the microscope slides compare to the internet images in Figure 5.3, Figure 5.4, and Figure 5.5? How are you convinced that the chromoplast pigments were lipids? [SP 3, Visual Representations; SP 3, Questions and Methods]
2. Differentiate between the pigment functions in chromoplasts and in chloroplasts. [SP 1, Concept Explanation]
3. Iodine (or Lugol's solution) is not a very water-soluble stain. What type of molecules do you think the iodine solution is staining in the potato slice? [SP Concept Explanation; SP 3 Questions and Methods]

[Answer Key]

1. Depending on the student's microscope skills, the internet images may be higher magnification. Students should note that the plastids are colored in the carrot (carrots have carotenoids—lipid soluble pigments) that range from yellow to red and are visible to the human eye but hard to see in the potato without the iodine stain. (Iodine stain is not very water soluble, since starch is non-polar the iodine does stain the starch as well as other lipids in the cell membrane and other subcellular membranes).

2. Chloroplasts are a specialized chromoplast that contain the pigment chlorophyll, which captures light energy for use in photosynthesis. Other chromoplasts have lipid soluble pigments, such as carotenoids that are also used in photosynthesis and provide orange color to flowers and leaves. Chloroplasts can change into chromoplasts when fruit ripens.
3. Iodine (or Lugol's solution) is not very water soluble and primarily used to detect non-polar starches and lipids. Therefore, iodine can also stain cell membranes and other membrane-bound organelles.

Activity 2: Pre-Assessment

1. **Answer the following question in your notebook:** How do plant cells and animal cells capture energy that they use to survive?
2. **Answer the following question in your notebook.** How do the different ways that plant and animal cells acquire energy reflect the different ways in which they live?
3. **Discuss the answers to questions 1 and 2 with a partner and then the class.**

Activity 2: Central Vacuole and Anthocyanins

Plant cells are eukaryotic; they have subcellular organelles. Like bacteria they have a **cell wall** to help keep the cell rigid—although in plants the cell wall is composed of a complex carbohydrate called cellulose. Plant cells also have a **nucleus** with DNA and a **large central vacuole** (see Figure 5.7) full of water and other important substances (such as carbohydrates, non-nutrients, wastes) for maintaining life and cell pressure.

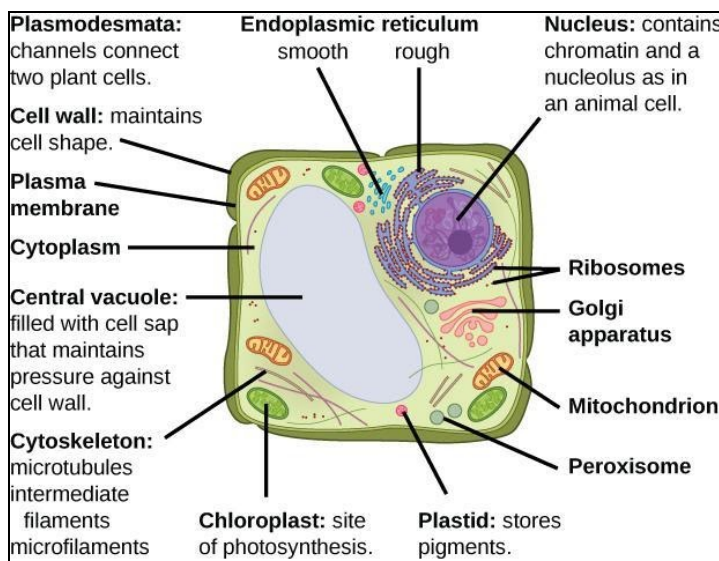


Figure 5.7: Structures of a generalized plant cell.

The second major type of pigment found in plastids is water soluble. These pigment molecules, called **flavonoids**, are stored in the central vacuole of plants. The specific pigment found in the central vacuole that is examined in this activity is called **anthocyanin** (meaning *flower blue*) the addition of a sugar group to its structure makes it water soluble. If cations (positively charged ions or polyatomic ions) are added or removed from the anthocyanin structure the pigment color will change, showing the anthocyanins are sensitive to pH changes. Anthocyanins are found in most plant tissues as well as algae and some bacteria. Anthocyanins are hypothesized to protect plant tissues from harmful UV radiation and are also used as camouflage from herbivores.

Safety Precautions

- Be careful handling glass slides, the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Wear goggles when handling pH solutions.

- If any pH solutions get on your hands, flush with water to remove.
- Dispose of pH solutions according to teacher instructions and local regulations.
- Wash your hands with soap and water after handling pH solutions.

For this activity, you will need the following:

- Red onion
- Clean microscope slides and cover slips
- Water and dropper
- Forceps
- pH solutions in dropper bottles (pH 3.0, pH 7.0, and pH 8.5)
- Notebook to observe and draw features of plant cells.

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 2:

- Show a diagram of a plant cell and to point out the nucleus, central vacuole, chloroplasts, and cell wall. Ask students to describe the function of each of these structures.
- Show a picture of flavonoid-containing fruits and vegetables, including strawberries, blueberries, cherries, and tomatoes.
- Show the structure of anthocyanin. Explain that the structure of anthocyanin changes with pH, which causes a change in color.
- Demonstrate that anthocyanin can be used as a pH indicator. To prepare a demonstration, boil red cabbage in water. Dilute the cabbage juice enough to see through and add the diluted juice to a series of test tubes. Add substances that differ in pH to each test tube (it helps if the substances are colorless) and ask students to note the color. Substances that can be used include white vinegar; lemon-lime flavored soft drinks, tap water, baking soda, and ammonia.
- Remind students to handle the microscope with care, and to use care with glass slides and coverslips.

Structured Inquiry

Step 1: Hypothesize/Predict: What subcellular features do you expect to see in red onion cells? Will the central vacuole, nucleus, and chromoplasts be obvious? Will any features differ

between red and white onion cells? If not, hypothesize how you would be able to see them? What do you hypothesize will happen to the red onion cells in various pH solutions? Record your ideas and hypothesis in your notebook.

Step 2: Student-led planning: Prepare your plant cell slides as shown in Figure 5.8. Each partner is expected to prepare one sample. Each of you should view, draw, state the size and magnification, and label each sample. To prepare the onion skin, cut the onion in quarters, take the outer red peel of an onion section, and place it on the microscope slide. Experiment with a few different cuts to determine the best technique to get the thinnest possible slice. Spread the onion skin on the microscope slide, put one small drop of water and top with a coverslip. Record your observations (drawings, color if present, labels, magnification, and size of cell) in your notebook, keeping in mind your hypotheses from above.

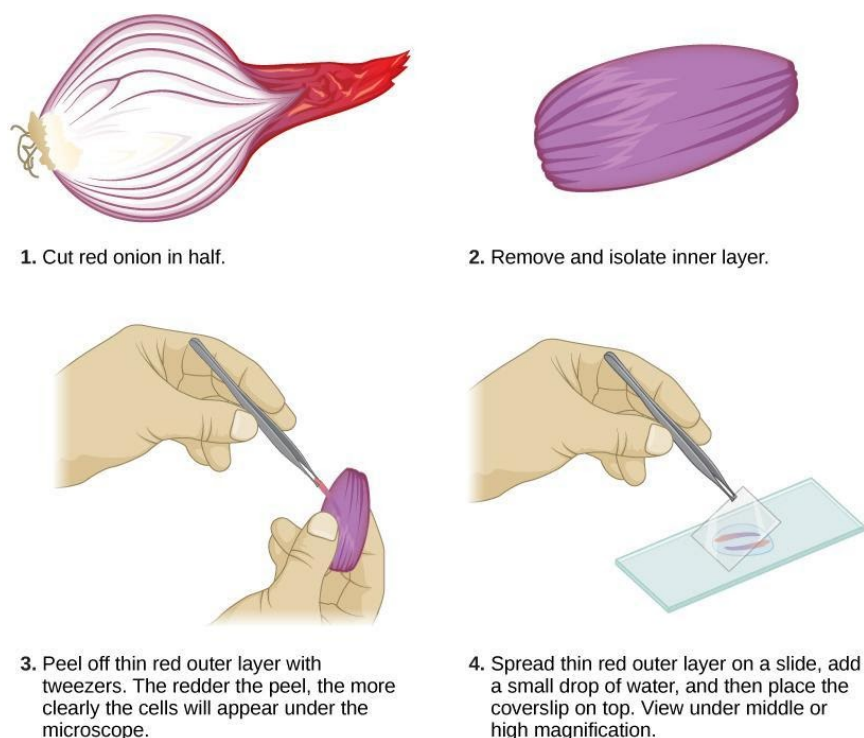


Figure 5.8: Process for preparing red onion wet mounts.

Step 3: Critical Analysis: Were the predictions you made supported by your data (observations)? Why or why not? Are there any methods you could have used that would improve your results? Discuss with your partner and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: A change in pH can often alter the function of macromolecules and cell structures. How do you think a change in pH will affect the pigment of the red onion

cells? Write one hypothesis each describing how an increase and a decrease in pH would affect the onion cell pigment.

Step 2: Student-led planning. Your teacher has provided you with solutions of different pH. Discuss, with your partner, how you will examine each onion skin under the different pH solutions (e.g., How long should you observe each slide? How many times should you repeat your observations?). Record your plan in your notebook and create data tables in which to record your results.

Step 3: Carry out the experiment you designed in Step 2, preparing your slides using the technique in Figure 5.8. Create drawings in your notebook to record all of your observations.

Step 4: Critical analysis: Did the results you predicted match what you observed as the pH changed? In a paragraph, describe how increasing and decreasing pH affected the red onion cells. Include an explanation of why you think pH affected the cells in the way you observed. Discuss your analysis with your partner and write it in your notebook.

Assessments

1. How are you able to find the vacuole of the red onion cells? What should you be looking for in the cell? [SP 3, Questions and Methods]
2. Red wines range in color from pink, red, and even violet-blue. If red grapes are used to make red wine, what part of the grape would explain that coloration? Predict the pH of wine based on the color. [SP 1, Concept Explanation]

[Answer Key]

1. Anthocyanins are contained in the central vacuole. Anthocyanins are water-soluble and the central vacuole solvent is water. The vacuole is full of anthocyanins and the outline of the vacuole between the cell wall/cell membrane and the clear cytoplasm should hopefully stand out. Note this picture shows the vacuole in a hypertonic solution.



2. Students may or may not know that the skin of the grape provides color. If the grapes are crushed with their skin, then the solution will contain anthocyanins that were located in the vacuoles of the skin cells. The activity above had students explore the

relationship between pH and the color of the red onion cells. The wine coloration is linked to the pH of the wine. Red would indicate a lower pH than violet.

Activity 3: Pre-Assessment

1. **Answer the following question in your notebook.** What structures would you expect to find in plant cells but not animal cells?
2. **Answer the following question in your notebook.** Do you expect that the subcellular structures will be easy to see? Why or why not?
3. **Discuss the answers to questions 1 and 2 with a partner (think, pair, and share) and then the class.**

Activity 3: Observation of Subcellular Structures in Animal Tissues

Animal cells (Figure 5.9) are eukaryotic and possess subcellular components in common with the plant cells you observed earlier. Some example structures include, nuclei (contains DNA, controls cell function), **Golgi apparatuses** (sorting, packing, and modification of proteins), **mitochondria** (energy production from organic molecules such as glucose), **ribosomes** (translation of messenger RNA into proteins), and the **endoplasmic reticulum** (folding of proteins and manufacture of lipids). Animal cells are predominantly colorless. Exceptions include the red of hemoglobin in red blood cells, red myoglobin in striated muscle, and melanin in skin cells. Thus; SPeCific biological stains are required to make visible cellular features under a light microscope.

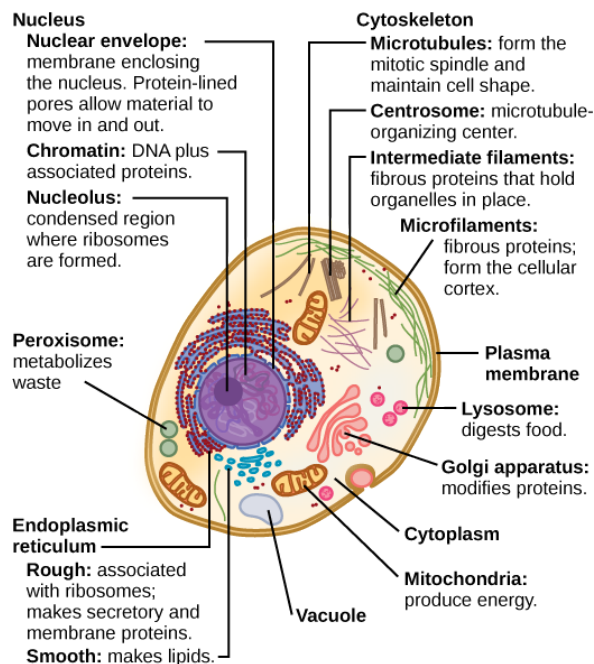


Figure 5.9: Structures of a generalized animal cell.

Nissl bodies, shown in Figure 5.10, present in neurons can be stained and allow you to see the rough endoplasmic reticulum and rosettes of free ribosomes. The **cytoskeleton** includes thin and thick filaments which are involved in cell shape, movement, and muscle contractions.

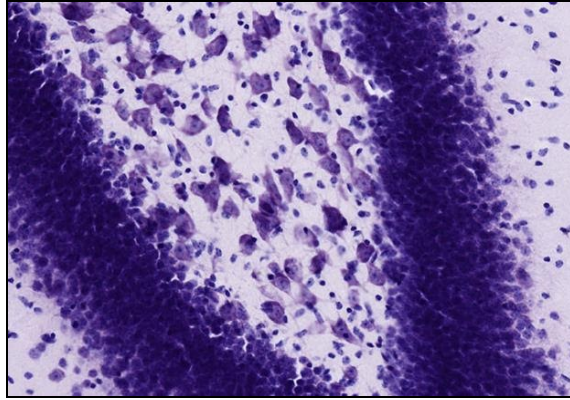


Figure 5.10: In this cross section from a rodent brain cells containing Nissl bodies are stained purple.

Safety Precautions

- Be careful handling glass slides, the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Wear goggles when using staining solutions.
- Dispose of staining solutions according to teacher instructions and local regulations.
- Wash your hands with soap and water after handling live organisms and biological stains.

For this activity, you will need the following:

- Prepared slides of Nissl bodies and striated muscle
- *Elodea anacharis*
- Clean microscope slides, cover slips
- Janus Green B stain
- Dropper
- Small squares of paper towels
- Notebook to observe and record data.

For this activity, you will work *in pairs*.

Activity 3 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 3:

- Show a diagram of an animal cell and to point out the nucleus, mitochondria, and endoplasmic reticulum (ER). Ask students to describe the function of each of these structures. Response should indicate that the nucleus carries genetic information, mitochondria provide energy to the cell by breaking down food, and the rough ER makes fats, steroids, membrane proteins, and secretory proteins.
- Show students a micrograph of an aniline-stained neuron. Ask students to identify the function of neurons and to think about how the long, thin axons and dendrites might help the cell accomplish this function. Response should indicate that neurons are involved in cell-cell communication, and that the long extensions allow the neuron to form connections with other cells. Explain that Nissl bodies, which are stained purple by aniline stain, are rough ER.
- Show students a diagram of a striated muscle cell. Explain that these cells are found in skeletal muscle, and that bands called sarcomeres give the cells a striated appearance. Ask students to think about why the cells form long thin bands. Response should indicate that the bands form muscle fiber, and all the cells act together to cause the muscle to contract.
- Ask students to think about how animal cells differ from plant cells. Response should indicate that plant cells have a cell wall, plastids, and a central vacuole and animal cells do not.

Structured Inquiry

Step 1: Hypothesize/Predict: Predict how big the structures of the Nissl bodies, thin and thick filaments, and mitochondria will appear when you look at them under the microscope. Which structures do you think you can see without staining?

Step 2: Observe the prepared slides and record (draw, label any visible parts, use color if visible, magnification, and size of cells) in your notebook.

Step 3: Critical analysis: How visible were the Nissl bodies, thin and thick filaments, and mitochondria before and after staining? Write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Given the function of mitochondria, predict how a biological stain would help you visualize mitochondria. Record your prediction in your notebook.

Step 2: Prepare a wet mount microscope slide with *Elodea anacharis* (each partner is expected to prepare one sample and each of you should view, draw, state the size and magnification, and label each sample). Experiment with a few different cuts to determine the best technique to get the thinnest possible slice. Observe your prepared slide, record (draw, label any visible parts, use color if visible, magnification, and size of cells) in your notebook.

Step 3: Prepare a second wet mount, this time with Janus Green B stain (Figure 5.12). No Figure 12 is provided. Observe over a period of time. Record your observations, drawings, label any visible parts, use color if applicable, and magnification in your notebook.

Step 4: Critical Analysis: Were the structures smaller or larger than you thought? How do the structures compare to the general size of prokaryotes? There should be small blue dots visible on the *Elodea* cells—much smaller than the chloroplasts. Hypothesize what those blue dots might be. Why are some of the dots turning pink over time? Come up with an experimental design that would allow you to test your idea. Record the answers to these questions in your notebook.

Assessments

1. Explain how your experimental design allowed you to identify a mitochondrion versus a nucleus in terms of function in the *Elodea* cell slide. [SP 1, Concept Explanation]
2. Describe some of the functions of thin and thick filaments in the muscle cells, based on your observations of their structure. [SP 1, Concept Explanation]

[Answer Key]

1. Methylene blue stains nucleic acid, and will therefore stain the nucleus, while Janus Green B stains the components of mitochondria. Therefore, the nucleus and chloroplasts will appear different in color due to the staining. The nucleus is also much larger than a single mitochondrion.
2. In muscle cells, thin and thick filaments combine their efforts to pull on the edges of the muscle cells, allow them to contract and relax. This allows muscles to move various parts of the body.

Lab 6: Diffusion and Osmosis

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
2.3 Cell Size	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.B Explain the effect of surface area-to-volume ratios on the exchange of materials between cells and the environment. ENE-1.C Explain how specialized structures and strategies are used for the efficient exchange of molecules to the environment.	ENE-1.B.1 Surface area-to-volume ratios affect the ability of a biological system, including cells, to exchange nutrients, wastes, and energy with the environment. ENE 1.B.2 The surface area of the plasma membranes must be large enough to adequately exchange materials; smaller cells typically have a larger SA:V ratio. ENE-1.C.1 Organisms have evolved efficient strategies to obtain nutrients and eliminate wastes, using specialized exchange surfaces.
2.4 Plasma Membranes	ENE-2 Cells have membranes that allow them to establish and maintain internal environments that are different from their external environments.	ENE-2.A Describe the roles of each of the components of the cell membrane in maintaining the internal environment of the cell. ENE-2.B Describe the Fluid Mosaic Model of cell membranes.	ENE-2.A.1 Cell membranes consist of a phospholipid bilayer with hydrophilic and hydrophobic regions, with fatty acid regions facing each other within the interior of the membrane. ENE-2.A.2 Proteins embedded in the bilayer can be hydrophilic or hydrophobic. ENE-2.B.1 Cell membranes consist of a structural framework (bilayer) of phospholipid molecules with embedded proteins, steroids such as cholesterol in eukaryotes, glycoproteins, and glycolipids.

2.5 Membrane Permeability	ENE-2 Cells have membranes that allow them to establish and maintain internal environments that are different from their external environments.	ENE-2.C Explain how the structure of biological membranes influences selective permeability.	ENE-2.C.1 The structure of cell membranes results in selective permeability. ENE-2.C.2 Cell membranes separate the internal environment of the cell from the external environment. ENE-2.C.3 Selective permeability is a direct consequence of membrane structure. ENE-2.C.4 Small, nonpolar molecules freely pass across the membrane, whereas larger polar molecules and ions move across through the embedded channel and transport proteins. ENE-2.C.5 Polar uncharged molecules, including water, pass through the membrane in small amounts.
2.6 Membrane Transport	ENE-2 Cells have membranes that allow them to establish and maintain internal environments that are different from their external environments.	ENE-2.E Describe the mechanisms that organisms use to maintain solute and water balance.	ENE-2.E.1 Passive transport is the net movement of molecules from high concentration to low concentration without the direct input of metabolic energy. ENE-2.E.2 Passive transport plays a primary role in the import of materials and the export of wastes.
2.7 Facilitated Diffusion	ENE-2 Cells have membranes that allow them to establish and maintain internal environments that are different from their external environments.	ENE-2.G Explain how the structure of a molecule affects its ability to pass through the plasma membrane.	ENE-2.G.1 Membrane proteins are required for facilitated diffusion of charged and large polar molecules. Large quantities of water pass through aquaporins.
2.8 Tonicity and Osmoregulation	ENE-2 Cells have membranes that allow them to establish and maintain internal environments that are different from their external environments.	ENE-2.H Explain how concentration gradients affect the movement of molecules across membranes. ENE-2.I Explain how osmoregulatory mechanisms contribute to the health and survival of organisms.	ENE-2.H.1 External environments can be hypotonic, hypertonic, or isotonic to internal environments of cells, with water moving by osmosis from areas of high water potential/low solute concentration to areas of low water potential/high solute concentrations. ENE-2.I.1 Growth and homeostasis are maintained by the constant movement of molecules across membranes. ENE-2.I.2 Osmoregulation maintains water balance and allows organisms to control their internal solute composition/water potential.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation <i>Explain biological concepts, processes, and models presented in written format.</i>	1.A Describe biological concepts and/or processes. 1.B Explain biological concepts and/or processes. 1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations <i>Analyze visual representations of biological concepts and processes.</i>	2.B Explain relationship between difference characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Representation relationships within biological models, including mathematical models and diagrams.
SP 3 Questions and Methods <i>Determine scientific questions and methods.</i>	3.A Identify or pose a testable question based on an observation, data, or a model. 3.C Identify experimental procedures that are aligned to the questions, including variables and controls. 3.D Make observations, or collect data from representations of laboratory setups or results. 3.E Propose a new/next investigation based on a previous experiment.
SP 4 Representing and Describing Data <i>Represent and describe data.</i>	4.B Describe data from a table or graph, describing patterns and/or relationships.
SP 5 Statistical Tests and Data Analysis <i>Perform statistical tests and mathematical calculations to analyze and interpret data.</i>	5.A Perform mathematical calculations, including equations in the curriculum, rates, and percentages.
SP 6 Argumentation <i>Develop and justify scientific arguments using evidence.</i>	SP 6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. SP 6.C Provide reasoning to justify a claim by connecting evidence to biological theories.

Cells are like miniature factories, using materials and energy to break down and manufacture substances that are important to their survival. The movement of materials into and out of the cell is important for proper cell functioning. This movement is regulated by the plasma membrane, which determines what goes in and what comes out of the cell. The plasma membrane is said to be **selectively permeable** which means that it only allows certain molecules to cross the membrane while others cannot (Figure 6.1). This is largely because the membrane's phospholipid bilayer has a hydrophobic (water-fearing) core, so hydrophilic (water-loving) substances and large, polar molecules need assistance to cross the membrane. Conversely, molecules that are small, nonpolar and hydrophobic can cross the membrane easily

without using energy through a method called **passive transport**. Passive transport involves the diffusion of molecules down a concentration gradient. Recall that **diffusion** is the natural tendency of particles (e.g., atoms, molecules, ions) to move from an area of high concentration to an area of low concentration. **Osmosis** is a special type of diffusion that involves the movement of water across a selectively permeable membrane. In this lab, you study both diffusion and osmosis.

The selective permeability of the cell membrane allows the cell to control what substances enter or leave. Polar molecules, such as water, must enter the cell through membrane proteins that facilitate their diffusion across the membrane. This is called **facilitated diffusion**. Other molecules, such as those that are very large or are already in a high concentration within the cell, must be *pumped* across the membrane with the assistance of a membrane protein that uses energy (ATP). Membrane transport that requires energy is called **active transport**.

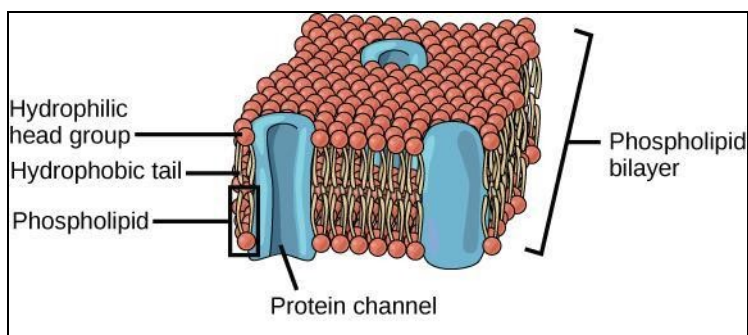


Figure 6.1: The plasma membrane is mainly made up of phospholipids which have hydrophobic and nonpolar tails in the interior and polar and hydrophilic heads toward the exterior.

Instructor Introduction Notes

The following are recommendations for introducing the lab's background content to your students.

- Show students a labelled diagram of a cell while introducing the lab.
- Review the terms *polar*, *nonpolar*, *hydrophilic*, and *hydrophobic* by showing a diagram that distinguishes the properties.
- Reinforce the distinctions between passive transport, active transport, and osmosis with short videos or animations from the internet or YouTube.

In this lab, you will learn

- how simple diffusion works;
- how various factors affect the rate of diffusion;
- to calculate diffusion rates of substances based on molecular weight;
- how different media affect the rate of diffusion;
- how surface area-to-volume ratio influences the effectiveness of diffusion;
- how the process of osmosis works.

Activity 1: Pre-Assessment

1. **Answer the following questions in your notebook:** What happens when an air freshener is sprayed in a corner? What is the name of the process that causes the molecules to move?
2. **Answer the following questions in your notebook:** Do you think that the rate of the air freshener molecules moving would change if the room temperature was warmer or colder? Why or why not?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 1: Diffusion

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry Activity 1:

- Tell students that certain dyes will not completely wash out of their clothing.
- Ask the students to hypothesize temperatures for what they think very cold or very hot water is.
- Tell students that they should not stir the solution or move the setup while diffusion is taking place.
- Instruct students to avoid touching the surface of agar with their fingers because it could affect diffusion.

The following are recommendations for the Structured Inquiry for the Guided Inquiry for Activity 1:

- Have students identify the skills learned in the structured inquiry, such as quantifying the rate of diffusion. They will need to carry out the guided inquiry.
- Place students in work groups to expedite the activity. Encourage each group to assign specific tasks to group members.
- Ask students to write their own paper written in their words. Check for plagiarism by comparing the answers between group members.

Diffusion is the movement of molecules from an area where the molecule is highly concentrated to an area of low concentration, as illustrated in Figure 6.2. The rate of diffusion is dependent upon the temperature of a system, molecular size, and the medium through which diffusion is occurring (i.e., semi-solid, liquid, air). In this activity, we will be observing the diffusion of a dye through a beaker of water and through agar (a gelatinous substance), diffusion as a function of temperature, and diffusion as a function of molecular weight.

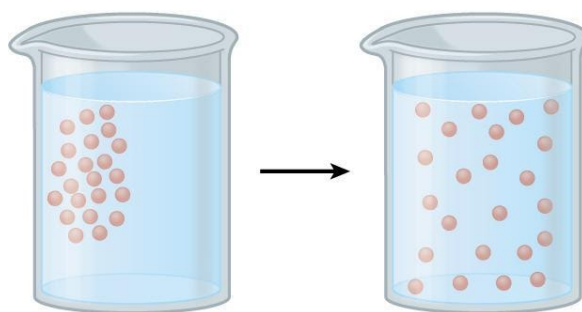


Figure 6.2: In diffusion, molecules move from areas of high concentration to areas of low concentration.

Safety Precautions

- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent other people from slipping.
- Be careful with the dye as it can stain your clothes, and it should not be ingested.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Three 250 mL beakers
- Water
- Food coloring
- Agar plates
- Potassium permanganate
- Congo red
- Methylene blue
- Thermometer
- Incubator
- Refrigerator
- Clock or timer

For this activity, you will work in *groups of four*.

Structured Inquiry

Step 1: Measure 200 mL of room temperature water in a beaker. Put three drops of food coloring into the water. Time how long it takes for the dye to completely diffuse throughout the water. Record the time and describe in your notebook what you observe. Create a data table for your observations.

Step 2: Hypothesize/Predict: Predict what would happen to the rate of diffusion if you had beakers with both very hot and very cold water in them. Add your predictions to the data table you created in Step 1.

Step 3: Student-led Planning: Determine how diffusion of the food color would be affected when the water is either very hot or very cold. Use a thermometer and record the temperature for each. Use a timer to measure how long it takes for complete diffusion to occur in all scenarios.

Step 4: Critical Analysis: Create a graph that shows how the diffusion rate is affected because of temperature change. Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Guided Inquiry

Step 1: Gather four agar plates and the three dyes, provided by your instructor, that differ in molecular size: Congo red (mol. wt. 696.66 g/mol), methylene blue (319.85 g/mol), and potassium permanganate (mol. wt. 158.03 g/mol).

Step 2: Hypothesize/Predict: How would the rate of diffusion of a molecule through a gel compare to its rate of diffusion through water? How would the rate of diffusion differ between molecules of different molecular sizes? Write your ideas in your notebook.

Step 3: Student-led planning: Use 1 plate for determining how molecular size affects diffusion using the 3 dyes. Determine how best to measure movement of the dye in an agar plate. Be sure to keep the dyes far enough apart so that they do not touch once they start diffusing. Get your instructor's approval before proceeding with the experiment. Measure the distance that the dye spreads in 20-minute intervals for 1 hour.

Step 4: Examine the effect of temperature on the rate of diffusion for 1 dye of your choosing. With your group, determine 3 temperatures that would be appropriate. Measure the diameter of the dye spread for each. Write the results in your notebook.

Step 5: Critical Analysis: Rank all 3 dyes in terms of diffusion rate. What was the relationship between diffusion rate and molecular size? What is the relationship between temperature and diffusion rate? Discuss your answers with your group and write them in your notebook.

Assessments

1. In a system, there is a concentration of molecules. However, on the outside, there is little to no concentration of this particular molecule. In which direction would the molecules be moving more so than the other direction? [SP 1, Concept Explanation]
2. Diffusion is affected by what factors? [SP 1, Concept Explanation]
3. Dye tends to move faster in warmer temperatures. Why is this? [SP 1, Concept Explanation]

[Answer Key]

1. The molecules would move from the area of higher concentration to lower. So, the movement would be from inside to outside.
2. Temperature, molecular size, and the type of medium all affect the rate of diffusion.
3. Increased temperature means increased molecular motion.

Activity 2: Pre-Assessment

1. **Answer the following questions in your notebook:** What do you think happens to a plant when it is placed in salt water? Why might this occur?
2. **Discuss the answers to question 1 with the class.**

Activity 2: Measuring Osmosis

Osmosis is the diffusion of water across a selectively permeable membrane (i.e., cell membrane). Because of osmosis, water will move where water is less concentrated from an area of low solute (high water content) to an area with high solute concentration (low water content) (Figure 6.3). Dialysis tubing is used to model selectively permeable membranes because it will prohibit large molecules from crossing the membrane but will allow small molecules to cross. For example, water and glucose are small molecules that can easily cross the membrane. However, starch (a polymer of glucose), cannot cross due to its large molecular size. Additionally, cell surface area and volume can affect the rate of diffusion across a membrane. The **surface area-to-volume ratio** describes the relationship between the area outside the cell to the volume inside the cell (Figure 6.4). In this lab, we first will use dialysis tubing to model how the membrane selectively allows certain molecules to cross the membrane. Then, we will compare the diffusion rate of dialysis tubing with a small surface area-to-volume ratio to one that has a large surface area-to-volume ratio.

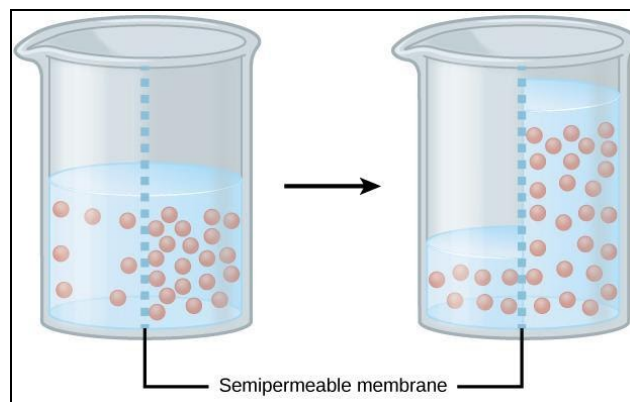


Figure 6.3: Osmosis is the diffusion of water across a semipermeable membrane. Water moves from an area of high water and low solute concentration to an area of low water and high solute concentration. Note that the solute does not move.

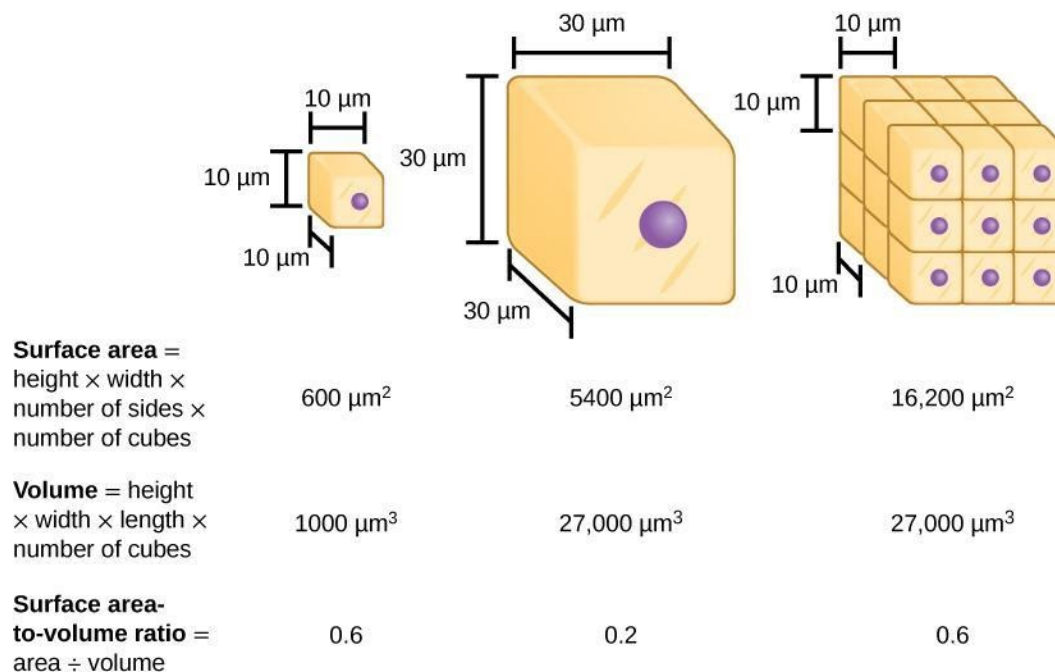


Figure 6.4: Smaller cells have higher surface areas compared to larger cells.

Safety Precautions

- Safety goggles/glasses should be worn when chemicals or solutions are heated.
- Handle all chemicals safely.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent other people from slipping.
- Dispose of all chemicals per local regulations.
- Use caution when performing the Benedict's reaction which involves the use of a hot water bath.

For this activity, you will need the following:

- Dialysis tubing, two pieces, about 15 cm each long and narrow (pre-soaked)
- Dialysis tubing, one piece, short and wide (pre-soaked)
- Six Dialysis tubing clamps (optional)
- Three 500 mL beaker
- Water
- 5 percent Glucose solution, 10 mL

- Starch suspension, 10 mL
- Iodine
- Benedict's reagent
- Graduated cylinder
- Test tube
- Hot water bath

For this activity, you will work in *groups of four*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry Activity 2:

- Remind students that the minimum molecular weight of starch in solution is likely 50,000 MW. The best dialysis tubing for retaining starch has a molecular weight cutoff of 12,000 to 14,000 MW.
- Demonstrate how to properly clamp the dialysis tubing. You can have an image of the setup or a model setup where the students can view it.
- Discuss with students that the dyes used in class may stain the skin for a few hours to a couple of days.

The following are recommendations for the Guided Inquiry for Activity 2:

- Have students identify the skills learned in the structured inquiry, such as detecting the presence of glucose, they will need to carry out the guided inquiry.
- Place students in work groups. Encourage each group to assign specific tasks to group members.
- Ask students to write their own paper written in their words. Make sure to check for plagiarism by comparing answers between group members.

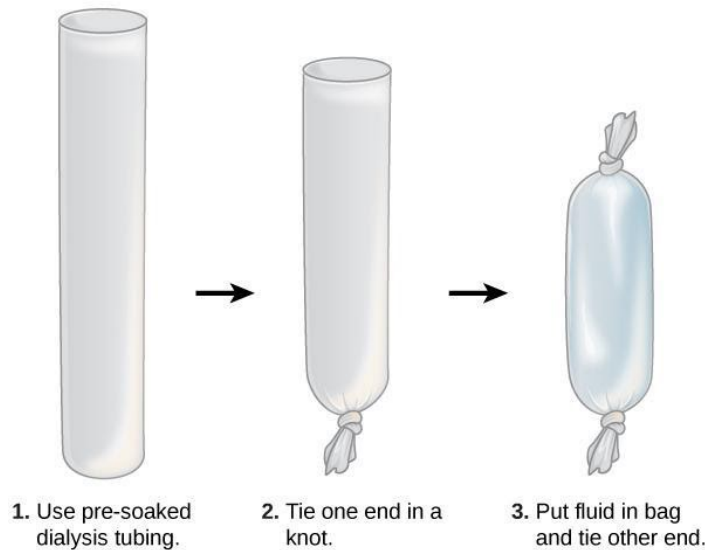


Figure 6.5: Directions for how to seal a dialysis tube.

Structured Inquiry

Step 1: Use a graduated cylinder to measure 250 mL of room temperature water in a beaker. Set aside. Obtain 1 piece of long and narrow dialysis tubing. Seal 1 end of the tube by tying a secure knot or attaching a dialysis tubing clamp to the end of the tubing. Put 10 mL of starch suspension and 10 mL of glucose solution in the tube. Then tie or clamp the other end of the tube. Place the sealed tube into the beaker (Figure 6.6). Develop and implement the use of an appropriate controlled experiment to go alongside your experiment. Allow both to sit for 20–30 minutes.

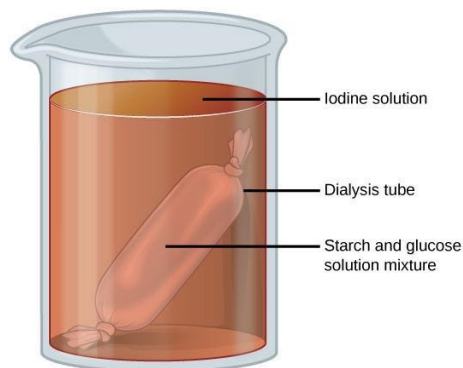


Figure 6.6: Your dialysis tube should be placed into the beaker and allowed to sit for the time specified.

Step 2: Hypothesize/Predict: Consider the molecular sizes of glucose and starch. What do you think will happen with the starch and the glucose inside of the dialysis tube? Predict whether starch and/or glucose will remain inside of the tube or if they will move out.

Step 3: Student-led planning: Briefly remove the dialysis bag from the beaker and set aside. Pour 2 mL of the water from the beaker into a test tube. Put 10 drops of Benedict's reagent into the tube (note the initial color of the solution) and place into the hot water bath for 5 minutes. Note whether there is a color change. Place the bag back into the beaker. Put 20 drops of iodine into the water in the beaker. Allow this to sit for about 10 minutes. Determine whether starch is present in the bag and/or the beaker. Describe in your notebook what you observe. Create a data table for your observations that shows whether starch and glucose were inside and/or outside of the dialysis tubing.

Step 4: Critical Analysis: Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Predict what would happen to the rate of osmosis if the surface area-to-volume ratio was large (as it would be with small cells compared to larger ones). Record your prediction.

Step 2: Student-led planning: Determine how the rate of osmosis of water using a dialysis bag with starch solution is affected if surface area-to-volume ratio is altered. Use dialysis tubing of 2 different lengths and widths to determine this. Record weights of each bag at different time intervals. Discuss with your group the time points at which you will check the bags for differences in weight over a 20-minute period. Record your data for each. Discuss with your group how best to set up these experiments.

Step 3: Critical Analysis: Create a graph that shows how osmosis rate is affected over time because of alterations in surface area-to-volume ratios. Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Assessments

1. Would a large protein have difficulty crossing the membrane of a dialysis tube? Why or why not? [SP 1, Concept Explanation; SP 3, Questions and Methods]
2. Why do smaller cells have more efficient diffusion compared to larger ones? [SP 1, Concept Explanation; SP 5, Statistical Tests and Data Analysis]

[Answer Key]

1. Yes. Large molecules cannot cross the membrane.
2. Smaller cells have a larger surface area-to-volume ratio compared to larger cells.

Activity 3: Pre-Assessment

1. **Answer the following question in your notebook:** Saline solutions are given to patients in an IV. Why might the salt concentration in the solution need to be the same as that in the blood cells?
2. **Discuss the answer to question 1 with your group.**

Activity 3: Osmosis in Living Cells

The inside and the outside of a cell is mainly composed of water with dissolved solutes. The differences in solute concentration direct the movement of water across the plasma membrane of the cell. This difference between the two solutions (i.e., the cytoplasm and the extracellular fluid) is known as **tonicity**. Solutions are said to be **isotonic** if they both have equal concentrations of solute. The extracellular fluid is **hypertonic** to the intracellular fluid if it has a higher solute concentration. On the other hand, a lower solute concentration in the extracellular fluid compared to the inside of the cell would mean that the outside of the cell is **hypotonic** to the inside.

We can observe tonicity in *Elodea* leaves by placing them into different solutions with various solute concentrations.

Safety Precautions

- Be careful handling glass slides; the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent other people from slipping.
- Handle all chemicals safely.

For this activity, you will need the following:

- Two glass slides
- *Elodea* leaves
- Two coverslips
- Distilled water
- 10 percent NaCl solution
- 30 percent NaCl solution

- Microscope
- Potato cubes
- Electronic balance
- Weigh boat
- Three 50 mL beakers

For this activity, you will work in *groups of four*.

Activity 3 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry Activity 3:

- Tell students to handle the slides and coverslips carefully to prevent breaking slides and cutting their fingers.
- Project or make available an image of *Elodea* cells to help students understand what they are viewing under the microscope.
- Reinforce that students must be consistently measuring the actual weight of the potato cubes during their experiment.
- Use freshly cut potato cells that do not show brown discoloration. The cut surfaces of potatoes oxidize and dehydrate which affects diffusion and osmosis results.

The following are recommendations for the Structured Inquiry for the Guided Inquiry for Activity 3:

- Have the students identify the skills, such as identifying how to measure changes in the *Elodea* leaf, they will need to carry out the guided inquiry. These skills were learned in the structured inquiry.
- Place students in work groups. Encourage each group to assign specific tasks to group members.
- Ask students to write their own paper written in their words. Make sure to check for plagiarism by comparing answers between group members.

Structured Inquiry

Step 1: Obtain 2 glass slides and 2 cover slips. On 1 slide, place an *Elodea* leaf and put 1 drop of distilled water. Make a second slide with an *Elodea* leaf but put a drop of 10% NaCl. Allow these to sit for 2–3 minutes.

Step 2: Hypothesize/Predict: Predict what will happen with the leaf that has been placed into NaCl. What do you think will happen to the leaf in water? Record your predictions.

Step 3: Student-led planning: Determine what happens to the *Elodea* leaf cells in both solutions by observing them under the microscope. Record your observations for each. Draw pictures that demonstrate what you observe.

Step 4: Critical Analysis: Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: What happens to a cell in an isotonic solution? Hypertonic? Hypotonic? Predict what might happen to a potato in these solutions. Will it gain, lose, or maintain its weight? Write your ideas in your notebook.

Step 2: Student-led planning: Design a study that would test your hypothesis in Step 1. Plan which solutions you will use and how to determine if osmosis happened in each. Show your design to a teacher for approval then create data tables that you would need to test your hypothesis. Write your results in your notebook and create a graph or table to summarize your results.

Step 3: Critical Analysis: Discuss your results with your group. Are your results what you expected? How can you improve your experiment? Write your ideas in your notebook.

Assessments

1. Have you ever seen a slug hanging out on the steps? Oftentimes, people use salt to get rid of them. What do you think this does to the slug in terms of osmosis? [SP 1, Concept Explanation]
2. How can you predict whether osmosis will occur into or out of a cell? Explain in terms of water potential. [SP 5, Statistical Tests and Data Analysis; SP 6, Argumentation]
3. Explain what is meant when we say that a cell membrane is selectively permeable. What types of molecules might have a difficult time crossing the membrane? [SP 1, Concept Explanation]

[Answer Key]

1. Putting salt on a slug is like placing a cell into a solution with a higher salt concentration. This causes water to leave out of the cell to balance the environment and equalize the concentrations of the two environments.
2. You can determine the direction of osmosis by knowing the solute concentration inside relative to the outside of the cell. Water potential is a measure of potential energy in a sample of water, and describes the tendency of water to move from one place to another, from high water potential to low water potential. Water potential, which can be mathematically calculated, is influenced by solute concentration and pressure.

3. The cell membrane is selectively permeable which means that it determines what goes in and what comes out of the cell. Basically, it selects what it will allow to cross the membrane. Large molecules, as well as polar molecules, have a difficult time crossing the membrane due to the makeup of the membrane (i.e., phospholipids).

Lab 7: Factors Affecting the Enzymatic Activity of Lactase

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topics	Enduring Understanding	Learning Objective	Essential Knowledge
3.1 Enzyme Structure	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.D Describe the properties of enzymes.	ENE-1.D.1 The structure of enzymes includes the active site that specifically interacts with substrate molecules. ENE-1.D.2 For an enzyme-mediated chemical reaction to occur, the shape and charge of the substrate must be compatible with the active site of the enzyme.
3.2 Enzyme Catalysis	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.E Explain how enzymes affect the rate of biological reactions.	ENE-1.E.1 The structure and function of enzymes contribute to the regulation of biological processes by acting as catalyzes and lowering the activation energy of a chemical reaction.
3.3 Environmental Impacts on Enzyme Function	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.F Explain how changes to the structure of an enzyme may affect its function. ENE-1.G Explain how the cellular environment affects enzyme activity.	ENE-1.F.1 Change to the molecular structure of a component in an enzymatic system resulting from denaturation, or less than optimal temperature and/or pH, may result in a change of the function or efficiency of the system. ENE-1.G.1 – 1.G.4 Factors that affect enzyme activity include pH, temperature, concentration of substrates, and competitive and noncompetitive inhibitors.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including mathematical models and diagrams.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on an observation, data, or a model. 3.B State the null and alternative hypotheses, or predict the results of an experiment. 3.C Identify experimental procedures that are aligned to the questions, including variables and controls. 3.D Make observations, or collect data from representations of laboratory setups or results. 3.E Propose a new/next investigation.
SP 4 Representing and Describing Data Represent and Describe Data.	4.A Construct a graph, plot, or chart. 4.B Describe data from a table or graph.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including equations in the curriculum (pH) and rates. 5.B Use data to evaluate a hypothesis/prediction.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. 6.E Predict the causes or effects of a change in one or more components in a biological system based on data.

Enzymes are biological **catalysts**, substances that serve to increase the rate of chemical reactions. Most enzymes are proteins, but some RNA molecules have also been shown to demonstrate catalytic activity. Enzymes typically work by binding to starting chemicals, called **substrates**, and holding them in a way that will break chemical bonds and form new bonds more readily. In doing so, enzymes lower the **activation energy**, the energy needed to form or break chemical bonds (Figure 7.1) and convert the substrate(s) to **product(s)**. These are the molecules that result from such chemical reactions (Figure 7.2). After facilitating the transition from substrate(s) to product(s), an enzyme releases the product(s) and becomes available for reuse.

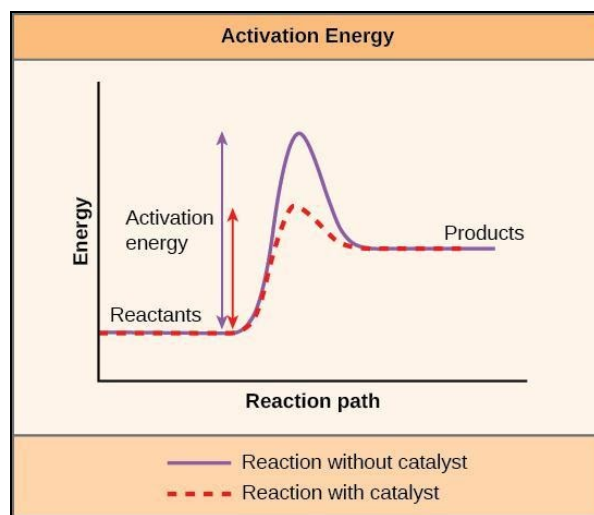


Figure 7.1: Enzymes lower the activation energy of chemical reactions. Here, we can see that the activation energy of a chemical reaction in the presence of an enzyme (red) is lower than the same reaction without an enzyme (purple).

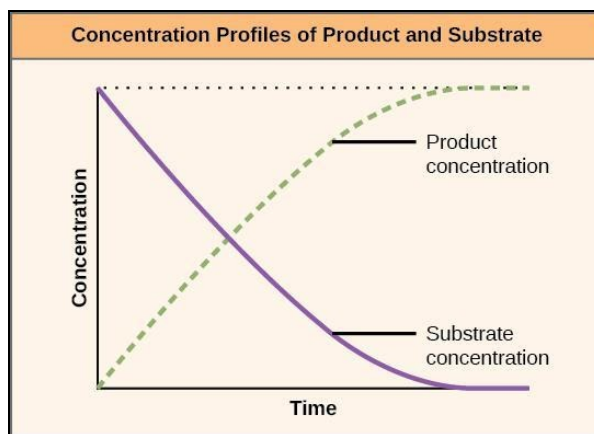


Figure 7.2: Over the course of a chemical reaction, the substrate concentration (purple) will decrease as the product concentration (green) increases.

Because enzymes are normally proteins, they are composed of amino acids, each containing a **side chain** or **R group** with distinct chemical properties. Side chains may be hydrophobic or hydrophilic (polar, acidic, or basic). The combination of side chains within an enzyme and the chemical interactions between them contribute to the enzyme's unique three-dimensional structure. This structure also determines the shape of its **active site**, the structural portion of the enzyme that binds to the substrate(s). There are a variety of factors that may influence enzymatic activity; including temperature, pH, and substrate concentration.

In this lab, you will learn

- how to measure enzymatic activity of the enzyme lactase over time and represent it graphically
- how to monitor the effects of environmental conditions on enzymatic activity
- how to determine the specificity of the enzyme lactase

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students.

- Show a brief animation or video on enzyme action as a review of enzyme function.
- Project and draw a diagram showing the primary through quaternary structures of proteins as a review of protein structure.
- Do a quick inquiry to see what information students have retained about the terms *hydrophobic* and *hydrophilic*.
- Ask students what it means to be lactose intolerant. Do not define it immediately because lactose intolerance is briefly described in Activity 1.

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** How would the concentration of an enzyme, its substrate(s), and its product(s) change over time as an enzymatic reaction takes place?
2. **Answer the following questions in your notebook:** What molecule(s) would you measure to monitor the progress of an enzymatic reaction? How might you observe the relative concentrations of these molecules over the course of the enzymatic reaction?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 1: Measuring the Enzymatic Activity of Lactase

How would we measure the activity of a specific enzyme? One easy way to measure the concentration of a chemical is by using a colored indicator. As an enzymatic reaction proceeds, the color of the solution changes, and can be monitored visually.

In this lab, you will measure the enzymatic activity of lactase, an enzyme that breaks down lactose, a disaccharide found in milk and other dairy products, into the monosaccharides galactose and glucose (Figure 7.3). While human infants naturally produce lactase, most adults do not, making them lactose intolerant, or unable to produce enough lactase to digest ingested lactose. Interestingly, in the past 10,000 years, several populations of humans have developed lifelong lactase activity. These human populations are often people who raise livestock and drink animal milk. For most individuals who are lactose intolerant, lactase may be purchased in tablet form from pharmacies.

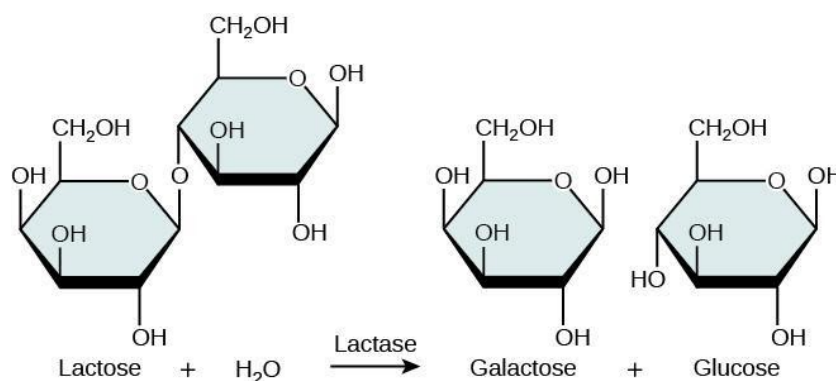


Figure 7.3: The enzyme lactase degrades the disaccharide lactose into the monosaccharides galactose (1) and glucose (2).

Glucose, one of the products of lactose degradation, can be detected visually using glucose detection strips, which can also be purchased from pharmacies. One type of glucose detection strip contains the dye toluidine blue and the enzyme glucose oxidase (Figure 7.4). Glucose oxidase within the strip converts the glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide then interacts with the toluidine blue within the strip, bringing about a color change from blue to green to yellow to brown.



Figure 7.4: Diastix glucose detection strips turn from blue to green to yellow to brown with increasing concentrations of glucose.

Safety Precautions

- Measure fluids carefully using graduated cylinders to avoid breakage and spillage.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.

For this activity, you will need the following:

- Graduated cylinder
- Beaker
- Water
- Milk
- Lactase (obtained from laboratory supply company)
- Stirring rod
- Test tubes
- Glucose (powdered)
- Balance
- Labeling pencil
- Glucose test strips

- Graph paper
- Timer

For this activity you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry 1:

- Reduce the number of glucose test strips used in this activity by carefully slitting them in half lengthwise. Make sure that the indicator patch remains intact on each side.
- Store lactase solution in a refrigerator to reduce a loss of activity. The solution should be used at room temperature.
- Tell students not to touch the indicator patch on the glucose test strips. Oils, sweat, and particles on their fingertips can contaminate the strip and give false results.
- You could use a Benedict's test in place of the glucose test strips in this activity to measure glucose, if desired.
- Confirm that test strips are actually measuring glucose by comparing a test strip result to a Benedict's test result on the glucose solution. They should be more familiar with Benedict's test.
- Project an unlabeled image of lactose and have the class identify the two subcomponents.
- Look for glucose powder in grocery stores or health food shops.
- Purchase lactase in grocery stores, health food shops, and pharmacies. Perform a test run of this activity before using it in class.

The following are recommendations for the Guided Inquiry for Activity 1:

- Have the students identify the skills, such as determining the breakdown of lactose, they will need to carry out the guided inquiry. These skills were learned in the structured inquiry. Place students in work groups. Encourage each group to assign specific tasks to group members.
- Ask students to write their own paper written in their words.

Structured Inquiry

Step 1: Make 100 mL of a 20 mg/mL glucose solution or obtain this from your instructor. Set up tubes as follows:

Negative control: 3 mL of water

Positive control: 3 mL of 20 mg/mL glucose solution

Insert a glucose test strip into each tube, and compare the color you observe to the color chart that comes with the test strips. What is the concentration of glucose in each tube? Are your results what you expected?

Step 2: Hypothesize/Predict. Based upon your knowledge of enzymes and lactase function, predict whether glucose will be detected in milk in the presence of lactase enzyme compared to milk lacking the lactase enzyme. Predict how the glucose concentration of milk will change over time under each of these two conditions. Write your predictions in your lab notebook.

Step 3: With your partner, prepare two tubes each containing 2 mL of milk. Additionally, make your lactase enzyme solution per your teacher's instructions.

Step 4: Student-led Planning. Your experiment should last for 15 minutes. Create a data table to record glucose concentrations in each of the two tubes of milk at 3-minute intervals. To start the experiment, add 1 mL of water to one of the tubes of milk and 1 mL of the lactase solution to the other. Then, every 3 minutes for 15 minutes, record the concentrations of glucose in each tube using the color chart that came with the test strips and record this in your data table.

Step 5: Critical Analysis. Using graph paper, graph your data of glucose concentration versus time. Which is the independent variable? Which is the dependent variable? Calculate the rates of glucose production for each tube. Are the predictions you made in Step 2 supported by your data? Explain how you know in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict. How do you think increasing the amount of lactase will affect glucose formation? How do you think decreasing the amount of lactase will affect glucose formation? Formulate a hypothesis involving the effect of the lactase amount on glucose formation.

How do you think increasing the amount of milk will affect glucose formation? What would happen if you decrease the amount of milk? Formulate a hypothesis involving the effect of the amount of milk on glucose formation.

Write your hypotheses and predictions in your notebook.

Step 2: Student-led Planning. Select one of the hypotheses above to discuss with your lab partner. Design an experiment to address this hypothesis and submit it to your teacher for approval. Once your teacher approves, create a table to record your data, prepare your test tubes per your design, and record data on glucose production every 3 minutes for 15 minutes. Graph your data and calculate the rate of glucose production for each tube.

Step 3: Critical Analysis. Are the predictions you made in Step 1 supported by your data? Is there any way you could improve your experiment? Discuss your answers with your lab partner and write it in your notebook.

Assessments

1. In this experiment, assume that all the lactose provided is broken down to glucose and galactose. Based upon this assumption, if one diluted the amount of milk by half in the tube containing milk and the lactase enzyme, what would happen to the amount of glucose produced? Explain why in terms of molecular interactions of the substrate with the enzyme and enzyme function. [SP 1, Concept Explanation; SP 3, Questions and Methods; SP 6, Argumentation]
2. In this experiment, describe the role of each of the following: [SP 3, Questions and Methods]
 - a. The water-only tube
 - b. The tube of 20 mg/mL glucose solution

[Answer Key]

1. One would expect the amount of glucose produced to be halved. This is because the number of interactions between substrate and enzyme would be cut in half if the amount of substrate (milk) is diluted by half. Thus, the rate of the enzymatic reaction would be cut in half.
2.
 - a. The water-only tube serves as the negative control for the glucose test strips.
 - b. The tube of 1 percent glucose solution serves as the positive control for the glucose test strips.

Activity 2: Pre-Assessment

1. **Answer the following questions in your notebook:** Which environmental conditions could alter the rate at which an enzymatic reaction takes place? Why would this occur?
2. **Answer the following questions in your notebook:** Which environmental conditions could affect an enzyme's active site? Why would this occur?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 2: The Effects of Temperature and pH on Enzymatic Activity

What types of environmental factors may affect enzymatic activity? Why? Several factors known to affect enzymatic activity are temperature, pH, and substrate concentration. In a typical chemical reaction, increasing temperature causes the substrates to become more energetic and hence more likely to bump into each other in solution. However, changes in temperature can cause an enzyme to **denature**, which changes the three-dimensional structure of the enzyme molecule. In addition, cellular enzymes each work within a certain pH range because the side chains within their active sites are optimized for efficient catalysis and are thus quite sensitive to changes in pH. Different enzymes may have different pH ranges and **pH optima**, conditions under which they work maximally; while many enzymes work best around a neutral pH, some are adapted to an acidic pH, while others are adapted to a basic pH.

Safety Precautions

- Measure fluids carefully using graduated cylinders to avoid breakage and spillage.
- Be careful not to touch solutions of concentrated acids and bases directly.
- Take precautions when using a hot plate and touching hot glassware.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.

For this activity, you will need the following:

- Graduated cylinder
- Beaker
- Water
- Buffer solutions of pH 3.0, pH 4.0, pH 5.0, pH 6.0, pH 7.0, pH 8.0, and pH 9.0
- Milk
- Lactase (obtained from laboratory supply company)
- Test tubes

- Stirring rod
- Labeling pencil
- Hot plate
- Ice bath
- Refrigerator set to approximately 4 °C
- Incubator set to 37 °C
- Thermometers
- Glucose test strips
- Timer
- Graph paper

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 2:

- Reduce the number of glucose test strips used in this activity by carefully slitting them in half lengthwise. Make sure that the indicator patch remains intact on each side.
- You could use a Benedict's test in place of the glucose test strips in this activity to measure glucose, if desired.
- Store lactase solution in a refrigerator to reduce a loss of activity. The solution should be used at room temperature.
- Perform a quick demonstration of protein denaturation can be shown by placing a drop of 1N HCl on liquid egg white in a flat container. Have the students explain what happened to the egg white solution.
- Reinforce student safety when they are handling the different pH solutions and working with the hot plates.

The following are recommendations for the Guided Inquiry for Activity 2:

- Have the students identify the skills, such as determining the rate of glucose production from lactose, they will need to carry out the guided inquiry. These skills were learned in the structured inquiry.
- Place students in work groups. Encourage each group to assign specific tasks to group members.
- Ask students to write their own paper written in their words.

Structured Inquiry: Temperature

Step 1: Prepare a large beaker of boiling tap water on a hot plate. Prepare five identical test tubes, each containing 2 mL of milk. Label five test tubes accordingly with each of the following temperatures: 0°C (ice bath), 4°C (refrigerator), room temperature, 37°C, and 100°C (boiling temperature). Place one tube of milk at each of the five temperatures. Create a data table to enter your results for each of these test tubes over time. Measure the room temperature using a thermometer.

Step 2: Hypothesize/Predict. Based upon your knowledge of enzymes and the effects of temperature on their activity, rank the tubes from fastest (1) to slowest (5) glucose production predicted over time after the addition of lactase. Add your predictions to the data table you created in Step 1.

Step 3: Student-led Planning. Discuss with your partner how you could use the data you collect to calculate a rate of lactase activity for each temperature.

Step 4: Make your lactase enzyme solution per your teacher's instructions. Add 1 mL of the lactase enzyme solution to each of the five tubes listed above and immediately start timing. Immediately after adding the lactase enzyme solution, determine the glucose concentration in each tube using glucose test strips and the color chart that came with the test strips. Record this in your data table.

Monitor the temperatures of each of these locations, both before and after the experiment using thermometers.

Step 5: Every 3 minutes for 15 minutes, record the concentrations of glucose in each tube using the color chart that came with the test strips and record in your data table.

Step 6: Critical Analysis. Calculate the rate of enzymatic activity for lactase at each temperature using the method you devised in Step 3. Using graph paper, graph your data of rates of lactase activity versus temperature. Which is the independent variable? Which is the dependent variable? Are the predictions you made in Step 2 supported by your data? Explain how you know in your notebook.

Guided Inquiry: Temperature

Step 1: Hypothesize/Predict. Based upon the data already collected, predict a temperature range that includes the optimal temperature for lactase activity. How do you think you could more finely pinpoint the optimal temperature for lactase activity? Write your ideas in your notebook.

Step 2: Student-led Planning. Determine how you could change the set-up of your test tubes to determine the optimal temperature for lactase activity. Once your teacher approves, create a table to record your data, prepare your test tubes per your design, and record data on glucose production every 3 minutes for 15 minutes. Determine the rates of lactase enzyme activity under each of your chosen conditions as you did in the Structured Inquiry. Graph the rates of lactase enzyme activity versus temperature and estimate the optimal temperature.

Step 3: Critical Analysis. Are the predictions you made in Step 1 supported by your data? Is there any way you can improve your experiment? Discuss your answer with your lab partner and write it in your notebook.

Structured Inquiry: pH

Step 1: Prepare three test tubes, each containing 2 mL of milk and label the three tubes as follows: 4.0 (acidic), 7.0 (neutral), and 9.0 (basic). To the first test tube, add 1 mL of pH 4.0 buffer. To the second test tube, add 1 mL of pH 7.0 buffer. To the third test tube, add 1 mL of pH 9.0 buffer. Create a data table to enter your results for each of these test tubes over time.

Step 2: Hypothesize/Predict. Based upon your knowledge of enzymes and the effects of pH on their activity, order the tubes from highest (1) to lowest (3) glucose production predicted over time. Add your predictions to the data table you created in Step 1.

Step 3: Student-led Planning. Discuss with your partner how to calculate a rate of lactase activity for each pH.

Step 4: Make your lactase enzyme solution per your teacher's instructions. Add 1 mL of the lactase enzyme solution to each of the three tubes listed above and immediately start timing. Immediately after adding the lactase enzyme solution, determine the glucose concentration in each tube using glucose test strips and the color chart that came with the test strips. Record this in your data table.

Step 5: Every 3 minutes for 15 minutes, record the concentrations of glucose in each tube using the color chart that came with the test strips and record in your data table.

Step 6: Critical Analysis. Calculate the rate of enzymatic activity for lactase at each pH. Using graph paper, graph your data of rates of lactase activity versus pH. Which is the independent variable? Which is the dependent variable? Are the predictions you made in Step 2 supported by your data? Explain how you know in your notebook.

Guided Inquiry: pH

Step 1: Hypothesize/Predict. Based upon the data already collected, predict a pH range that includes the optimal pH for lactase activity. How do you think you could more finely pinpoint the optimal pH for lactase activity? Write your ideas in your notebook.

Step 2: Student-led Planning. Determine how you would change the set-up of your test tubes to determine the optimal pH for lactase activity. Once your teacher approves, create a table to record your data, prepare your test tubes per your design, and record data on glucose production every 3 minutes for 15 minutes. Determine the rates of lactase enzyme activity under each of your chosen conditions. Graph the rates of lactase enzyme activity versus pH and estimate the optimal pH.

Step 3: Critical Analysis. Are the predictions you made in Step 1 supported by your data? Is there any way you could improve your experiment? Discuss your answer with your lab partner and write it in your notebook.

Assessments

1. If an enzyme has an optimal activity at 25°C, what do you think will happen to the enzyme's activity if the temperature is raised to 37°C? Why? [SP 1, Concept Explanation; SP 6, Argumentation]
2. If an enzyme has a largely acidic active site, what do you think will happen to the enzyme's activity if the pH is made basic? Why? [SP 1, Concept Explanation; SP 6, Argumentation]

[Answer Key]

1. Since 37°C is well above the optimum, one would expect a decrease in enzyme activity, possibly due to denaturation.
2. A basic solution would neutralize the active site, likely reducing the activity of the enzyme.

Activity 3: Pre-Assessment

1. **Answer the following question in your notebook:** How could we determine experimentally the specificity of an enzyme to its substrate?
2. **Discuss the answer to question 1 with the class.**

Activity 3: Substrate Specificity of the Enzyme Lactase

An enzyme's active site contains side chains of the amino acids that can only bind to certain molecules. For example, if the active site is largely positively charged, then negatively charged substrates will be attracted. Additionally, the three-dimensional shape of the active site within the enzyme is key in determining which substrates will fit into the active site (Figure 7.5). What does this suggest about how specific enzymes are to their substrates?

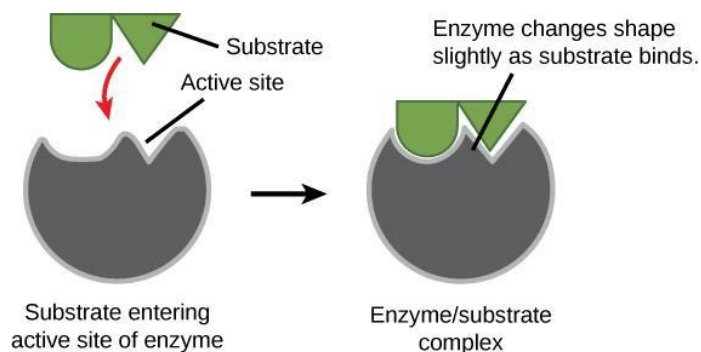


Figure 7.5: The three-dimensional shape of the active site is also important in determining which substrates will bind to the active site.

Safety Precautions

- Measure fluids carefully using graduated cylinders to avoid breakage and spillage.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.

For this activity, you will need the following:

- Graduated cylinder
- Beaker
- Water
- 20 mg/mL lactose solution
- 20 mg/mL sucrose solution
- Various foods for testing for presence of lactose, including both dairy products and those marketed as lactose-free
- Lactase (obtained from laboratory supply company)
- Test tubes
- Stirring rod

- Timer
- Labeling pencil
- Glucose test strips
- Graph paper

For this activity, you will work *in pairs*.

Activity 3 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 3:

- Provide students with images of lactose and sucrose. Have the students compare and contrast the structure of the two molecules.
- A short video or animation of enzyme-substrate interactions provides a good review for students.
- You can reduce the number of glucose test strips used in this activity by carefully slitting them in half lengthwise. Make sure that the indicator patch remains intact on each side.
- A Benedict's test can be used in place of the glucose test strips in this activity to measure glucose, if desired.
- Lactase solution must be stored in a refrigerator to reduce a loss of activity. The solution should be used at room temperature.
- Take precautions to prevent redundancy if you are requesting students to bring in food for this activity. Assign students to particular food groups and have them at first hypothesize about the foods they are selected as containing or not containing lactose.

The following are recommendations for the Guided Inquiry for Activity 3:

- Have the students identify the skills, such as determining the relative concentration of lactose, they will need to carry out the guided inquiry. These skills were learned in the structured inquiry.
- To expedite the activity, it is best to place the students in work groups. Encourage each group to assign specific tasks to group members.
- To facilitate individual assessments, students should be asked to write their own paper written in their words.

Structured Inquiry

Step 1: Prepare two test tubes, one with 2 mL of 20 mg/mL lactose and the other with 2 mL of 20 mg/mL sucrose. Label one tube as lactose-containing and the other as sucrose-containing. Create a data table to enter your results for each of these test tubes over time.

Step 2: Hypothesize/Predict. Based upon your knowledge of enzymes and their specificity, predict whether you will observe enzyme activity for each of the two tubes indicated above. Add your predictions to the data table you created in Step 1.

Step 3: Student-led Planning. Discuss with your partner how to calculate a rate of lactase activity for each substrate.

Step 4: Make your lactase enzyme solution per your teacher's instructions. Add 1 mL of the lactase enzyme solution to each of the two tubes listed above and immediately start timing. Immediately after adding the lactase enzyme solution, determine the glucose concentration in each tube using glucose test strips and the color chart that came with the test strips. Record this in your data table.

Step 5: Every 3 minutes for 15 minutes, record the concentrations of glucose in each tube using the color chart that came with the test strips and record in your data table.

Step 6: Critical Analysis. Calculate the rate of enzymatic activity for lactase in each substrate. Using graph paper, make a bar graph showing the rates of lactase activity in each of the two substrates. Are the predictions you made in Step 2 supported by your data? Explain in your notebook how you know.

Guided Inquiry

Step 1: Hypothesize/Predict. Based upon what you have learned, how could we use this assay for lactase activity to determine the relative amounts of lactose in various food products, including typical dairy products as well as those that are advertised as lactose-free? Write your ideas in your notebook.

Step 2: Student-led Planning. With your lab partner, choose two food products to test—one that you suspect contains lactose and one that is supposed to be lactose-free. Determine how you would change the setup of your test tubes above to assay for lactase activity in food products. Once your teacher approves your experimental design, create a table to record your data, prepare your test tubes per your design, and record data on glucose production every 3 minutes for 15 minutes. Determine the rates of lactase enzyme activity for each food product. Make a bar graph showing the lactase activity for each of the two foods chosen.

Step 3: Critical Analysis. Are the predictions you made in Step 1 supported by your data? Is there any way you could improve your experiment? Discuss your answers with your lab partner and write it in your notebook.

Assessments

1. How can substrate specificity of an enzyme be used to determine the presence of its substrate in a sample? Explain in terms of the active site. [SP 1, Concept Explanation]
2. What might you conclude if you observed glucose in a lactose-free food at the start of the experiment? [SP 3, Questions and Methods]
3. How could enzyme specificity be used to determine the concentration of a substrate in a food product? [SP 3, Questions and Methods]

[Answer Key]

1. Because enzymes are specific, an enzyme should only exhibit activity if its specific substrate is present within the sample. Due to the three-dimensional shape of the active site, the active site will only fit its specific substrate. Other substrates will not be of the appropriate shape to fit into the active site, so they will be unable to interact with the enzyme's active site and the enzyme will not function with incorrect substrates.
2. One would conclude that there was glucose present in the food product prior to lactase addition.
3. One could determine the rate of enzyme activity in the presence of a fixed amount of enzyme but different known concentrations of substrate to develop a standard curve of enzyme activity versus substrate concentration. Because a higher substrate concentration would lead to more interactions of the substrate with the enzyme, one would expect higher rates of enzyme activity at higher substrate concentrations. Then, one could add the fixed amount of enzyme to a food product, monitor the concentrations of product made over time, determine the rate of enzyme activity, and then compare that rate of enzyme activity to the standard curve to determine the initial amount of substrate in the food product.

Lab 8: Cellular Respiration

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
2.1 Cell Structure: Subcellular Components	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.D Describe the structure and/or function of subcellular components and organelles.	SYI-1.D.4 Mitochondria have a double membrane. The outer membrane is smooth, but the inner membrane is highly convoluted, forming folds.
2.2 Cell Structure and Function	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.E Explain how subcellular components and organelles contribute to the function of the cell. SYI-1.F Describe the structural features of a cell that allow organisms to capture, store, and use energy.	SYI-1.E.1 Mitochondrial double membrane provides compartments for different metabolic reactions. SYI-1.F.1 The folding of the inner membrane increases the surface area, which allows for more ATP synthesis. SYI-1.F. 8 The Krebs cycle (citric acid cycle) reactions occur in the matrix of the mitochondria. SYI-1.F.9 Electron transport and ATP synthesis occur on the inner mitochondrial membrane.
3.2 Enzyme Catalysis	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.E Explain how enzymes affect the rate of biological reactions.	ENE-1.E.1 Enzymes are biological catalysts that facilitate chemical reactions in cells and contribute to the regulation of biological processes.
3.4 Cellular Energy	ENE-1 The highly complex organization of living systems requires constant input	ENE-1.H Describe the role of energy in living organisms.	ENE-1.H.1 All living systems require constant input of energy.

	of energy and the exchange of macromolecules.		ENE-1.H.2 Life requires a highly ordered system and does not violate the second law of thermodynamics. ENE-1.H.3 Energy-related pathways in biological systems are sequential to allow for a more controlled and efficient transfer of energy.
3.6 Cellular Respiration	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.K Describe the processes that allow organisms to use energy stored in biological macromolecules. ENE-1.L Explain how cells obtain energy from biological molecules in order to power cellular functions.	ENE-1.K.1 Fermentation and cellular respiration, characteristics of all forms of life, use energy from biological molecules to produce ATP. ENE-1.K.2 Cellular respiration in eukaryotes involves a series of enzyme-catalyzed reactions. ENE-1.K.3 The electron transport chain transfers energy from the electron in a series of coupled reactions that establish an electrochemical gradient across membranes. ENE-1.L.1 Glycolysis releases energy in glucose to form ATP, NADH, and pyruvate. ENE-1.L.2 Pyruvate is transported from the cytosol to the mitochondrion, where further oxidation occurs. ENE-1.L.3 In the Krebs cycle, CO₂ is released, ATP is synthesized, and electrons are transferred to NADH and FADH₂. ENE-1.L.4 Electrons are transferred by NADH and

			FADH₂ to the electron transport chain. ENE-1.L.5 As electrons pass through the ETC, a proton gradient is established across the inner mitochondrial membrane. ENE-1.L.6 In fermentation, glycolysis occurs in the absence of oxygen. ENE-1.L.7 The conversion of ATP to ADP releases energy necessary to power metabolic processes.
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SCIENCE PRACTICES

Science Practices	Skills
SP 1 Concept Explanation <i>Explain biological concepts, processes, and models presented in written format.</i>	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations <i>Analyze visual representations of biological concepts and processes.</i>	2.A Describe characteristics of a biological concept, processes, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams and flowcharts.
SP 3 Questions and Methods <i>Determine scientific questions and models.</i>	3.A Identify or pose a testable question based on an observation, data, or model. 3.B State the null and alternative hypotheses, or predict the results of an experiment. 3.C Identify experimental procedures aligned to the question, including variables and controls. 3.D. Make observations and collect data. 3.E Propose a new/investigation based on evaluation of evidence from a previous experiment.
SP 4 Representing and Describing Data	4.A Construct a graph, plot or chart. 4.B Describe data from a table or graph.

<i>Represent and describe data.</i>	
SP 5 Statistical Tests and Data Analysis <i>Perform statistical tests and mathematical calculations to analyze and interpret data.</i>	5.A Perform mathematical calculations, including equations from the curriculum and rates. 5.B Use confidence level intervals and/or error bars to determine whether sample means are statistically different. 5.C Use data to evaluate a hypothesis.
SP 6 Argumentation <i>Develop and justify scientific arguments using evidence.</i>	6.A. Make a scientific claim. 6.B Support a claim with evidence. 6.C. Provide reasoning to justify a claim by connecting evidence to biological theories. 6.D Explain the relationship between experimental results and larger biological concepts, processes, or theories. 6.E Predict the causes of effects of a change in one or more components in a biological system based on data.

Living organisms require an external source of energy to sustain growth, metabolism, and reproduction. Where does this energy come from? **Phototrophs** can tap solar energy and convert it into chemical energy through the process of photosynthesis. **Chemotrophs** obtain chemical energy from food molecules and store it in the small molecules ATP and NADH. Cells release the energy of glucose and other food molecules through stepwise **redox reactions** in which electrons and energy are transferred from one compound to another. **Oxidation** describes the loss of electrons by a molecule referred to as an electron donor. Where do these electrons go? The accompanying reaction, **reduction**, is the gain of electrons by another molecule which serves as an electron acceptor. The overall chemical reaction of aerobic respiration is summarized below:



In the process of aerobic respiration, electrons are oxidized from glucose molecules ($\text{C}_6\text{H}_{12}\text{O}_2$) to create **ATP (adenosine triphosphate)**, the energy molecule of the cell. The electrons oxidized from glucose undergo a series of redox reactions, each of which releases energy that drives the formation of ATP. Some of these redox reactions occur during **glycolysis** and the **Krebs cycle**, but most occur during the **electron transport chain** of aerobic respiration. Eventually, the electrons are accepted by oxygen atoms and removed from the system. This allows additional electrons from other glucose molecules to enter the chain of redox reactions, synthesizing more ATP. During the multi-step oxidation of glucose, carbon dioxide is formed and released as a waste product. Another waste product of aerobic respiration is heat, as the redox reactions are not 100% efficient at transferring energy to ATP. Fortunately, this heat is used to maintain our body temperature, explaining why exercise increases heat production in our bodies.

Aerobic respiration can be divided in three main phases which are localized in different compartments of the eukaryotic cell, as shown in Figure 8.1.

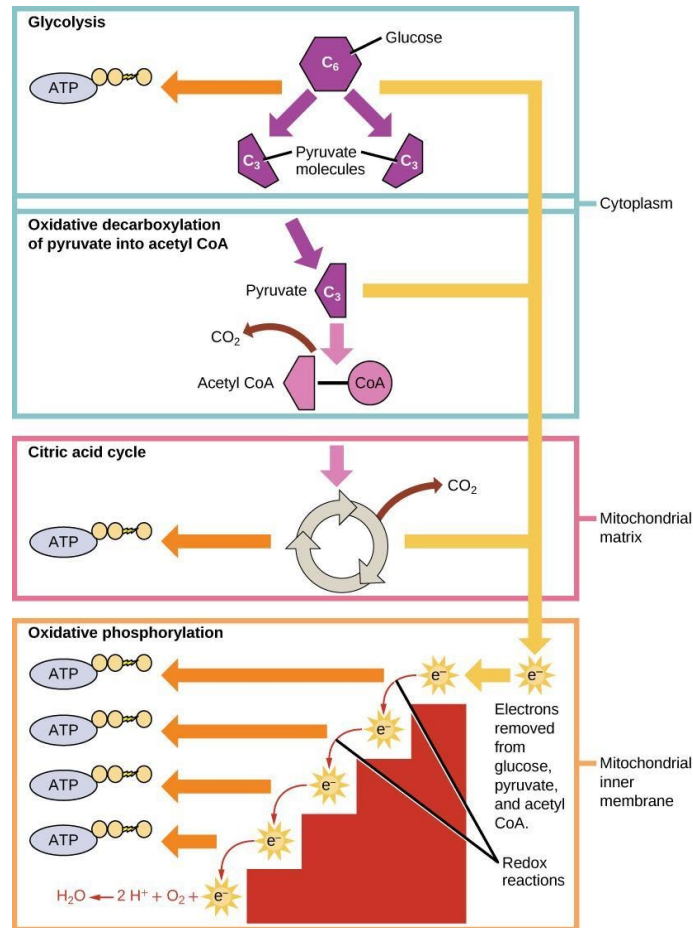


Figure 8.1: The three phases of cellular respiration are localized in different compartments of the eukaryotic cell.

Glycolysis, the partial breakdown of glucose to pyruvate with production of NADH and ATP, takes place in the cytoplasm. The intermediate step is the oxidation of pyruvate into acetyl CoA followed by the citric acid cycle in the matrix of the mitochondrion. In the **citric acid cycle**, glucose is completely oxidized to CO_2 . Electrons are transferred to small electron carrier molecules, NADH and $FADH_2$.

The final step, oxidative phosphorylation takes place in the inner membrane of the mitochondrion, as shown in Figure 8.2. **Oxidative phosphorylation** is the process by which reduced electron carriers generated in glycolysis and the citric acid cycle are re-oxidized by an electron transport chain embedded in a membrane. The energy released by the oxidation of the electron carriers fuels the production of ATP by chemiosmosis across the membrane (Figure 8.2).

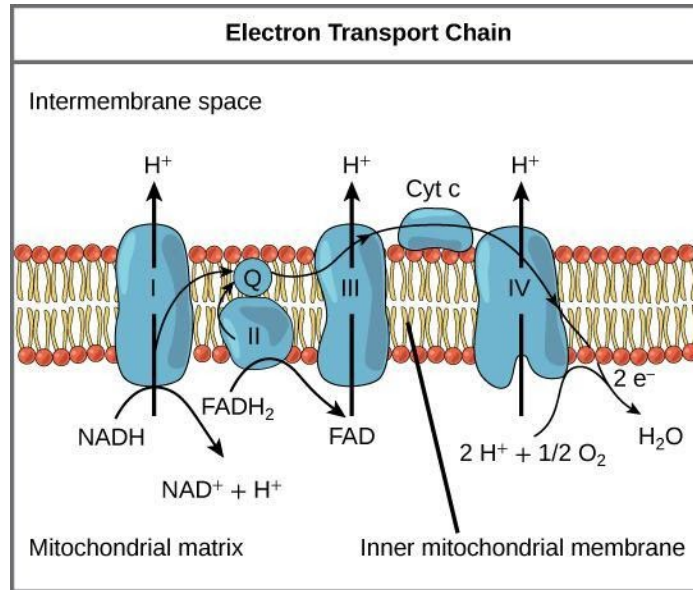


Figure 8.2: Electron transport chain embedded in mitochondrial membrane. Oxygen acts as the final electron acceptor.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Begin the first lab by discussing the background of this topic by including a brief PowerPoint lecture on the topic or even an animation of the various chemical reactions of cellular respiration. This will be especially helpful if your students have not had a general chemistry lab class in a long time or if this is their first time being in a biology lab.
- Use a single-edge razor blade or knife with caution to prepare the specimen slices. Do not leave blades exposed on the bench.
- Emphasize the importance of cellular respiration, the locations of where the reactions occur in cells, and the end results (products) that are produced by these chemical reactions. A *circle of life* type of illustration to show the interconnectedness of what happens in plants and animals will be useful for further guidance (i.e., discuss or include an illustration of how carbon dioxide released from animals is used by plants for carrying out photosynthesis and likewise discuss how Oxygen produced by plants is used by animals for the cellular respiration). This information would be helpful to assist students in seeing the entire *bigger picture* of why these processes are important.
- Remind students of lab safety guidelines and proper disposal of sharps items such as razors, slides, and coverslips.

- Remind students that human biological samples (i.e., cheek cells) may be used for part of this experiment. Highlight the proper disposal of this potentially hazardous waste (i.e., bleach or biohazard container). Be sure to have one of these items on hand as part of the preparation for the lab setup.
- Supply students with either lab coats or aprons to protect their clothing and/or skin from exposure. It will be useful to also require the students to wear gloves/goggles when handling the dyes.

In this lab, you will learn

- how to determine the site of respiration in the cell;
- how to measure the consumption of oxygen during respiration;
- how to measure the effect of environmental conditions on respiration in pea seeds.

Activity 1: Pre-Assessment:

1. **Answer the following questions in your notebook:** What function do mitochondria fulfill in the cell? What kind of staining would allow visualization of mitochondrial activity?
2. **Answer the following questions in your notebook:** Which plant tissue would you choose to stain mitochondria? Explain your choice.
3. **Discuss the answers to questions 1 and 2 with the class.** Activity 1: Staining Mitochondria with Janus Green B

You will investigate the site of oxidative phosphorylation and the effect of environmental conditions on the mitochondria. Janus Green B is a stain that appears blue-green when oxidized (that is, when it loses electrons) and is colorless or light pink when reduced (when it gains electrons).

Safety Precautions

- Dispose of coverslips in a broken glass container.
- Be careful handling glass slides, the edges may be sharp.
- If using cheek cells, dispose of flat toothpicks and slides in a jar containing 10 percent bleach.
- Handle dyes with care and be careful not to ingest.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.

- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Onion; alternatively slices of celery branch or cheek epithelial cells may be used
- Toothpicks and 10 percent bleach container if using epithelial cells
- Forceps
- Water and dropper
- 0.001 percent solution of Janus Green B
- Absorbent paper such as a paper towel or filter paper
- Microscope
- Slides and coverslips
- Mounting fluid to be chosen by students (7 percent sucrose, 0.9 percent salt, vinegar, ammonia)

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Assist students in performing a wet mount by doing a proper demonstration in front of the entire class prior to letting them commence with this activity (i.e., demonstrate how to safely use the razor to slice the onion).
- Remind students of the potential changes in color they will see upon adding the Janus Green B dye indicator. Remind them to write their hypotheses first prior to commencing the experiment. Additionally, discuss the various mounting solutions that are available that they will have to choose from.
- Having a brief discussion of the proper use and handling of the microscope may also be useful to discuss prior to letting the students begin their experiments. Tips to consider discussing include instructions on how to properly carry the microscope, the locations and uses of the various parts (i.e., oculars, condenser, fine and coarse adjustment knob, stage, etc.), and how to store the microscopes will be very helpful in assisting your students in conducting this part of their lab exercises. Keep in mind that if they are beginners, you may have to instruct them on how to focus their specimen as well as on how to correctly record the total magnification of the specimen they are viewing (i.e., $\text{Total Magnification} = \text{Magnification of Ocular lens} \times \text{Magnification of Objective lens}$).

The following are recommendations for the Guided Inquiry for Activity 1:

- Students could remain in groups of two while doing the Guided Inquiry project, but you may wish to have each student write their own hypotheses to ensure that they are submitting their own original work.
- Although an illustration has been provided in Figure 8.3, you may wish to demonstrate how to perform the wicking method prior to letting the students perform this part of the experiment. Be sure to provide the students with a timer so that they can accurately check their results at the designated times.
- If time allows, you may want to instruct the students to try redoing their experiment by changing the mounting solutions they are using for their wet mounts and have them compare the results yielded and/or observations made when utilizing the different types of mounting solutions. Remind the students that both should be observing their results microscopically and writing their observations in their respective notebooks to ensure that everyone is doing their fair/equal share of the work.
- In Step 4, clarify to students how to analyze their results. Providing a grading rubric for this activity with criteria describing each of the mandatory parts of this activity can help guide students to include the proper components in their experiment for this activity.

Structured Inquiry

Step 1: Preparation and observation of wet mount:

1. Grab the edge of the layer with the forceps peeling back a thin transparent layer of epidermal tissue. The thickness of the layer is one or a few cells which will allow you to visualize clearly the inside of the cells.
2. Add a drop of water and place a cover slip over the onion slice; do not remove air bubbles if they form.

Step 2: Hypothesize/Predict: Janus Green B is an indicator of the redox state. Knowing that it appears blue/green in its oxidized state, and loses its color when it is reduced, allows you to predict which organelle will show a progressive change in color because it is the active site of oxidation-reduction. Discuss with your lab partner how you would expect Janus Green B color to reflect active respiration. Which experimental conditions would you choose to investigate with your current setup? Record your prediction in your notebook.

Step 3: Student-Led Planning: Decide which mounting solution(s) you will choose to observe changes in respiration in the epidermal layer. View the sample first under low magnification to focus on the cells. Proceed to the highest magnification available to you (highest dry objective or oil immersion) and observe internal structures.

Stain with Janus Green B by using the wicking method as shown in Figure 8.3. Place a piece of filter paper on one side of the cover slip. Add one or two drops of Janus Green on the opposite side of the coverslip close to the edge. The stain solution will flow in by capillary action.

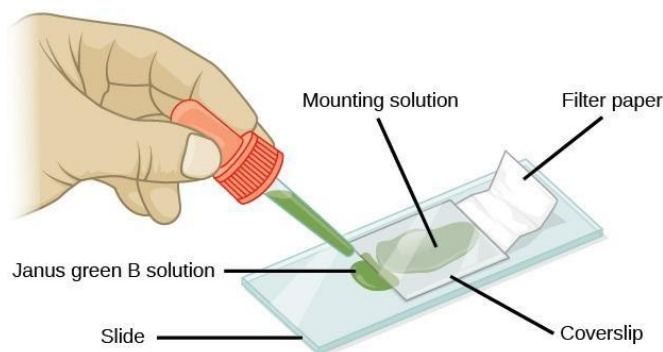


Figure 8.3: Staining a wet mount by capillary action.

Monitor the changes in stain appearance for five to 10 minutes, taking turns observing the slide. Record your observation in your lab notebook.

Step 4: Critical Analysis: Draw and label all the structures that you can identify. Do not forget to add a title and the final magnification to all the drawings. Draw only what you observe. Do not copy from existing micrographs from published or online work. Compare the effect of different mounting solutions to distilled water. Did you observe a change in the color of mitochondria? Did it change over time? If there was an effect, how can you explain your observations? Can you think of other experiments that would support your conclusions?

Guided Inquiry

Step 1: Hypothesize/Predict: Janus Green B is a vital stain and an indicator of redox state. Knowing that it appears blue/green in its oxidized state and loses its color when it is reduced, allows you to predict which organelle will show a progressive change in color because it is the active site of oxidation-reduction and which organelle will appear colorless. What environmental conditions would be essential to observe a blue color of stain?

Step 2: Student-led planning: Prepare a wet mount of tissue under the microscope. Stain with Janus Green B, using the wicking method described earlier, while observing under the microscope. Which organelles can you distinguish? Are there any changes with time? Record your observations in your notebook. Repeat your experiment with a different mounting medium, staining with Janus Green B according to the wicking method. Record your observations in your notebook.

Step 3: Critical analysis: How did the various mounting fluids you used influence the response of the mitochondria to Janus Green B? How can you explain the effect that you observed? Compare the effect of different mounting solutions to distilled water. Was there an effect? If there was an effect, how can you explain your observations? Can you think of other experiments that would support your conclusions? Write your ideas in your notebook.

Assessments

1. What do you predict would be observed if the epidermal layer of an onion is incubated in a solution of rotenone, an inhibitor of respiration? [SP 3, Questions and Methods; SP 6, Argumentation]
2. A student carefully mounts a specimen of onion epidermal layer, pushing out all the air bubbles. She is very disappointed that she does not observe a change in the color of Janus Green B. Can you explain her observation? [SP 1, Concept Explanation; SP 3, Questions and Methods]
3. Cyanide is a known metabolic poison that acts mainly by blocking cytochrome oxidase, an enzyme embedded in the inner membrane of mitochondria, and preventing the reduction of oxygen. If cyanide were added to an onion layer stained with Janus Green B, what you would observe and why? [SP 1, Concept Explanation; SP 3, Questions, and Methods; SP 6, Argumentation]

[Answer Key]

1. Janus Green B loses its stain only if active mitochondria are present and use up oxygen. In an oxidizing atmosphere, where oxygen is present. The mitochondria would look green.
2. The student created an anaerobic environment. The stain cannot be oxidized by the mitochondria because there is no oxygen.
3. The mitochondria would stay green because respiration is inhibited.

Activity 2: Pre-Assessment

1. **Answer the following questions in your notebook:** Students stain corn seeds over a period of several days after the seeds are soaked with water to promote germination with iodine. Iodine stains starch blue. The students observe that the amount of starch decreases during germination. Can you explain this observation? Which metabolic process uses up starch?
2. **Answer the following questions in your notebook:** What kind of biological catalysts are involved in the reactions of respiration? If the rate of a chemical reaction doubles with the temperature, would you expect that rate of respiration to increase continuously with temperature?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 2: Measurement of Respiration and Effect of Temperature on Respiration Rate

Imagine that you plan to monitor respiration in a whole organism, such as a small invertebrate or a seedling. You may decide to follow the disappearance of the reactants, either glucose or oxygen. Your second choice is to measure the formation of the products, either water or carbon dioxide. In this laboratory, you will design experiments to assess the effect of environmental conditions on the process of cellular respiration.

In respiration, oxygen is consumed and CO₂ is released. In this experiment, we will measure the disappearance of oxygen. A **respirometer** consists of an enclosed chamber in which the studied organism is placed and a graduated pipette with which we measure changes in the gas volumes. The CO₂ gas which forms will be removed by adding Ca(OH)₂ that reacts with carbon dioxide to form the insoluble salt CaCO₃, calcium carbonate.

While measuring the changes in the amount of gas produced, you will consider the ideal gas law equation which can be stated as

$$PV = nRT$$

P represents the atmospheric pressure in mmHg, V is the volume of the gas in liters, n is the number of moles of gas, R is the ideal gas constant, and T is the temperature in degrees Kelvin. In the respirometer, pressure remains constant as the gas produced displaces water in the tube. We will set up the respirometers in a water bath to minimize fluctuations in temperature.

In this experiment, you will use pea seeds. In a seed, like the yellow peas shown in Figure 8.4, a tough coat protects the plant embryo. Nutrients in the form of starch and lipids surround the embryo and support its **germination**, or growth from seed, until the appearance of photosynthetic structures. Seeds are normally **dormant**, that is metabolically inactive, until the environmental conditions helpful for growth are available. In order to bring the seeds to an active state, (out of dormancy), the seeds you will use were soaked in water via a process called imbibition, for 6 to 8 days.



Figure 8.4: Dried yellow peas are the seeds of a variety of the plant *Pisum sativum* and are considered a staple food. They contain yellow cotyledons and a large reserve of starch.

Cellular respiration involves three major sequential stages: glycolysis, the citric acid cycle, and oxidative phosphorylation. Oxygen serves as a terminal electron acceptor. Glycolysis takes place in the cytoplasm whereas mitochondria are the site of the citric acid cycle and the electron transport chain.

All the steps of respiration are mediated by **enzymes**, biological catalysts—mainly proteins—that lower the **activation energy**, the energy required to be available in a system before a chemical reaction can take place. Enzymes are not used up by the reactions they catalyzed. The process of respiration responds to the same environmental factors that affect the activity of enzymes. In this activity, you will measure the effect of temperature on respiration rates.

Safety Precautions

- Handle test tubes or glass containers with care; insert the plug by holding the container in a paper towel.
- Use plastic pipettes rather than glass pipettes.
- Wear goggles or safety glasses.
- Wear gloves when working with KOH or lime [$\text{Ca}(\text{OH})_2$] that are corrosive chemical compounds.
- Use care while handling hot water. Wear mitts and do not leave boiling water or a hot plate unattended.
- Protect your clothes with an apron.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Dried yellow peas
- Glass beads
- Balance and weigh boats
- Paper towels to imbibe seeds
- KOH or lime water
- Food coloring
- Absorbent and nonabsorbent cotton

- Drilled rubber stoppers which fit the opening of the test tubes or bottles
- 1-ml plastic pipettes
- Top loading balance
- Thermometers
- Water baths
- Weights such as clamps or hex keys
- Wide glass test tubes or bottles
- Stirring rod
- Ice
- Hot plate to boil water
- Mitts

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 2:

- Remind students of the importance of lab safety and that they should wear appropriate clothing as well as shoes to the lab for each period. Additionally, since a hot plate is being used for this activity, it is important to also underscore the importance of the proper use of this piece of equipment to avoid burn injuries and possible fire hazards.
- Provide the students with instructions on the proper handling of mercury thermometers as well as what to do in the event that one breaks during lab.
- Be sure to have any extra materials available while prepping for this experiment. Students working in pairs may require a bucket or other carrying container for their materials (i.e., pipettes, seeds, ice, etc.)
- Since the first part of this activity requires students to utilize a respirometer, it will be useful to demonstrate the proper use and setup of this equipment to the students prior to letting them begin their experiments.
- Be sure to have three water baths set up ahead of time at the following temperatures: 10°C, room temperature (see Structured Inquiry), and 50°C. You will want to check the water baths periodically to ensure that the temperatures remain stable.

The following are recommendations for the Structured Inquiry for Activity 2:

- Students could remain in groups of two while doing the Guided Inquiry project, but you may wish to have each student write their own redesign of the experiment. This is to ensure that each student contributes equally to a group project.
- You will want to check the student's hypothesis and initial experimental design during the Structured Inquiry before allowing them to proceed with performing their experiment. This will help to ensure that they are performing an experiment that can actually be conducted in your lab, given the available equipment and time you have allotted for this activity.
- Students often will not include all vital components of an experiment due to their inexperience. Therefore, they will also likely need advice on how to determine what controls to use for their experiment as well as identifying their independent and dependent variables.
- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.
- In Step 4, clarify to students how to analyze their results. Often students don't understand the importance of why they must perform experiments multiple times or have multiple variables, controls, etc., therefore remind them of the importance of the reproducibility of data when trying to analyze results for scientific significance. Instruct them on how to determine if any human error could have been introduced into the experiment that could have skewed the results.
- Providing a grading rubric for this activity with criteria describing each of the mandatory parts of this activity can help guide students to include the proper components in their experiment for this activity.

The following are recommendations for the Guided Inquiry for Activity 2:

- You will want to check the water baths periodically to ensure that the temperatures remain stable.

To ensure that the respirometers are working properly, you may want to instruct the students on how to test for leaks by immersing the respirometer with the plug and pipette *before* filling it with reagents and cotton.

- Students often will not include all vital components of an experiment due to their inexperience. Therefore, they will also likely need advice on how to determine what controls to use for their experiment as well as identifying their independent and dependent variables during the guided portion of the exercise.
- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.

- In Step 4, clarify to students how to analyze their results. Often students don't understand the importance of why they must perform experiments multiple times or have multiple variables, controls, etc., therefore remind them of the importance of the reproducibility of data when trying to analyze results for scientific significance. Instruct them on how to graph the changes in gas volumes from the data in your table for all three temperatures for the experimental and control set-up.
- Providing a grading rubric for this activity with criteria describing each of the mandatory parts of this activity can help guide students to include the proper components in their experiment for this activity.

Structured Inquiry

Step 1: Obtain 25-30 germinating peas, dry peas and glass beads to start your experiment. Place the germinating peas in a weigh boat and measure their weight. Record the weight in your notebook and then repeat for the dried peas and glass beads.

Step 2: In this activity, you will indirectly measure the rate of respiration of the peas by monitoring the decrease in gas when the peas are placed in the respirometer chamber. What gas will decrease in the chamber as the peas undergo respiration? Hypothesize how much the gas levels will likely change for the germinating seeds, dry seeds, and glass beads. Record your hypotheses and predictions in your notebook.

Step 3: Student-Led Planning: Which of your treatments serve as a control? Is this a positive or negative control? How will this control reveal whether or not the experiment is functioning properly? Write your answers in your notebook.

Step 4: Assemble a respirometer using Figure 8.5 as a guide and following the steps below.

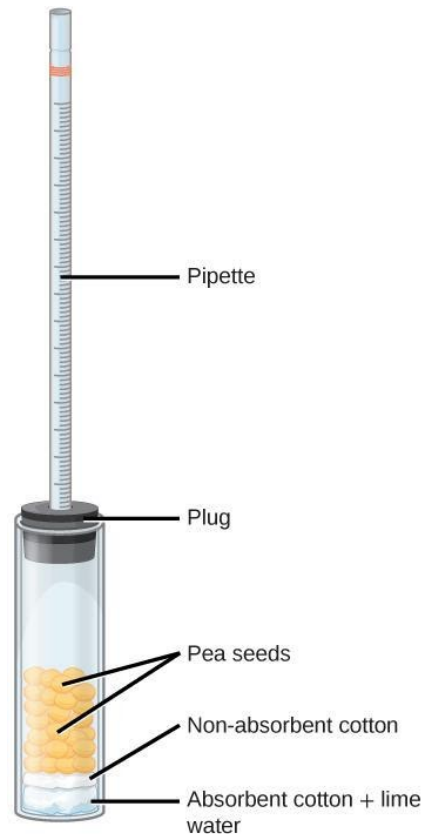


Figure 8.5: Assembled respirometer.

- a. In a wide test tube (or bottle), drop a pad of absorbent cotton. Pack down the cotton with a stirring rod. Add lime water $\text{Ca}(\text{OH})_2$, being careful not to oversaturate the pad or drip the lime water on the side of the tube.
- b. Insert a thin layer of nonabsorbent cotton, pushing down with the glass rod. The cotton protects the seeds from lime water; however, if it is too thick, it will interfere with the diffusion of CO_2 .
- c. Plug the test tube with a bored rubber stopper. Add a drop of colored water in a 1-ml graduated pipette and insert the pipette in the hole of the stopper. Adjust the position of the drop by inserting a syringe in the stopper until you can easily read the position of the dye. (The syringe is not shown in Figure 8.5.) Rub some petroleum jelly where the pipette comes into contact with the rubber stopper. The respirometer must be water tight to yield reliable results. It is also possible to wrap the openings with stretchable plastic film.
- d. You may want to test for leaks by immersing the respirometer with the plug and pipette *before* filling it with reagents and cotton.

Step 5: Assemble the respirometer containing the control sample in the same manner.

Step 6: Immerse the respirometers with the experimental sample and the control in the water bath. Lining the water bath with a white paper towel will make it easier to read the markings on the pipettes. Make sure that the pipettes are resting across a piece of ribbon or string that spans the width of the water bath, as illustrated in Figure 8.6. The goal is to keep the pipettes out of the water while the test tubes remain submerged.

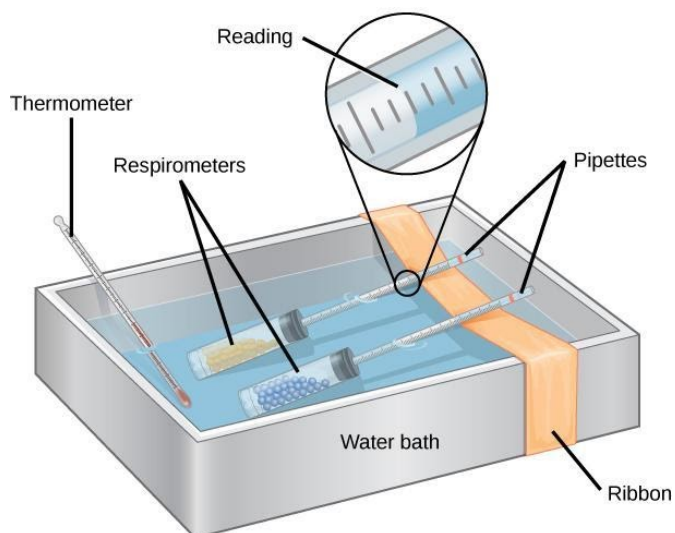


Figure 8.6: Respirometers in water bath.

Step 7: Let the respirometers equilibrate for 5–10 minutes.

Step 8: Read the starting volume on the pipette. This is time 0 min. Record the displacement of the colored bead for all samples every 2 minutes for 20 minutes and enter data in a table of measurements.

Step 9: Critical Analysis: Calculate the changes in volume where the reading at time 0 is subtracted from every subsequent reading. Subtract the rate of volume change measured in the control samples to obtain a corrected rate of respiration. Graph the changes in volume in respirometers as a function of time and calculate the rate of change from the slopes of the line plots. Calculate the rate of change per gram of seed. This will allow you to compare values obtained from different samples. Draw a plot of changes in gas volumes from the data in your table. What measurements will you enter on the x-axis? What measurements will you enter in the y-axis? Determine the rate of respiration in your experiment. How did you use the data from your control or controls? Did volumes change during the experiment? Which gas caused the change in volume? Do the results support your hypothesis? Can you explain unexpected results? Were the respirometers water-tight at all times? How could you modify the experiment in the future? Write your answers in your notebook.

Guided Inquiry

Step 1: Repeat the steps to set up the respirometers described in the Activity 2 Structured Inquiry. Use three water baths at the following temperatures: 10°C, room temperature (see Structured Inquiry), and 50°C.

Step 2: Hypothesize/Predict: Discuss with your partner what kind of influence temperature might have on metabolic processes. How would respiration rate measured at 10°C compare to the rate measured at room temperature? Will the rate of respiration be higher at 30°C than room temperature? Do you predict that the rate of respiration will be higher at 50°C than at room temperature or 30°C? Enter your hypotheses in your notebook.

Step 3: Student-Led Planning: You will now measure the rate of respiration at three different temperatures. Discuss with your partner if you need to run the experiment at room temperature again. Decide which control you will set up for this experiment. Make a note of all the steps you will perform, as you did in Activity 2, and create tables for your observations in your lab notebook. You will take readings of the colored water bubble at 2-minute intervals for 20 minutes. Have your teacher approve your experimental procedure before proceeding.

Step 4: Once approved, carry out your experimental procedure, closely monitoring the temperature as you take measurements.

Step 5: Critical Analysis: Graph the changes in gas volumes from the data in your table for all three temperatures for the experimental and control set-up, as you did for the Structured Inquiry. Determine the rate of respiration for each temperature. Because the gas law shows that differences in temperature affect volumes, you must correct for any changes in volume that are a consequence of temperature variations rather than respiration. To do this, subtract changes in volumes measured in the respirometer containing glass beads from the changes in volume measured in the tubes containing germinating seeds held at the same temperature. Do the results support your hypothesis? Explain whether your results support or refute your hypothesis. How could you modify the experiment in the future? Write your ideas in your notebook.

Assessments

1. Students record changes in gas released from respirometers containing germinating seeds and dry seeds. They set up their tubes in air rather than in a water bath. A thermometer probe is inserted in each respirometer. The tube that contains germinating seeds shows an increase in temperature. No such increase is recorded in a respirometer that contains dry seeds. What is the reason for the difference in temperature? [SP 1, Concept Explanation; SP 3, Questions and Methods; SP 6, Argumentation]
2. The ideal gas law shows that volume depends on temperature as well as pressure. Why do you set your respirometers in a water bath? [SP 1, Concept Explanation; SP 3, Questions and Methods]

3. A classmate insists that there are no mitochondria in leaves because chloroplasts produce ATP through photosynthesis. How would you experimentally disprove this claim? [SP 3, Questions and Methods; SP 6, Argumentation]

[Answer Key]

1. Respiration is an exergonic reaction. The metabolically active seeds release heat as well as produce ATP.
2. Water has a high heat capacity. By immersing the respirometer in a comparatively large volume of water, we buffered the change in temperature.
3. Set up a respirometer with leaves in the chamber. Tightly wrap the respirometer in aluminum foil to block all light. Measure the changes in gas over time. The changes are due to oxygen consumption because there is no photosynthesis. You may also use Janus Green B to make visible mitochondria which are noticeably smaller than chloroplasts.

Lab 9: Fermentation in Yeast

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
3.2 Enzyme Catalysis	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.E Explain how enzymes affect the rate of biological reactions.	ENE-1.E.1 Enzymes are biological catalysts that facilitate chemical reactions in cells and contribute to the regulation of biological processes.
3.4 Cellular Energy	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.H Describe the role of energy in living organisms.	ENE-1.H.1 All living systems require constant input of energy. ENE-1.H.2 Life requires a highly ordered system and does not violate the second law of thermodynamics; energy input must exceed energy loss. ENE-1.H.3 Energy pathways are sequential for a more efficient transfer of energy.
3.6 Cellular Respiration	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.K Describe the processes that allow organisms to use energy stored in biological macromolecules. ENE-1.L Explain how cells obtain energy from biological macromolecules in order to power cellular functions.	ENE-1.K.1 Fermentation and cellular respiration, characteristic of all life, use energy from biological macromolecules to produce ATP. ENE-1.L.6 Fermentation allows glycolysis to proceed in the absence of oxygen and produces organic molecules, including alcohol and lactic acid.

SCIENCE PRACTICES

Science Practices	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe characteristics of a biological concept, processes, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams and flowcharts.
SP 3 Questions and Methods Determine scientific questions and models.	3.A Identify or pose a testable question based on an observation, data, or model. 3.B State the null and alternative hypotheses, or predict the results of an experiment. 3.C Identify experimental procedures aligned to the question, including variables and controls. 3.D. Make observations and collect data. 3.E Propose a new/investigation based on evaluation of evidence from a previous experiment.
SP 4 Representing and Describing Data Represent and describe data.	4.A Construct a graph, plot or chart. 4.B Describe data from a table or graph.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including equations from the curriculum and rates. 5.B Use confidence level intervals and/or error bars to determine whether sample means are statistically different. 5.C Use data to evaluate a hypothesis.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.A. Make a scientific claim. 6.B Support a claim with evidence. 6.C. Provide reasoning to justify a claim by connecting evidence to biological theories. 6.D Explain the relationship between experimental results and larger biological concepts, processes, or theories. 6.E Predict the causes of effects of a change in one or more components in a biological system based on data.

Non-photosynthetic organisms extract and store energy from organic molecules, such as glucose. The chemical energy in these molecules is used to make ATP, the energy source that allows cells to function. The transfer of the chemical energy in food to ATP defines the process of **cellular respiration**. Two types of cellular respiration are aerobic respiration and fermentation. **Aerobic respiration** depends on the presence of oxygen as a final electron acceptor and yields large quantities of ATP from each organic molecule. Due to its dependence on oxygen, it is called an aerobic mechanism. **Fermentation** also yields ATP from organic molecules but occurs without the presence of oxygen. Fermentation is therefore called an anaerobic mechanism. In the presence of increased oxygen, cells of organisms capable of both processes will transition from fermentation to cellular respiration, because it is a more efficient way to obtain larger quantities of energy. The scientist, Louis Pasteur, was the first to observe this pattern, and therefore, it is called the Pasteur Effect.

Some organisms, such as yeast, can utilize both processes but it is more efficient for them to perform aerobic respiration as opposed to fermentation, as aerobic respiration produces more ATP per organic molecule. However, when oxygen levels are low or inadequate, these cells can switch to fermentation in order to keep producing ATP. In addition to yeast, some human cells, such as our muscle cells, can switch from aerobic respiration to fermentation for brief periods of time. You may be familiar with sore muscles or cramps from exercise, which are caused by the lactic acid produced by fermentation in our muscle cells. In this lab you will explore fermentation in yeast cells.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Begin the lab by giving the students a brief overview on the topic of the two types of cellular respiration, along with examples of each. You can utilize real life examples such as on the ones mentioned in the lab's introduction to make it more understandable to the students. For instance, you can have the students do a quick one-minute workout in class (i.e., jumping jacks) that may exert them to the point of producing lactic acid to illustrate your point further.
- Underscore the importance of proper lab safety for your students since they will be working with a living microorganism (dry yeast).
- Instruct students on the proper way to dispose of their yeast samples, Petri dishes (if disposable) into a biohazard container, if you have one present in the lab.

In this lab, you will learn

- how different carbon compounds affect the rate of fermentation;
- how temperature affects the rate of fermentation.

Activity 1: Pre-Assessment

1. **Answer the following questions in your notebook:** What is the difference between real sugar and a sugar substitute? Could either substance be used during cellular respiration?
2. **Answer the following questions in your notebook:** How could you tell that an organism, such as yeast, has switched from aerobic respiration to fermentation?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 1: Comparing Rates of Fermentation in Yeast

Yeast carry out fermentation as a means to access the chemical energy from their food, which, in this case, will be sugars such as glucose. In this activity, you will be comparing fermentation between these food sources and a control group of water. Yeast can exist in a state of **dormancy**, where they are alive, but their physical activity has temporarily stopped to minimize energy use. This adaptation allows these organisms to survive extended periods of drought and other harsh environmental conditions. As you add water to the dry yeast, you will be activating the cells out of dormancy, and they will resume physical activity and begin eating. Once the yeast absorbs the food molecule, it will first break the molecule down in a process called **glycolysis**. The word glycolysis literally translates into the breaking apart of glucose and this is the first step of the metabolism of sugars (Figure 9.1).

Shortly after mixing the activated yeast with the sugar molecules, fermentation will begin, and you will be able to observe gas bubbles being produced. This gas is carbon dioxide, one of the products of fermentation.

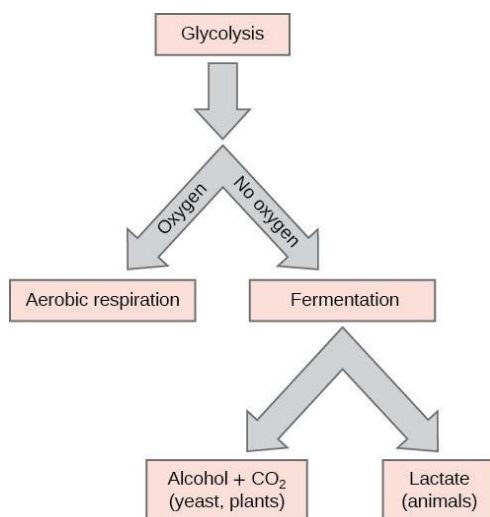


Figure 9.1: Glycolysis is the first step of both aerobic respiration and fermentation. Aerobic respiration only produces carbon dioxide as a toxic byproduct, but fermentation also produces alcohol or lactate.

Safety Precautions

- Use care when using glassware.
- Be careful when inverting the graduated cylinder into a Petri dish.
- Inform your teacher immediately of any broken or cracked glassware, as it could cause injuries.
- Clean up any spilled liquids to prevent people from slipping.

For this activity, you will need the following:

- Graduated cylinder (50 ml)
- Petri dishes
- Beaker/container for yeast solution (150–200 ml)
- One package of dry yeast
- Warm water
- Room temperature water
- Glucose
- A sugar substitute
- Pipette or droppers
- Paraffin wax paper
- Paper towels

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Demonstrate how and where to properly dispose of the reconstituted yeast and its metabolic byproducts prior to letting students begin their experiments. Continue to remind the students of the importance of lab safety. Since pipettes are being used, remind them to *never* pipet anything by mouth.
- Since the yeast will be producing alcohol as a byproduct of fermentation, it may be interesting to have the students smell the alcohol produced as a result of the anaerobic respiration process. If you choose to do this, it is highly recommended to teach students how to do the waft test in lieu of directly smelling the chemical agent. Please demonstrate this prior to initiation of the experiments, as you may have students who may be learning how to do this for the first time.
- Remind students of the locations of the following lab safety items in your lab in the event they need them during this activity: first aid kit, emergency exits, shower, fire extinguisher, eyewash, waste containers (broken-glass waste), safety glasses bin, gloves, and any other safety equipment and supplies in the lab.

- Have students create their data tables prior to beginning the lab exercise, to ensure that they are including the appropriate criteria in their lab reports for later analysis.
- Remind students that they are to perform the tests with each of the variables (i.e., sugar vs sugar substitute) multiple times, per the Scientific Method.

The following are recommendations for the Structured Inquiry for the Guided Inquiry for Activity 1:

- Have each student write their own hypotheses regarding the proposed outcomes of the experiment. This is to ensure that each student contributes equally and submit their own original content.
- Check the students' hypotheses and results from the initial experimental design during the Structured Inquiry before allowing them to proceed with performing their experiment. This will help to ensure that they are on the right track and will not waste too many materials not needed for the activity.
- Offer students advice on how to determine what controls to use for their experiment as well as identifying their independent and dependent variables. Students often will not include all vital components of an experiment due to their inexperience.
- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.
- In Step 3, clarify to students how to analyze their results. Additionally, providing a grading rubric for this activity with criteria describing each of the mandatory parts of this activity can help guide students to include the proper components in their experiment for this activity.

Structured Inquiry

Step 1: Add the yeast packet to the beaker and add 100 ml of warm water to activate it. Swirl slightly until water and yeast are mixed. Allow 10 minutes for activation. During this time, create a data table to measure the volume in milliliters of the carbon dioxide gas bubbles as a function of time. Include space for two trials for each variable of water, glucose, and the sugar substitute, as well as predictions of the amount of respiration that will occur for each sugar.

Step 2: Hypothesize/Predict: Based on what you know about the differences between water, glucose, and the sugar substitute, predict how much carbon dioxide concentrations will differ in the presence of 1) glucose, 2) the sugar substitute, and 3) water. Add your predictions to the data table created in Step 1.

Step 3: Student-Led Planning: You will now start with the first trial of measuring carbon dioxide gas bubbles produced from fermentation. Choose a time interval to take down measurements. After the first trial, adjust the time intervals if necessary for taking down measurements. These should be in your data table. Determine and record the quantity of glucose and sugar substitute that you will use, as you should perform more than one trial of each test.

Step 4: Pour 15 ml of yeast solution into the graduated cylinder. Then add in the water and fill up the rest of the cylinder. For the other trials, glucose or sugar substitute will be added instead of water. As soon as you add the water, cover the cylinder with paraffin wax paper and invert it, to avoid spilling the fluid. One person can hold the graduated cylinder in place displacing it carefully and completely onto the Petri dish, as shown in Figure 9.2. As fermentation takes place, bubbles will rise into the cylinder, and the volume of the bubbles can be recorded. To do this, you will read the measurement markings at either end of the bubbles to get a total volume reading.

Step 5: Student-Led Planning: Discuss with your partner how the first trial worked and whether any adjustments need to be made to the set-up to ensure more accurate results. If the original trial did not go well due to measurement issues, repeat after adjusting the procedure. Then use the corrected trial as the first one. All subsequent trials will then use the same exact procedure.

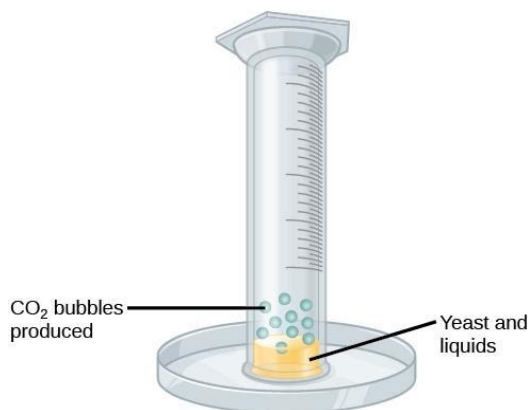


Figure 9.2: As fermentation takes place, carbon dioxide bubbles will rise into the cylinder. Volume measurements can be taken at specific time increments.

Step 6: Critical Analysis: Record the amount of carbon dioxide gas produced in your data table. Are the predictions made in Step 2 supported by the data? Why or why not? What is the control in this experiment? Is it a positive or negative control and why? What changes to the quantities of liquids, time period, or set up can you make to improve your results? Discuss with your partner and write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: How do you think the chemical make-up of the compound added to the yeast solution affects the rate of fermentation? How different do you think the results will be with the glucose and sugar substitute trials? Write your hypotheses in your notebook.

Step 2: Student-Led Planning: Make any adjustments to your first trial set up and re-do if necessary. Then, carry out the second trial for water and two additional trials each using glucose and sugar substitute. Record your measurements in the data table.

Step 3: Critical Analysis: Which compound led to the highest rate of fermentation? Was there a significant difference between the glucose and sugar substitute trials? Discuss your answers with your partner and write them in your notebook.

Assessments

1. What does data from the experiment show about the energy needs of yeast? [SP 5, Data Analysis; SP 6, Argumentation]
2. What do you think would happen to the activated yeast if no carbon-based molecules were provided? [SP 6, Argumentation]
3. What environmental reasons would cause yeast to go dormant? [SP 1, Concept Explanation; SP 6, Argumentation]

[Answer Key]

1. Possible answer: Yeast will absorb and process carbon-based food molecules to access energy that they require to survive and carry out all cellular functions.
2. Possible answer: Without carbon-based molecules, the yeast could not undergo cellular respiration. Eventually, the water would evaporate and the yeast would return to a dormant state.
3. Yeast may go dormant if there is a lack of food molecules available, which would prevent them from undergoing a high rate of cellular respiration.

Activity 2: Pre-Assessment

1. **Answer the following questions in your notebook:** How does temperature affect the rate of chemical reactions? Explain your answer.
2. **Answer the following questions in your notebook:** What would likely influence the optimum temperature of cellular respiration for an organism? Do you think there is a temperature that cellular respiration would cease and what would determine this upper temperature limit for cellular respiration?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 2: Dependence of Fermentation on Temperature

All cellular processes, like fermentation, consist of an interconnected series of chemical reactions. However, temperature can influence the rate of chemical reactions by affecting how quickly the reactants move, and therefore, how often they collide with each other. If the temperature gets too high, enzymes or other cellular proteins involved with cellular respiration can break apart, or **denature**, rendering them inactive. Therefore, it is very important for cells and organisms to regulate their internal temperature to ensure that cellular respiration and other chemical reactions can continue at the proper rate.

Safety Precautions

- Use protection when handling hot glass and materials.
- Do not mix very hot water with very cold water in glass containers.
- Clean up any spilled liquids to prevent slipping.

For this activity, you will need the following:

- Graduated cylinders
- Water
- Ice
- Thermometer
- Beakers
- Sugar substitute solution
- Yeast solution (one packet of yeast with 100 ml of water; can use from Activity 1)
- Hot plate
- Droppers or pipettes
- Medium-size, un-inflated balloons
- Cloth measuring tape or pieces of string and a ruler
- Balloons

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 2:

- Demonstrate how to properly utilize this equipment prior to letting students begin their experiments. Continue to remind the students of the importance of lab safety and first aid in the event of a burn injury.
- Demonstrate how to apply the balloons to the graduated syllabus. If students are not familiar with the concept of denaturation, a brief overview on this topic should also be given prior to the experiment, preferably during the initial lab lecture/setup discussion.
- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.
- Demonstrate how to measure the circumference of the balloons prior to beginning the lab exercise, to ensure that students are utilizing proper technique for their data yields.
- Remind students that they are to perform the tests with each of the variables (i.e., sugar vs. sugar substitute) multiple times, per the Scientific Method.
- Since students are recording results at different time intervals, it will be useful to have multiple timers present for this exercise.

The following are recommendations for the Structured Inquiry for the Guided Inquiry for Activity 2:

- Have each student write their own hypotheses regarding the proposed outcomes of the experiment. This is to ensure that each student contributes equally and submit their own original content.
- Advise the students to develop their proposed experimental design prior to allowing them to proceed with performing their experiment. This will help to ensure that they are on the right track and will not waste too many materials not needed for the activity.
- Remind students to include controls for their experiment as well to identify the independent and dependent variables.
- Instruct students to create their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.
- Remind students to wait until the temperature measurements stabilize before placing their beakers into the various water baths for acquisition of their results.
- In Step 3, clarify to students how to analyze their results. Additionally, providing a grading rubric for this activity with criteria describing each of the mandatory parts of this activity can help guide students to include the proper components in their experiment for this activity.

Structured Inquiry

Step 1: Hypothesize/Predict: Unlike Activity 1, you will measure the volume of carbon dioxide gas by placing balloons over the graduated cylinders after the reactants are added. Use 10 ml of each reactant. As the balloons fill with gas, you can measure their circumference to find the volume. How do you think temperature will affect the rate of fermentation? How can this be tested? Write your hypotheses in your notebook.

Step 2: Student-Led Planning: In this lab, you will measure the changes in the circumference of a balloon. Decide, with your partner, how you will take this measurement using a practice balloon that you inflate with your mouth. Make sure that your measurement is repeatable regardless of the size of the balloon. Create a data table for your measurements of balloon circumference versus solution temperature. You should record the volume of the gas in the balloon every 2 minutes for 10–15 minutes per trial.

Step 3: Prepare the first beaker with 100 ml of water at room temperature, approximately 21 °C. Add cold or hot water to get the water as close to 21 °C as possible. Once the temperature is stable, place the graduated cylinder containing the yeast and sugar substitute directly into the water beaker. Be sure to record the actual temperature of your room temperature solution in your notebook.

Step 4: Repeat Step 3 for the cold and hot water beakers one at a time. For the cold water treatment, fill the beaker with cold water and then add ice. Wait until the temperature stabilizes before beginning the timer. For the hot water beaker, bring the water to a boil using a hot plate. Then, turn down the hot plate to maintain the water at a low boil. Record the stabilized temperatures for both treatments before immersing the graduated cylinders containing the yeast into the beakers.

Step 5: Critical Analysis: Does this set up seem to capture the carbon dioxide gas effectively? Should you adjust the quantities of any of the liquids and/or the time intervals? Discuss with your partner and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: How do you think the more extreme temperatures, such as (~ 0–4 °C) and (55–60 °C) will affect the rate of fermentation? Are there any new safety concerns to consider with such changes to the temperature?

Step 2: Student-Led Planning: Pick two additional temperatures to test within the range (~ 0–4 °C) and (55–60 °C). Use the water, ice, hot plate, and thermometer to make these baths before adding the graduated cylinders containing the yeast and sugar substitute solutions. Then perform your trials as in the structured inquiry, measuring the volume of gas in the balloon. Create appropriate data tables before beginning your trials.

Step 3: Critical Analysis: How did temperature affect the rate of fermentation for this species of yeast? Is it likely any of the proteins involved in the reaction denatured and at what temperature did this occur? Graph the circumference of the balloon versus the temperature of the reaction. Based on your graph, what is the best estimate of the optimal temperature for fermentation in yeast? Discuss your answers with your partner and write them in your notebook.

Assessments

1. What likely caused the differences in reaction rate at the three temperatures examined in Activity 2? [SP 1, Concept Explanation; SP 6, Argumentation]
2. How could this experiment better measure the effect of temperature changes in the natural habitat of the yeast? [SP 6, Argumentation]
3. What other biotic and abiotic factors would likely affect the rate of cellular respiration in yeast? Explain your answers. [SP 6, Argumentation; SP 1, Concept Explanation]
4. What patterns can you identify in the data you collected between the yeast and the abiotic factor of temperature? [SP 4, Representing and Describing Data]

[Answer Key]

1. The reaction rate was slow at the coldest temperature because the reactants were moving slowly and had a low rate of collision. The reaction rate was fastest at room temperature because the reactants were moving fast and the enzymes were still functioning. At the highest temperature, no reaction occurred because the enzymes had likely denatured.
2. Rather than exposing the yeast to a single temperature at a time, the same sample of yeast could be exposed to a steady temperature change, during which fermentation rates are constantly monitored.
3. Anything that affects reaction rate would affect the rate of cellular respiration, including the concentration of the reactants and the concentration of the enzymes
4. Student answers will vary, but will most likely show an increase in fermentation rates with warmer temperatures, to a point where protein denaturing took place.

Lab 10: Plant Pigments

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
2.1 Cell Structure: Subcellular Components	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.D Describe the structure and/or function of subcellular components and organelles.	SYI-1.D.4 Chloroplasts are specialized organelles that are found in photosynthetic algae and plants and have a double outer membrane.
2.2 Cell Structure and Function	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.F Describe the structural features of a cell that allow organisms to capture, store, and use energy.	SYI-1.F.1 The folding of the inner membrane increases the surface area, which allows for more ATP to be synthesized. SYI-1.F.2 Within the chloroplasts are thylakoids and the stroma. SYI-1.F.3 The thylakoids are organized in stacks. SYI-1.F.4 Membranes contain chlorophyll pigments and electron transport proteins that comprise the photosystems.
3.4 Cellular Energy	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.H Describe the role of energy in living organisms.	ENE-1.H.1 All living systems require constant input of energy.
3.5 Photosynthesis	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.I Describe the photosynthetic processes that allow organisms to capture and store energy. ENE-1.J Explain how cells capture energy from light and transfer it to biological molecules for storage and use.	ENE-1.I.1 Photosynthesis captures energy from the sun and produces sugars. ENE-1.J.1 During photosynthesis, chlorophylls absorb energy from light.

SCIENCE PRACTICES

Science Practices	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe characteristics of a biological concept, processes, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams and flowcharts.
SP 3 Questions and Methods Determine scientific questions and models.	3.A Identify or pose a testable question based on an observation, data, or model. 3.B State the null and alternative hypotheses, or predict the results of an experiment. 3.C Identify experimental procedures aligned to the question, including variables and controls. 3.D. Make observations and collect data. 3.E Propose a new/investigation based on evaluation of evidence from a previous experiment.
SP 4 Representing and Describing Data Represent and describe data.	4.B Describe data from a table, diagram, or graph.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including equations from the curriculum.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.A. Make a scientific claim. 6.B Support a claim with evidence. 6.C. Provide reasoning to justify a claim by connecting evidence to biological theories. 6.E Predict the causes of effects of a change in one or more components in a biological system based on data.

Photoautotrophs such as plants, algae, and photosynthetic bacteria transform the energy from sunlight into chemical energy that is used to power life activities. The sun emits a wide range of electromagnetic waves. However, photosynthetic pigments only absorb within the narrow range of visible light, from 380 nm to 700 nm, which is referred to as **photosynthetically active radiation (PAR)**.

Photons must be absorbed before light energy can be converted into chemical energy. Arrays of **pigments**, which are brightly colored chemical compounds, absorb specific wavelengths of photons while other wavelengths are reflected. The color we see is determined by the wavelengths of light that are not absorbed, but reflected off the structure, as shown in Figure 10.1. For example; Spinach leaves look green because they reflect green wavelengths of visible light.

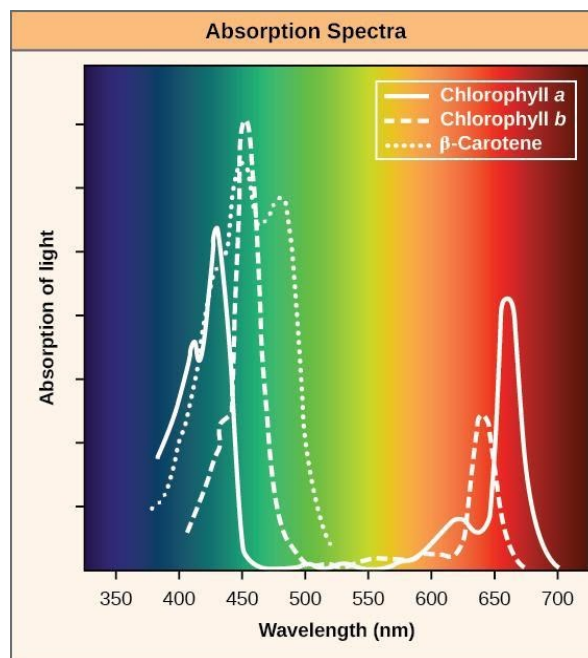
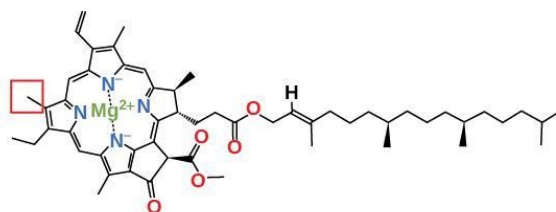


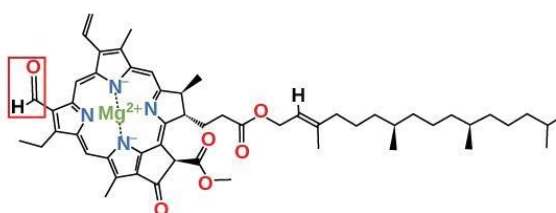
Figure 10.1: Absorption spectra of chlorophyll *a*, chlorophyll *b*, and beta-carotene, which are photosynthetic pigments in land plants. Which color of light do you expect to be the most efficient for photosynthesis?

Chlorophyll *a* is the molecule responsible for the transformation of light energy into chemical energy in photosynthetic plants. Most pigment molecules in photoautotrophs are not actually involved in the photosynthesis. These **accessory pigments** can be compared to the satellite dishes that collect radio waves. They capture photons from a broad range of wavelengths and re-emit them to special chlorophyll-protein complexes called **reaction centers**. The photons are eventually absorbed by chlorophyll *a* in the reaction center, exciting its electrons to a higher energy state. The ensuing flow of high-energy electrons through an electron transport chain produces the ATP and NADPH that fuels the conversion of carbon dioxide into organic molecules.

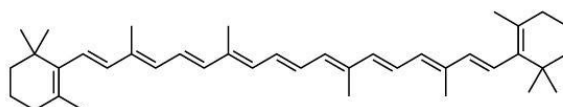
As shown in Figure 10.2, many types of photosynthetic pigments are found in nature giving plants and algae their characteristic colors. They promote photosynthesis by widening the spectrum of visible light that can be used for photosynthesis. Chlorophylls and carotenoids are present in all land plants, algae, and cyanobacteria. Carotenoids fulfill the dual role of accessory and protective pigments. They prevent photo damage caused by intense light energy.



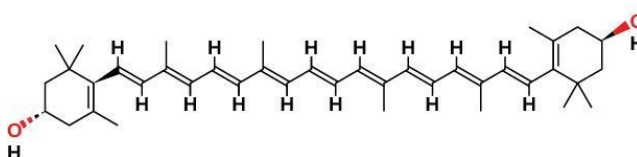
(a)



(b)



(c)



(d)

Figure 10.2: Molecular structures of plant pigments. The pigments display characteristic colors as follows: (a) chlorophyll *a* is green-blue, (b) chlorophyll *b* is olive green, (c) carotenoids are yellow-orange, (d) and xanthophylls are lemon yellow

What is the main environmental factor that determines which colored pigments are present in a photosynthetic organism? In this lab, you will make predictions about the pigments that are present in a plant leaf and study the pigments found in leaves by separating them on paper per their solubility in a solvent system.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Show a diagram of a chloroplast. Explain that energy from sunlight is captured in the light reactions, which take place in the thylakoid membrane. This energy is used to make sugars in the dark reactions, which take place in the stroma.
- Show a diagram of the thylakoid membrane. Explain that light is captured by accessory pigments and re-emitted to chlorophyll *a* in the reaction center. Chlorophyll *a* shuttles electrons into the electron transport chain, generating ATP and NADPH.
- Ask students why accessory pigments are needed. Response should indicate that these pigments can capture different wavelengths of light than chlorophyll *a*, increasing the total amount of sunlight that the plant can use.
- Explain that all photosynthetic tissue contains chlorophyll *a*, but the amount of other pigments present can vary. As a result, the color of photosynthetic tissue can vary.

In this lab, you will learn

- how to extract pigments from plant material;
- how to separate pigments by paper chromatography;
- how to measure R_f (retention factor) values for pigments.

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** Think about what colors you have seen in leaves of plants. What determines the color of a leaf? What color or colors do you expect to see if you extract pigments from leaves and separate them chemically?
2. **Answer the following question in your notebook:** Other structures in plants besides leaves are brightly colored. Fruit, flowers, and some roots display colors. Are these colors associated with photosynthesis? What are the purposes of those colors?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 1: Extracting Pigments from Plant Material

The chloroplast, as illustrated in Figure 10.3, is the photosynthetic organelle in the plant cell. The chloroplast is surrounded by a double membrane. Within the inner membrane is a fluid compartment called the stroma. The **thylakoids** form a complex network of membranes that appear as stacked disks called grana (singular, granum). The photosystems, and photosynthetic pigments they contain, are embedded in the thylakoid membrane. Carbon fixation takes place

in the stroma. What chemical properties would you expect photosynthetic pigments to display and how can these chemical properties be used to extract the pigments from tissues? How do the solubility properties enable scientists to separate individual pigments for analysis?

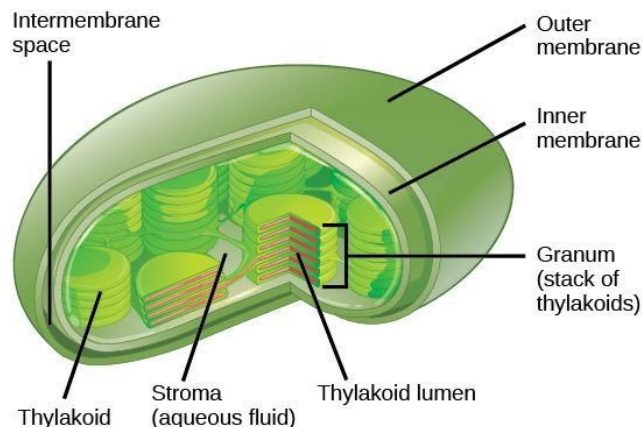


Figure 10.3: A section through a chloroplast shows the arrangement of the thylakoid membranes in stacks and single membranes. The thylakoids are pockets enclosed by membranes.

Safety Precautions

- Wash your hands after completion of this activity.

For this activity, you will need the following:

- Plant material: intact leaves of spinach and *Coleus* (one leaf of each per pair of students)
- Filter or chromatography paper
- Ruler (one per group)
- Coins
- Pencils
- Colored pencils
- Forceps
- Scissors

In this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Show examples of plant tissue that is colored by different pigments. For example, the orange color in carrots is produced by carotenoids; the red color in grapes is produced by anthocyanins; the green color in spinach is produced by chlorophyll.

- Ask students whether two colors are visible after paint has been mixed (no). Based on this answer and on what they know about plant pigments, ask students to predict whether more than one pigment will be present in the leaves they will be testing, and to predict the color of the pigments.
- Demonstrate how to rub the coin on the leaf.

Structured Inquiry

Step 1: Hypothesize/Predict: In this activity, you will extract pigments from spinach leaves. Discuss and predict what colored pigments you will observe after extraction. Write your hypotheses in your notebook.

Step 2: Student-led Planning: Assemble all the material needed for extracting pigments from spinach leaves. Read the procedure in Step 3 and Figure 10.4 to extract pigments from plant material. Throughout the procedure, you will need to avoid touching the paper with your fingers, because oils from your skin can interfere with the chromatography. Handle the paper by the edges. Devise a method for handling the paper with your partner. Use a few scraps of paper to practice extracting pigments from loose leaves. If you smear pigments all over the piece of paper while you apply pigment, the stains will also separate by chromatography and interfere with the interpretation of the results. Throw any smeared paper away and start over.

As pigments separate along the paper, the color intensity of each pigment decreases and makes it more difficult to identify each color. What approach will you use to obtain a dark line of concentrated pigment on the paper?

Step 3: Complete the steps below to extract pigments from plant material, as illustrated in Figure 10.4. Use the method you devised in Step 2 to avoid touching the paper with your fingers.

1. Measure the height and the diameter (D) of the container with your partner to determine the size of the piece of chromatography paper you need for the experiment. Subtract 1 cm from the height of the container. The width of the chromatography paper should be about 1 cm shorter than the circumference of the container. Record your calculations and show them to your instructor before you cut the paper.
2. With a pencil, draw a line across the entire width of filter/chromatography paper about 1.5 cm from the bottom. This line is called the **origin** or start of the separation. It is the place where the sample is applied.
3. Select 1 large spinach leaf and carefully blot it dry with paper towels. Note in your lab notebook why it is important to blot dry the leaf.
4. Position the leaf on top of the pencil line leaving a space on each end. The leaf should reach the edge of the paper.

5. Align a plastic ruler over the leaf with the edges of the pencil line that was left uncovered by the leaf.
6. Roll a coin pressing down firmly along the ruler edge without crushing the rest of the leaf and smearing pigment on the surface of the filter paper. You may find it helpful to lay the filter paper on top of the paper towels taking care not to cover the line where the pigments are deposited.

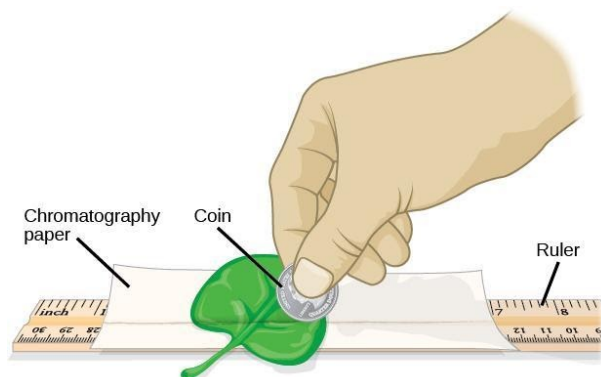


Figure 10.4: Extract pigments by rolling a coin along a ruler. Make sure that the leaf does not cover the entire paper and align the ruler with the starting line to avoid edge effects.

7. Let the pigment line dry for 3 to 5 minutes. Move to a different section of the leaf and roll the coin again over the same line. Keep the band of pigments as narrow as possible. The pigments will diffuse during separation and the bands will smear if they are laid down too thick.

Step 4: Critical Analysis: Record the results from your experiment. Try to identify the pigment or pigments you extracted. Can you state conclusively what types of pigments are present in your extract on the line? How could you determine which pigments are present in the extract without performing a chemical separation? Write your answers in your lab notebook. You may refer to Figure 10.1 while discussing the answer.

Guided Inquiry

Step 1: Hypothesize/Predict: If pigments are colored molecules, can you decide which pigments are present by looking at biological material? Do spinach leaves contain other pigments besides the green chlorophylls? Do *Coleus* leaves contain additional pigments? Do you see a red pigment on the line for *Coleus*? Write your hypotheses in your notebook.

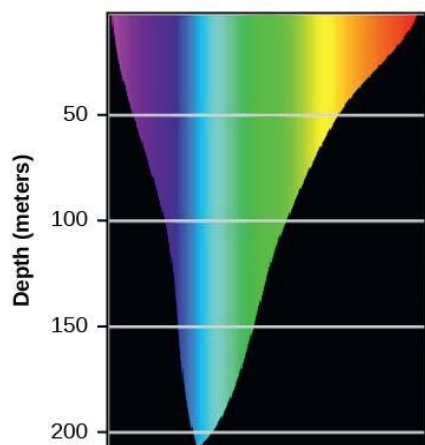
Step 2: Student-led Planning: Decide with your lab partner how you will test your hypotheses on the kinds of pigments you expect to find in your extracts of spinach and *Coleus* leaves. One lab partner should use spinach and the other one should use *Coleus*. Decide with your lab partner which plant material you will use to test your hypothesis. Extract pigments from leaves by the coin method described in Step 2 of the Structured Inquiry. Record all the steps you perform and draw the colors seen on the line in your lab notebook.

Step 3: Critical Analysis: Observe the pigment lines on the paper. Record in your lab notebook the color or colors you detected. Note the differences, if any, between the spinach and *Coleus* samples.

Assessments

1. Plants in the rainforest that live under the canopy of tall trees must adapt to low light conditions. Can you predict how the pigments of plants that receive low light levels will differ from the pigments of plants that receive high light levels? What would happen to the forest if the tall trees are removed for timber? [SP 6, Argumentation]
2. You analyze a pigment extract from a mutant plant and discover that the plant is defective in its carotenoid biosynthetic pathway. What would happen to that plant if it is grown in bright sunlight? Where would you expect it to thrive and how would such a plant compensate for the loss of carotenoids? [SP 6, Argumentation; SP 1, Concept Explanation]
3. The Rhodophyta are algae that can grow deeper in the ocean than other plants. Based on the information shown below in Figure 10.5, what color of the visible spectrum would you expect to be absorbed by an accessory pigment found in Rhodophyta? Why do you think plants have reddish colors? [SP 6, Argumentation; SP 1, Concept Explanation]

Light penetration in the open ocean



[Answer Key]

1. Leaves have wide surfaces and contain large amounts of light-harvesting pigments; however, the pigments are the same as for other land plants. The adaptive strategy is to increase the amount of light collected by leaves. If tall trees are removed, the ecosystem will be damaged, because the lower plants are adapted to low light intensity and will be bleached by sunlight.
2. The plant would likely survive in the shade, but undergo photobleaching, loss of color due to light, under intense sunlight. Carotenoids are mostly protective pigments. Plants will increase the synthesis of chlorophyll a and b and associated light-harvesting complex proteins.
3. The pigment would absorb in the blue and green region. The pigment itself would be red. Having red pigments would allow plants to have access to a larger region of the visible spectrum on land.

Activity 2: Pre-Assessment

1. The leaves of some plants change color in fall. Green foliage appears to turn to hues of yellow and brown. Does the yellow color appear because carotenoids replace the green chlorophylls? Explain your reasoning.
2. Examine the molecular structures of photosynthetic pigments in Figure 10.2. Photosynthetic pigments are hydrophobic molecules located in thylakoid membranes. Will these pigments dissolve in water?

Activity 2: Paper Chromatography of Plant Pigments

Paper **chromatography** is an analytical method that separates compounds based on their solubility in a solvent. The solvent is used to separate a mixture of molecules that have been applied to filter paper. The paper, made of cellulose, represents the **stationary** or immobile phase. The separation mixture moves up the paper by capillary action. It is called the **mobile** phase. The results of chromatography are recorded in a chromatogram. Here, the chromatogram is the piece of filter paper with the separated pigment that you will examine at the end of your experiment (see Figure 10.6).

We separate the compounds based on how quickly they move across the paper. Compounds which are soluble in the solvent mixture are more concentrated in the mobile phase and move faster up the paper. Polar compounds will bind to the cellulose in the paper and trail behind the solvent front. As a result, the different compounds will separate according to their solubility in the mixture of organic solvents we use for chromatography.

Safety Precautions

- Work under a hood or in a well-ventilated space and avoid breathing solvents.
- Do not have any open flames when working with flammable solvents.
- Wear aprons and eye protection.
- Do not pour any organic solvent down the drain.
- Dispose of solvents per local regulations.
- Use forceps to handle chromatography paper that has been immersed in solvent and wash your hands after completing this activity.

For this activity, you will need the following:

- Plant material: intact leaves of spinach and *Coleus* (one leaf of each plant per pair of students)
- Filter or chromatography paper
- Ruler (one per group)
- Coins
- Pencils
- Colored pencils
- Beakers (400 mL) (Mason jars are an acceptable substitute)
- Aluminum foil
- Scissors
- Forceps
- Freshly prepared solvent mixture
 - Petroleum ether: acetone: water in a 3:1:1 proportion
 - If no hood or well-ventilated place is available, the mixture can be substituted with 95 percent isopropyl alcohol. Note that, if isopropyl alcohol is used, the pigment bands will smear. You may not be able to separate and identify the chlorophylls or carotene from xanthophyll.

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Notes

The following are recommendations for the Structured Inquiry for Activity 2:

- Ask students why chromatography is conducted using a nonpolar solvent rather than water. Response should indicate that photosynthetic pigments are embedded in the membrane and are not soluble in water.
- If students are using petroleum ether: acetone solvent, remind them to use the hood and not to breathe the fumes. Make sure students know how to dispose of solvent.
- Emphasize that the pigment line must be above the liquid or it will get washed away. Explain that the solvent will pick up the paper and carry the pigment with it. Pigments that are more soluble will travel faster than pigments that are less soluble.
- Remind students to cover the chromatography container so liquid does not evaporate.
- Tell students to lay the chromatography paper on a paper towel when the experiment is done. Emphasize that they must record the results immediately as the solvent line and color bands will fade over time.
- Show a diagram of a chromatography strip, and to demonstrate how to calculate the R_f value.

Structured Inquiry

Step 1: Hypothesize/Predict: Discuss with your lab partner what color pigments will likely be present in the spinach leaves. Write your predictions in your lab notebook and draw a diagram of how you think the pigments will separate out on the chromatography paper.

Step 2: Student-led Planning: Read Step 3 below. Discuss with your lab partner the setup of the experiment. Then agree upon the dimensions of the filter/chromatography paper that you will use. To allow good separation, the paper should not touch the walls of the container. The paper must fit inside the container while being long enough for maximum separation. Write all your calculations in your lab notebook.

Step 3: Follow the steps below to set up your filter paper and perform the chromatography experiment.

1. Prepare the chromatogram by cutting a piece of filter paper. Transfer pigments from spinach leaves as in Activity 1. A heavy application line will yield stronger colors when the pigments separate, making it easier to read results. Allow the pigments to dry between applications. Wet extracts diffuse on the paper and yield blurry lines.
2. Form a cylinder with the filter paper without overlapping the edges (to avoid edge effects). The sample should face the outside of the cylinder. Secure the top and bottom of the cylinder with staples.

3. Pour enough separation mixture to provide a mobile phase while staying below the origin line on the chromatogram. The exact volume is not critical if the origin, the start line where you applied the solvent, is above the solvent. See Figure 10.6.



Figure 10.6: Chromatography can be set up in a container such as a Mason jar. The start line of the sample, the origin, is shown above the solvent.

4. Label the beaker with a piece of tape with your initials and your partner's initials.
5. Lower the paper into the container with the band from the extraction in the lower section. The paper must touch the solvent, but not reach the band of pigment you applied. Why must the band be above the solvent line? Write your answer in your notebook.
6. Cover the container tightly with a piece of aluminum foil.
7. Track the rising of the solvent front. Can you see a separation of colors on the paper?
8. When the solvent front is within 1 cm of the upper edge of the paper, remove the cylinder from the beaker using forceps. Trace the solvent front with a pencil before it evaporates and disappears! Draw the colored bands seen on your chromatography paper in your lab notebook immediately. The colors will fade upon drying. If no colored pencils are available, record the colors of the lines.
9. Let the paper dry in a well-ventilated area before making measurements because the wet paper is fragile and may break when handled. This is also a precaution to avoid breathing fumes from the chromatogram.
10. Discard solvent mixture per your instructor's directions. Do not pour down the drain.

Step 4: Critical Analysis: Open the dried cylinder by removing the staples. Measure distance from the first pencil line to the solvent front, as shown in Figure 10.7. This is the distance traveled by the solvent front. Measure the distance from the pencil line to the middle point of each color band and the original pencil line. Record your results in your notebook in a table modeled after Table 10.1. The retention factor (R_f) is the ratio of the distance traveled by a colored band to the distance traveled by the solvent front. Calculate R_f values for each pigment using the following equation:

$$R_f = \frac{\text{Distance traveled by colored band}}{\text{Distance traveled by solvent front}}$$

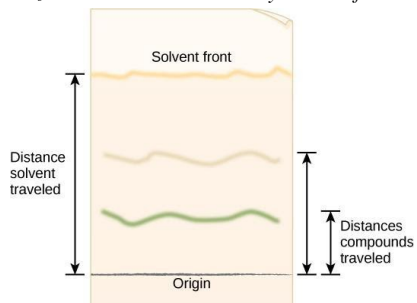


Figure 10.7: Chromatogram shows the distance traveled by the solvent front and the compounds separated by chromatography.

Step 5: After determining the color of the band, tentatively identify each band. Did your results support your hypothesis about the color of each band? Discuss which aspects of the experiments may have yielded inconclusive results. How could you improve the experiment?

Guided Inquiry

Step 1: Hypothesize/Predict: What type of pigments are present in *Coleus* leaves and where are the different colors located? Can you make a hypothesis based on the coloration of the variegated leaves? Write your hypothesis down in your lab notebook. Would there be a difference if you performed chromatography on pigment composition from different colored regions of the leaves?

Step 2: Student-led Planning: Cut the chromatography/filter paper to the dimensions needed. Apply pigments from different parts of the *Coleus* leaves following the procedure described under Activity 1, keeping in mind that a darker line will yield stronger colors when the pigments are separated, which will make it easier to read the results. Allow the pigments to dry between applications. Wet extracts diffuse on the paper and yield blurry lines.

Step 3: When the solvent front reaches 1 cm from the top of the filter paper, stop the procedure. Draw the pigment bands you see on the filter paper in your lab notebook. Clearly indicate the color you observed for each band.

Step 4: Let the cylinder dry and measure the distance the front traveled from the origin and the distances traveled by each of the pigments. If the bands broadened during separation, take measurements to the middle of each band.

Step 5: Critical Analysis: Calculate R_f for each of the bands and record them in a table in your notebook. Compare the R_f you obtained with those of other groups. Are the R_f values similar? What may have altered R_f values?

Assessments

1. Carotenoids and chlorophylls are hydrophobic molecules that dissolve in organic solvents. Where would you find these molecules in the cell? What would happen if you ran the chromatography in this lab with water as the solvent? [SP 6, Argumentation; SP 3, Questions and Methods]
2. All chlorophyll molecules contain a complexed magnesium ion. Your houseplant is developing yellow leaves. What may cause this, and how can you restore your plant's health? [SP 1, Concept Explanation]
3. Seeds that grow under dim light are said to be etiolated, which describes their pale and spindly appearance. They soon waste away after exhausting their food reserves. Can you explain this observation? [SP 1, Concept Explanation]

[Answer Key]

1. Carotenoids and chlorophylls are embedded in the thylakoid membranes of chloroplasts which, like all biological membranes, contain many phospholipids. If water is used as the separation mixtures, chlorophylls and carotenoids would stay on the origin line.
2. The plants developed chlorosis, loss of green color, because of a magnesium deficiency in the soil. The condition can be corrected by feeding the plant a fertilizer rich in magnesium. Horticulturalists often add Epsom salts, which contains magnesium, to garden soil.
3. Without the complete PAR, seedlings are deprived of the full spectrum of light and are forced to use their food storage until their reserves are depleted. This will cause the seedlings to wither and die. Plants growing under rocks and ledges show such spindly appearance.

Lab 11: The Light Reaction of Photosynthesis

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
2.2 Cell Structure and Function	SYI-1 Living systems are organized in a hierarchy of structural levels that interacts.	SYI-1.F Describe the structural features of a cell that allow organisms to capture, store, and use energy.	SYI-1.F.1 The folding of the inner membrane increases the surface, which allows for more ATP to be synthesized. SYI-1.F.2 Within the chloroplast are thylakoids and the stroma. SYI-1.F.3 The thylakoids are organized in stacks called grana. SYI-1.F.4 Membranes contain chlorophyll pigments and electron transport proteins that comprise the photosystems. SYI-1.F.5 The light-dependent reactions of photosynthesis occur in the grana.
3.4 Cellular Energy	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.H Describe the role of energy in living organisms.	ENE-1.H.3 Energy-related pathways are sequential to allow for a more controlled and efficient transfer of energy.
3.5 Photosynthesis	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.I Describe the photosynthetic processes that allow organisms to capture and store energy. ENE-1.J Explain how cells capture energy from light and transfer it to biological molecules for storage and use.	ENE-1.I.2 The light-dependent reactions of photosynthesis in eukaryotes involved a series of pathways that capture energy in light to yield ATP and NADPH, which power the production of organic molecules.

			ENE-1.J.1 During photosynthesis, chlorophylls absorb energy from light, boosting electrons to a high energy level in photosystems I and II. ENE-1.J.2 Photosystems I and II are embedded in the internal membranes of chloroplasts and are connected through an electron transport chain (ETC). ENE-1.J.3 When electrons pass through the ETC, an electrochemical gradient of protons (H^+) is established across the membrane. ENE-1.J.4 The formation of the H^+ gradient is linked to the synthesis of ATP. ENE-1.J.5 The energy captured in the light-dependent reactions is transferred to ATP and NADPH, which power the production of carbohydrates in the Calvin cycle.
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SCIENCE PRACTICES

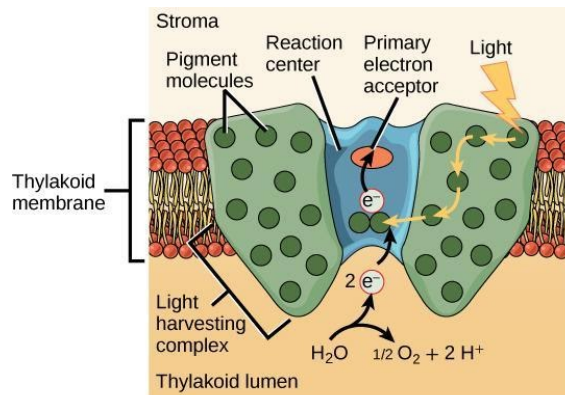
Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on an observation, data, or model. 3.B Predict the results of an experiment. 3.C Identify experimental procedures that are aligned to the questions, including variables and controls. 3.D Make observations, or collect data from representations of laboratory setups or results. 3.E Propose a new/next investigation based on evidence from a previous experiment.
SP 4 Representing and Describing Data Represent and describe data.	4.A Construct a graph, plot, or chart. 4.B Describe data from a table or graph.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including equations in the curriculum and rates.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. 6.C Provide reasoning to justify a claim. 6.E Predict the causes of effects of a change in components of biological systems based on data.

Sunlight is the main source of energy that powers life on Earth. **Photoautotrophs** convert light energy into chemical energy through the process of **photosynthesis**. Land plants, algae, and cyanobacteria are all photoautotrophs. The energy derived from the sun allows these organisms to fix carbon, in the form of CO₂, into sugars. In eukaryotes, photosynthesis takes place in a specialized organelle, the chloroplast, and can be divided into three main phases:

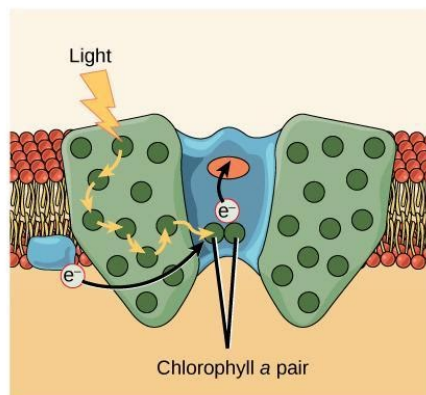
1. Light capture by chlorophylls and other pigments and release of high energy electrons from the pigment molecules

2. Electron flow through an electron transport chain embedded in the thylakoid membranes with concomitant production of NADPH and ATP
3. Fixation of carbon into sugars through a series of reactions comprising the Calvin cycle

The **light-dependent** reaction refers to the first and second stages, although only the first stage involves the absorption of light. The **light-independent** reaction describes the Calvin cycle. At one time, carbon fixation was called the dark reaction, but it proceeds in darkness or light. The primary light reaction occurs in the **reaction centers**; Special protein-chlorophylls at the center of a photosystem (Figure 11.1).



(a) Photosystem II (P680)



(b) Photosystem I (P700)

Figure 11.1: Photosystem II (a) and photosystem I (b) are formed by a cluster of proteins associated with a chlorophyll *a* pair embedded in the thylakoid membrane.

The **photosystem** (PS) is an aggregated array of pigments and proteins. Its primary function is to use light energy to excite electrons that travel to the electron transport chain. In cyanobacteria and eukaryotes, two photosystems, PSII and PSI operate in series (Figure 11.2).

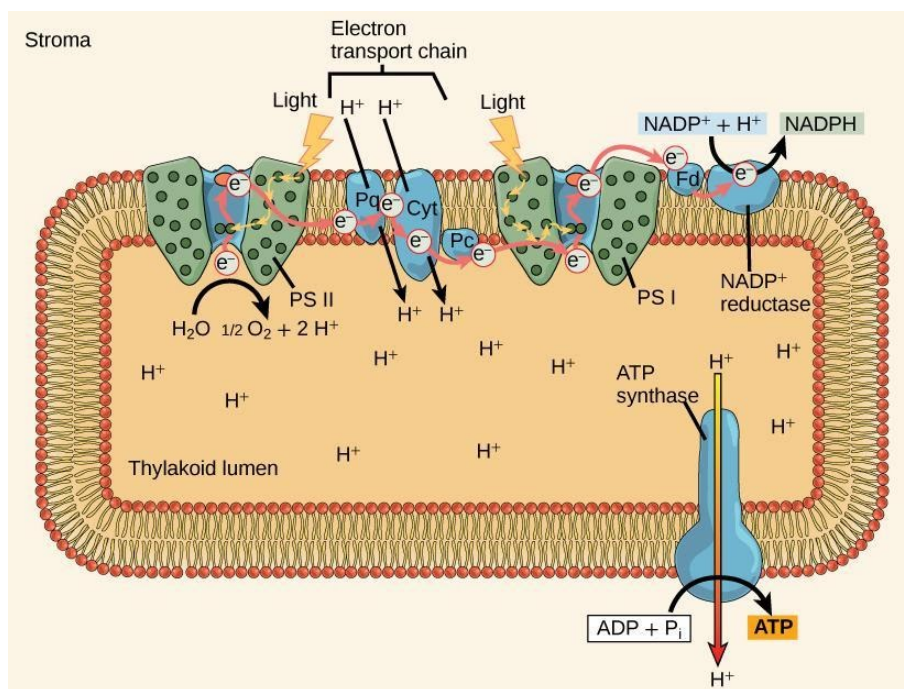
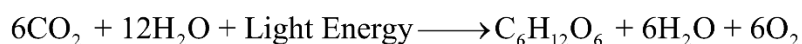


Figure 11.2: Electrons flow from PSII to PSI through several electron carriers embedded in the thylakoid membrane. Electrons extracted from water reduce PSII to maintain the flow through the electron transport chain.

As can be seen in Figure 11.2, the electrons lost by the reaction center chlorophylls are eventually replaced by electrons obtained by splitting water to form oxygen gas and protons.

The overall reaction of photosynthesis is summarized below.



A metabolic reaction can be studied by measuring the disappearance of reactants or the accumulation of its products. In the first activity, you will predict the effect of environmental conditions on photosynthesis by monitoring the disappearance of CO_2 . In the second activity, you will follow the production of oxygen under several conditions that affect photosynthesis.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content for your students:

- Discuss the various names used to describe the photosynthetic pathways, for example, the light-independent reactions being called the dark cycle or the Calvin cycle.
- Have students show a brief video or animation summarizing the photosynthesis pathways.

- Provide students with a flowchart or diagram of carbon fixation in the light-independent reactions. Have the students discuss its relationship to cellular respiration carried out by the plant cells.
- Have the class hypothesize different ways that they could try to measure whether a plant is carrying out photosynthesis.

In this lab, you will learn

- how to measure the dependence of CO₂ fixation on the light-dependent reaction
- how to explain the chemical principles of pH indicators and the bicarbonate-CO₂ equilibrium
- how to analyze the dependence of O₂ production on the color of light spectrum and other environmental variables

Activity 1: Pre-Assessment

1. **Answer the following questions in your notebook:** Which gas is released by plants during photosynthesis? Which gas is absorbed during photosynthesis? How does the overall chemical equation for photosynthesis compare to the overall chemical equation for respiration?
2. **Answer the following questions in your notebook:** What chemical reactions take place when CO₂ is released in water? What is the connection between an increase in CO₂ in the atmosphere and the acidification of oceans? Why are plants essential in mitigating the effects of climate change?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 1: Influence of the Light Reaction on the Concentration of CO₂

In this experiment, you will predict and test the effect of environmental conditions on the process of photosynthesis. Gaseous carbon dioxide dissolves in water per the reaction



Carbon dioxide and water combine to generate the unstable carbonic acid molecule that separates into hydrogen and bicarbonate ions effectively lowering the pH of the solution. The reaction is reversible. When CO₂ molecules are consumed by photosynthesis, the reaction shifts to the left. Hydrogen ions and bicarbonate ions combine to replace CO₂ with the net effect of increasing the pH of the solution.

A **pH indicator** is a chemical compound that changes color based on pH level. In this experiment, you will use phenol red, which appears yellow at pH below 7, orange at neutral pH, and bright red or pink at basic pH values (see Figure 11.3). How can the presence of phenol red allow measurement of the consumption of CO_2 by a plant submerged in water? Discuss the method with your partner and write your answer in your notebook.

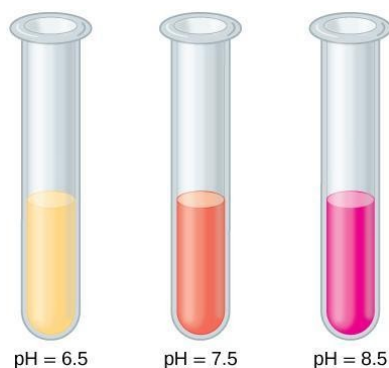


Figure 11.3: The pH indicator phenol red appears yellow at an acidic pH, pinkish orange at a neutral pH, and bright red or pink at a basic pH.

Safety Precautions

- Be careful when blowing into the straw inside the reaction vessels. Do not suck up the solution.
- Do not touch incandescent lamps, because they can be very hot.
- Wear aprons and eye protection.

For this activity, you will need the following:

- Plant material: *Elodea* or other aquatic plant
- Flasks
- Transfer pipettes
- Solution of phenol red
- New drinking straws
- Test tubes and test tube racks
- Lamp with LED bulb
- Beakers of water
- Hot plate
- Ice

- Aluminum foil
- Acetate colored filter (at a minimum, use blue, red and green filters)
- Thermometer
- Meter stick

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for Structured Inquiry for Activity 1:

- Provide the class with a pH scale diagram that shows the pH of various substances that are familiar with such as drain cleaner, lemon juice, and milk.
- Use healthy *Elodea* sprigs that have dark green leaves with few brown dead spots. The sprigs should be at least 10 centimeters long and have leaves on at least 2/3 of the stem.
- Substitute colored cellophane if colored filters are not available.
- Caution students to be careful phenol red solution. It should not remain on the skin if contamination occurs.
- Phenol red should not be disposed down the drain and must be placed in an appropriate waste container.
- Warn students that phenol red solution will stain clothing and this it is difficult to remove the stain through regular laundry washing. The stain remover used to eliminate wine stains is effective at cleaning phenol red.
- Reinforce the precautions of working with a hot plate.

The following are recommendations for the Structured Inquiry for the Guided Inquiry for Activity 1:

- Have students identify the skills, such as designing a setup to determine the presence of carbon dioxide, they will need to carry out the guided inquiry. These skills were learned in the structured inquiry.
- Place students in work groups. Encourage each group to assign specific tasks to group members.
- Ask students to write their own paper written in their words. Make sure to check for plagiarism by comparing writing of the answers between group members.

Structured Inquiry

Step 1: Hypothesize/Predict: In this experiment, you will measure CO₂ uptake in *Elodea* exposed to light compared to a control *Elodea* in a control tube where light is blocked. Make a hypothesis with your partner about the color changes you expect to see in the pH indicator when the test tubes are exposed to light. Write down your hypothesis in your notebook. Discuss what you predict will happen to the test tubes kept in the dark. Decide what you will do if you observe no change in the test tubes exposed to light within the time allocated.

Step 2: Student-led planning: Set up the materials described in Step 3 below. If you have access to a camera, you may take pictures of your set-up. Otherwise, draw the set-up in your lab notebook indicating the colors in words or by using colored crayons. What is the control treatment in this setup? Is it a positive control or a negative control? Explain your reasoning in your notebook.

Step 3: Monitor the photosynthetic response by following CO₂ uptake, as outlined in the following steps.

1. Prepare test tubes for the experiment. First, label all tubes with a piece of tape and a marker to identify the sample. The first tube will contain the sprig of *Elodea* exposed to light. This is the experimental sample that will be labeled *Light*. The second tube will contain a sprig of *Elodea* and will be wrapped in aluminum foil. Label this tube *Dark Control* because it will serve as a control. The third tube will be labeled *Light Control* and will contain water and indicator without plant material.
2. Prepare a dilute solution of phenol red (mixed with tap water) in sufficient quantity for the test tubes. For example, if you set up test tubes that will contain 25 ml each, prepare 120 ml of solution to make it easier to aliquot equal amounts of solution. If the color is close to red or pink, it means that the tap water is basic. In this case, it may be more practical to use distilled water; since it may be difficult to blow enough CO₂ to adjust the pH. The reason for using distilled water is that freshly distilled water has a pH of 7.0. As CO₂ from the atmosphere dissolves in the distilled water, the pH drops below 7.0.
3. If the starting phenol solution is a light shade of pink, take a clean drinking straw and gently blow bubbles into the solution until it turns bright yellow. What does the yellow color indicate? Write your answer in your notebook.
4. Fill test tubes with water as indicated by your teacher (see Step 2). Then add the pH indicator solution.
5. Cut leafy sprigs of *Elodea* with the scissors without crushing the material. To produce comparable results, the sprigs should be similar in length and number of leaves.
6. Place the sprigs in the test tubes you prepared for the experiment. Top with water to make sure that the sprigs are fully immersed.

7. Record the color of each solution in your lab notebook in a table like Table 11.1. Remember to modify the table to accommodate all your data.
8. Wrap a piece of aluminum foil around the tubes if you are measuring photosynthesis in the *Dark Control* tube. Make sure that there are no light leaks.
9. Place a lamp about 0.5 m from your experimental set up. Place a container of water between the lamp and the test tube to absorb the heat radiating from the bulb if you use an incandescent bulb, as shown in Figure 11.4. LED lamps radiate less heat making it possible to bypass the use of a heat sink.
10. Start your watch and record the color of the tap water with a phenol indicator solution every 30 minutes. To compare CO₂ production in the presence and absence of light, record the end-point color of the test tubes wrapped in aluminum foil after two hours of incubation.

Table 11.1: Change in Phenol Red Color

Time (min)	Tube with <i>Elodea</i> exposed to light	Tube without <i>Elodea</i> exposed to light	Tube with <i>Elodea</i> in the dark	Tube without <i>Elodea</i> in the dark
0				
30				
60				
90				
120				

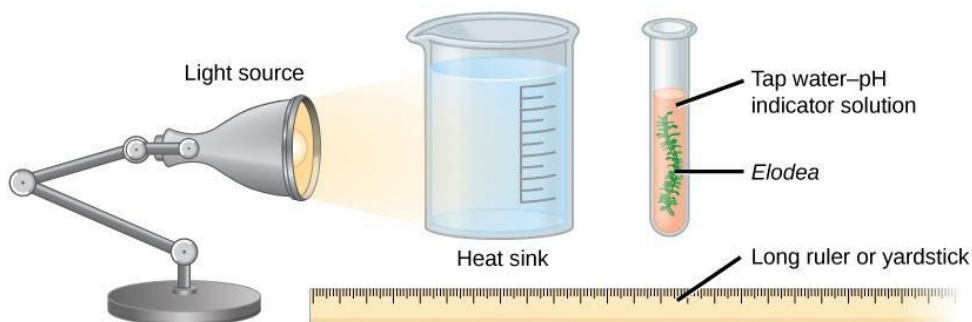


Figure 11.4: Experimental set up to measure release of CO₂ during photosynthesis.

Step 4: Critical Analysis: Record the colors you observe in the test tubes over time in the table you prepared in your lab notebook. What happened to the *Dark Control* test tubes compared to the *Light Control* tubes and what does this tell you about the validity of your experiment? Do the experimental results support your hypothesis?

Guided Inquiry

Step 1: Hypothesize/Predict: With your partner, review the process of photosynthesis and decide on an environmental condition you want to test. You may choose to investigate the influence of temperature, light intensity, or light color. Can you dim the room lights to minimize the effect of white light when experimenting with the effect of colored light? Decide with your lab partner how you will test the environmental condition and what you predict will happen to the pH in the solution. Include all necessary controls in your hypothesis. You should also devise a positive and negative control for your experiment. Check with your instructor on the availability of supplies to carry out your experiment.

Step 2: Student-led planning: Set up your experiment following the guidelines described under Step 2 of structured inquiry. You may need to shut off the room lights and cover the windows so there is only one light source. When measuring the effect of light intensity, use the equation $I=1/D^2$ where I is light intensity and D is distance. In every case, ensure that you measure only one variable in your set up. The temperature can be kept constant by placing the tube in a water bath. Prepare a table like Table 11.1 in your lab notebook to enter your data.

Step 3: Critical analysis: Record your measurements in your lab notebook. Analyze the results and compare them to your hypothesis. Did the environmental variable affect pH changes as you predicted? What do the changes in pH tell you about the changes in CO_2 production? What other factors may have contributed to the results? If you measured the effect of temperature, explain which reactions in photosynthesis would be dependent on temperature? How would you modify the experiment if you were to repeat it? Discuss your results with groups that tested different environmental variables.

Assessments

1. In an experiment, the water was first boiled, which drove out all of the existing gases. The water was then added to the test tube containing *Elodea*, which was then exposed immediately to light. The photosynthetic activity of the *Elodea* was then monitored but no photosynthetic activity was detected. How can you correct the situation by modifying the composition of the *Elodea* environment? [SP 3, Questions and Methods]
2. Scientists report that the levels of carbon dioxide are rising in the atmosphere and driving climate change. Another observation is that acidification of oceans is also a consequence of climate change. Can you explain what link may exist between the two phenomena and how it affects the marine ecosystem? [SP 1, Concept Explanation]

3. Farmers report that planting corn plants too closely can stunt growth even when the plants are heavily fertilized and receive plenty of light. Furthermore, the effect seems to be reduced in recent years. What is the growth-limiting factor under these conditions? What may be an explanation? How do plants respond to decreased availability of the limiting factor? [SP 2, Concept Explanation; SP 6 Argumentation]

[Answer Key]

1. Photosynthesis would slow down considerably because little to no CO_2 is present. There is no recycling of NADPH and without a final electron acceptor, the electron transport chain in the Z scheme would come to a halt. In a similar fashion, there would be no recycling of ATP to ADP to be phosphorylated. The pH indicator would stay pink-orange and not turn to yellow. If organisms such as water snails are introduced in the tube, they will respire and release CO_2 which, in turn, will be used for photosynthesis.
2. Excess CO_2 dissolves in ocean water and combines with water to form carbonic acid. Carbonic acid, in turn, dissociates into hydrogen ions and bicarbonate ions, driving the pH down and acidifying ocean water. Acidification of oceans does not have a negative impact on algae as measured so far, but it promotes overgrowth which may lead to eutrophication and spreading of invasive species.
3. The plants compete for CO_2 . Plants open their stomata to increase the intake of CO_2 losing water through evaporation. The levels of CO_2 are rising in the atmosphere, which increases plant growth and reduces opening of stomata in the short term. Because climate change is accompanied by heat, drought, and other ecological disturbances, it will negatively affect growth in the long term.

Activity 2: Pre-Assessment

1. **Answer the following questions in your notebook:** *Spirogyra* are thin, thread-like green algae that cluster up in rafts and float on the surface of ponds buoyed by bubbles of gas. What gas buoys the network of algae? What happens when there is no light?
2. **Answer the following questions in your notebook:** If chlorophyll absorbs maximally in the blue and red regions of the visible spectrum and carotenoids extend absorbance in the blue-green region, which color of irradiation will be the most effective: red, green or white?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 2: Dependence of Photosynthesis on Selected Wavelength in the Light Spectrum

Several environmental factors influence the light-dependent reaction of photosynthesis. In this activity, you will investigate the rate of the light-dependent reaction by measuring indirectly the production of oxygen. Leaf disks are suspended in a solution of bicarbonate. A small drop of dishwashing soap in the solution breaks the surface tension of the leaf disks and allows the bicarbonate to penetrate inside the leaf. The bicarbonate in the solution provides a source of CO_2 for photosynthesis. The inner leaf spaces in a leaf are normally filled with air. When a bicarbonate solution is forced into those air spaces, the leaf disks become heavier and sink to the bottom of the solution.

As photosynthesis proceeds, the oxygen produced by PSII pushes liquid out of the air spaces and eventually bubbles out of the disks, which float to the surface. By monitoring the time it takes the disks to float, the rate of photosynthesis can be estimated under various conditions. To compare results of experiments, you will use the estimated time for 50 percent of the disks to float to the surface, labeled as ET_{50} . This is a median value that allows you to discount outliers, such as a floating disk that never sank in the first place or a disk stuck to the bottom of the cup. Because ET_{50} values decrease when photosynthetic rates go up, you may choose to plot $1/\text{ET}_{50}$ as a function of your independent variable.

Safety Precautions

- Wear goggles and an apron
- Use caution while working with the lamp as light bulbs become very hot
- Use caution while handling glass

For this activity, you will need the following:

- Plant material: intact leaves of spinach and *Coleus*, and light-colored leaves such as iceberg lettuce, celery leaves or cabbage (one leaf per pair of students). Avoid fuzzy leaves that trap air on their surface and do not sink easily.
- 0.2 M sodium bicarbonate solution (0.4 M, 0.6 M, and 0.8 M should be available)
- Two to three plastic 10 mL syringes
- Clear plastic cups
- Dilute dishwashing soap solution
- Single-hole punch
- Lamps
- Acetate colored filters (at a minimum, use blue, red and green filters)
- Aluminum foil

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for Structured Inquiry for Activity 2:

- Have students demonstrate oxygen bubbling on a specimen of *Elodea* growing under a bright lamp. You can compare it to an *Elodea* that is shaded and was previously kept in the dark.
- Demonstrate the syringe setup as shown in Figure 11.5. This will eliminate many errors that can delay the experiment. Pictures of an appropriate initial setup and an incorrect initial setup would be valuable for students.
- Use fresh leaf samples that have few yellowish or brown dead spots.
- Substitute colored cellophane if colored filters are not available.

The following are recommendations for the Guided Inquiry for Activity 2:

- Have the students identify the skills, such as designing a setup for determining changing levels of carbon dioxide, they will need to carry out the guided inquiry. These skills were learned in the structured inquiry.
- Place students in work groups. Encourage each group to assign specific tasks to group members.
- Ask students to write their own paper written in their words. Make sure to check for plagiarism by comparing writing of the answers between group members.

Structured Inquiry

Step 1: Hypothesize/Predict: Discuss with your partner how different the wavelengths of light would affect the rate of photosynthesis. Write in your lab notebook the justification for your predictions. Include the controls you will need to run a meaningful experiment.

Step 2: Student-led planning: In this guided inquiry, you will measure the ET_{50} in white light, in the dark, and in different regions of the visible spectrum. Design your experiment so you change only one variable at a time. Can you devise a set-up to minimize stray white light when you test the effect of colored light? Some experimentalists build a cardboard box with an opening that can be covered by a filter. In this case, run all your experiments under the same condition using a transparent filter for white light and aluminum foil to block the light. Create a table for your data.

Step 3: Set up of experiment:

1. Using a single-hole punch, punch 10-15 disks from an intact leaf being careful not to damage the leaf in the process.
2. Transfer the disks to the barrel of a 10 mL syringe and replace the plunger.

3. Pull about 4–5 mL suspension solution in the syringe. Tapping gently on the barrel until all disks float freely
4. Push the plunger to expel excess air.
5. Create a vacuum in the syringe by holding your index finger on the tip of the syringe where the needle would be inserted and slowly draw the plunger down. You will feel the vacuum pulling on your finger. Do not draw the barrel out of the syringe. The solution should be flowing into the airspaces of the leaf disks. Wait two to three minutes and release the vacuum. The disks should sink after you repeat this procedure two to three times. See Figure 11.5.



Figure 11.5: Pulling the barrel of the syringe and capping the tip with a finger creates a vacuum inside the syringe.

6. Tap the syringe gently to re-suspend the disks and empty the content of the syringe into a clear cup. Cover the disks with additional solution if needed to submerge them.
7. Continue with your experimental setup. Illuminate the clear cups with the lamp and record the number of floating disks every minute for 20 minutes. For the dark control, check the disks after 20 minutes.

Step 4: Critical analysis: Plot the number of floating disks for each condition as a function of time and determine the ET_{50} for each condition. Compare the estimated photosynthetic rates as $1/ET_{50}$ at the wavelengths you tested. Do the results confirm your hypothesis? Plot your results in a graph.

Guided Inquiry

Step 1: Hypothesize/Predict: Discuss with your partner which conditions would impact the rate of photosynthesis in the disk. Conditions include the following:

1. **The effect of varying CO_2 concentration.** This is achieved by using different concentrations of a sodium bicarbonate buffer which releases CO_2 .
2. **Plant material differences,** such as comparing the deep green leaves of spinach to the pale leaves of cabbage or celery

3. **Light intensity differences**, such as by changing the distance between the light source and the cup

Recall that $I=1/D^2$, where I is the intensity of light and D is the distance between the light source and the target. You can use different areas of a variegated leaf from *Coleus*.

Choose a single variable you will study in your experiment. Write down in your notebook the justification for your experiment and your prediction. How will it improve your understanding of photosynthesis? Include the controls you will need to run a meaningful experiment. Once you have determined a condition, discuss the feasibility of the experiment with your instructor and be prepared to modify your experiment. After choosing a condition, design your experiment carefully to limit the effect of a single variable.

Step 2: Student-led planning: Sketch your set-up in detail and ask your teacher for approval. Modify your set-up per the feedback from your teacher if need be. Now you are ready to assemble all the materials that you will need to perform the experiment. Follow the instructions described in Step 3 under the structured inquiry. Count the number of disks that float over time to estimate the ET_{50} . Enter all data in a table like Table 11.2, which you will adapt to your experimental conditions.

Step 3: Critical analysis: Plot the number of floating disks for each experimental condition as a function of time and determine the ET_{50} for each condition. Plot the estimated photosynthetic rates using $1/ET_{50}$ as a function of your independent variable, either bicarbonate concentration or light intensity. Compare the $1/ET_{50}$ values for darkly pigmented leaves and pale leaves. In each case, discuss the validity of the data and how you would improve on the experiment. How reliable is it to use bicarbonate as a source of CO_2 ? Can you compare light intensities without actually measuring light flux? Is it a fair comparison to use the response of disks from different types of plants? What else could have been at play?

Assessments

1. In an experiment, water is labeled with the radioactive O^{18} isotope. The labelled water is then supplied to the leaf disks. In a second experiment, CO_2 labeled with the isotope O^{18} is supplied to the disks. In which of the two experiments will you detect O^{18} in the product of photosynthesis? Why is this so? [SP 3, Questions and Methods; SP 1, Concept Explanation]

2. DCMU (3-(3, 4-dichlorophenyl)-1, 1-dimethyl urea) is an herbicide that specifically inhibits transfer of electrons from PSII. What would you predict would happen to the production of oxygen in an *Elodea* twig submerged in a solution treated with DCMU? How would an animal such as a water snail be affected by the presence of DCMU in the same environment? You can assume that DCMU doesn't affect the snail directly. [SP 6, Argumentation]
3. What would happen to gas bubbles in *Elodea* if a test tube is exposed to light for a prolonged period without aeration? What would happen if water snails are added to the test tube? Illustrate your answer with a diagram showing gas and the interaction between the *Elodea* and the water snail. [SP 6, Argumentation; SP 3, Questions and Methods; SP 2, Visual Representations]

[Answer Key]

1. In the second experiment because CO₂ molecules combine to form glucose during photosynthesis, as opposed to water, which supplies hydrogen ions to the concentration gradient in the thylakoid space and resupplies electrons to the photosystems.
2. When DCMU blocks electron flow, there is no electron acceptor for the electron ejected from the reaction center chlorophyll. Chlorophyll must be in an oxidized state (missing an electron) to accept electrons from H₂O. No O₂ will be produced if chlorophyll cannot accept electrons. If there is no photosynthesis there will be less oxygen in the water, which will adversely affect the snail and may cause it to get sick or die.
3. Upon prolonged exposure to light, all the CO₂ gas is consumed and photosynthesis stops. No oxygen is released. If snails are present in the water and breathing, they release CO₂ that can be fixed by the plant. In turn, the snails breathe in the O₂ released by the plant. A diagram should show that CO₂ is absorbed by the plant and released by the snail. Oxygen is released by the plant and consumed by the snail.

Extension Activities

1. Measure the ET₅₀ of disks from plants maintained overnight in the light and in the dark. Do plants maintained in the dark perform photosynthesis faster, slower, or at the same rate? Write your prediction with an explanation and discuss your results.
2. Compare ET₅₀ at different temperatures, such as 10°C, room temperature, and 50°C. Which steps of photosynthesis may be temperature dependent and how do they affect the evolution of O₂?

Lab 12: Photosynthesis: The Dark Reactions

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
2.1 Cell Structure: Subcellular Components	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.D Describe the structure and/or function of subcellular components and organelles.	SYI-1.D.4 Chloroplasts are specialized organelles that are found in photosynthetic algae and plants.
2.2 Cell Structure and Function	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.F Describe the structural features of a cell that allow organisms to capture, store, and use energy.	SYI-1.F.2 Within the chloroplasts are thylakoids and the stroma. SYI-1.F.6 The stroma is the fluid within the inner chloroplast membrane and outside of the thylakoid. SYI-1.F.7 The carbon fixation (Calvin-Benson cycle) reactions of photosynthesis occur in the stroma.
3.4 Cellular Energy	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.H Describe the role of energy in living organisms.	ENE-1.H.3 Energy-related pathways in biological systems are sequential to allow for a more controlled and efficient transfer of energy.
3.5 Photosynthesis	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.I Describe the photosynthetic processes that allow organisms to capture and store energy. ENE-1.J Explain how cells capture energy from light and transfer it to biological molecules for storage and use.	ENE-1.I.1 Photosynthesis captures energy from the sun and produces sugars. ENE-1.J.5 The energy captured in the light reactions and transferred to ATP and NADPH powers the production of carbohydrates from carbon dioxide in the Calvin cycle.

8.2 Energy Flow Through Ecosystems	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.O Explain how the activities of autotrophs and heterotrophs enable the flow of energy within an ecosystem.	ENE-1.O.1 Photosynthetic organisms capture energy present in sunlight.
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SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on an observation, data, or model. 3.B Predict the results of an experiment. 3.C Identify experimental procedures that are aligned to the questions, including variables and controls. 3.D Make observations, or collect data from representations of laboratory setups or results. 3.E Propose a new/next investigation based on evidence from a previous experiment.
SP 4 Representing and Describing Data Represent and describe data.	4.A Construct a graph, plot, or chart. 4.B Describe data from a table or graph.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including equations in the curriculum and rates.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. 6.C Provide reasoning to justify a claim. 6.E Predict the causes of effects of a change in components of biological systems based on data.

Photosynthesis is the process that phototrophic organisms, like plants, algae, cyanobacteria, and other bacteria, use to convert the energy from sunlight into chemical energy that can then be used to make carbohydrates like glucose. The chemical equation for photosynthesis in plants, algae, and cyanobacteria is shown in Figure 12.1.

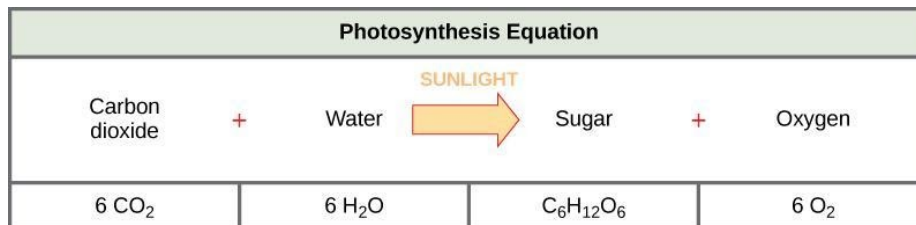


Figure 12.1: The chemical equation for photosynthesis in plants, algae, and cyanobacteria. Overall, light energy is used to fix CO₂ into organic carbon in the form of glucose (sugar).

In eukaryotic photosynthetic organisms like plants and algae, photosynthesis occurs in an organelle called a **chloroplast** (shown in Figure 12.2). Each chloroplast has both an inner and an outer membrane and numerous membrane-bound discs, called **thylakoids**, organized into stacks called **grana** (singular granum). Each thylakoid is composed of a folded **thylakoid membrane** that surrounds an inner **thylakoid space**. The fluid-filled space within the chloroplast that surrounds the thylakoids is called the **stroma**. In prokaryotic photosynthetic organisms like cyanobacteria, the photosynthetic membranes are infoldings of the cell's plasma membrane. In either case, the photosynthetic membranes are packed with photosynthetic pigments like **chlorophyll**, a type of photosynthetic pigment that appears green, which absorbs solar energy to initiate photosynthesis.

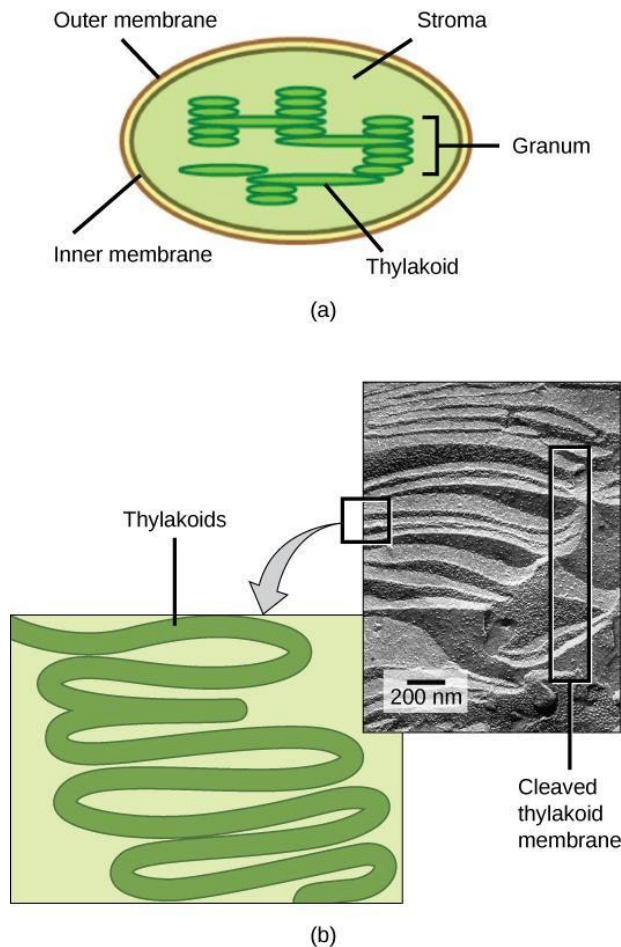


Figure 12.2: (a) Illustration of chloroplast structure showing the organization of thylakoids and grana within the chloroplast stroma. (b) Prokaryotic photosynthetic membranes are not contained with a distinct organelle, but instead are infoldings of the plasma membrane.

Photosynthesis is composed of two sets of reactions: the **light reactions** (or light-dependent reactions), which convert solar energy into chemical energy in the forms of ATP and NADPH, and the **dark reactions** (or light-independent reactions), which use the chemical energy formed from the light reactions to fuel the fixation of CO_2 to produce glucose (as shown in Figure 12.3). The light reactions occur in the thylakoid membranes, and the dark reactions occur within the stroma of chloroplasts or the cytoplasm of photosynthetic prokaryotes. Note that while the dark reactions do not require light to occur, they may take place in either the presence or absence of light, which is why they are often referred to as the light-independent reactions. Additionally, the dark reactions are dependent on the NADPH and ATP produced by the light reactions.

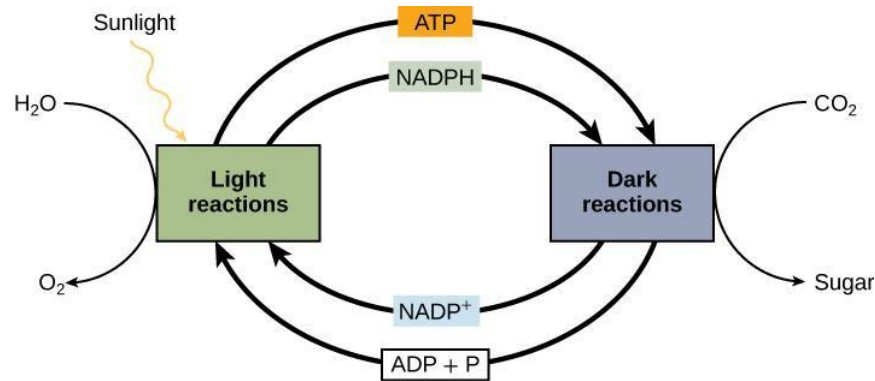


Figure 12.3: Photosynthesis is composed of both the light reactions and the dark reactions. The light reactions convert solar energy to chemical energy in the form of NADPH and ATP, which are then used by the dark reactions to fix CO_2 into carbohydrates like glucose.

The dark reactions refer to the various stages of enzymatic reactions called the **Calvin cycle** that serve to fix CO_2 using the energy of NADPH and ATP. The Calvin cycle can be split into three stages: fixation of CO_2 into organic carbon by the enzyme ribulose biphosphate carboxylase (RuBisCO), reduction of the organic carbon, and regeneration of the starting molecules. The stages of the Calvin cycle are summarized in Figure 12.4.

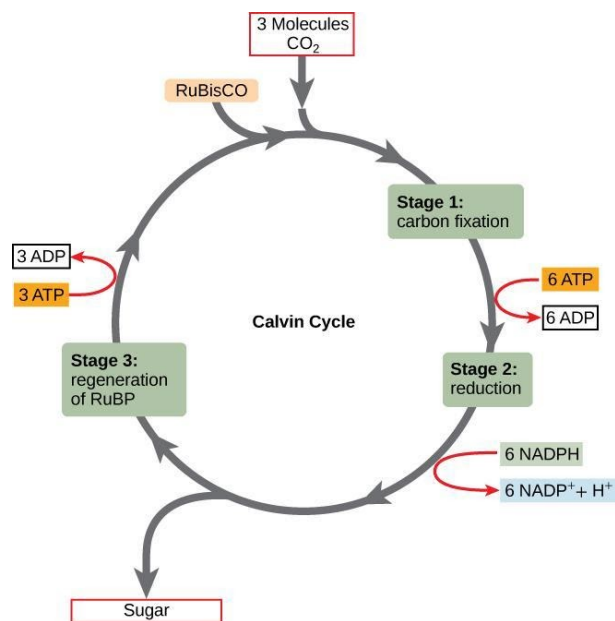


Figure 12.4: The Calvin cycle contains three stages. In the first stage, CO_2 molecules are fixed through the action of RuBisCO into organic carbon molecules. In the second stage, the organic carbon molecules are reduced, using NADPH and ATP. At this point, some of the reduced organic carbon leaves the cycle to form carbohydrates like the sugar glucose. The remaining organic carbon is used during the third stage to regenerate the starting molecules of the cycle, and ATP is used during this stage as well.

In photosynthetic organisms like plants, if the concentration of glucose exceeds the organism's immediate needs, it may be polymerized to form starch, a type of storage molecule that is insoluble. By storing starches, the organism bypasses the problem of increased osmotic pressure that may otherwise result when cells accumulate large concentrations of solute. This starch can then be stored until the organism has a need for glucose monomers, at which point the starch will be hydrolyzed to release individual glucose units.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Show a diagram of a chloroplast. Explain that energy from sunlight is captured in the light reactions, which take place in the thylakoid membrane. This energy is used to make sugars in the dark reactions, which take place in the stroma.
- Ask students where chlorophyll is located in the chloroplast, and what the role of chlorophyll is. Response should indicate that chlorophyll is located in the thylakoid membrane and captures energy from sunlight in the light reactions.
- Explain that besides chlorophyll, leaves contain other types of pigments. Some of these pigments are accessories in photosynthesis. Others act as sunscreen that protects the leaf from UV damage. The color of a leaf can vary depending on the type and amount of pigment that is present.
- Ask students to think about how stressors, such as lack of water or drought, might affect starch levels in plant tissue. Response should indicate that starch levels will drop when the plant is stressed.

In this lab, you will learn

- how to test for the presence of starch in plant leaves
- how to determine the effect of light on starch accumulation

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** Why might the presence of starch serve as a good indicator for the products of the dark reactions in plants?
2. **Answer the following questions in your notebook:** Leaves contain chlorophyll, but the amount can vary. For example, stems and veins may contain less chlorophyll and appear lighter in color. Some leaves are also variegated, with lighter and darker sections. Would you expect starch to accumulate more in the lighter-colored or darker-colored sections of a leaf? Why?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 1: Testing for the Presence of Starch in Plant Leaves

How might we test for the presence of starch in leaves? We could use a colorimetric indicator for starch that changes color to detect any color change when starch is present. However, this may be problematic since plant leaves, like those of spinach, are quite green due to the presence of chlorophyll, and this may mask any color formation if we use a colorimetric indicator for starch.

In this lab, to circumvent the problem mentioned above, you will first boil leaves in a solution of 70 percent ethanol to remove chlorophyll. Following this, you will then stain the now chlorophyll-free leaves with iodine, a dark red indicator that complexes with starch, turning bluish-black. This bluish-black color can be seen in Figure 12.5, where a starchy seed has been stained with a drop of iodine.



Figure 12.5: Iodine complexes with starch to form a bluish-black color, as seen here after a drop of iodine was added to this cross section of a starchy seed.

Safety Precautions

- Ethanol is highly flammable and should not be exposed to open flames.
- Wear eye protection when working with solutions of 70 percent ethanol and iodine.
- Use caution when using iodine solution as it may stain skin and/or clothing.
- Use caution when handling hot liquids.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent people from slipping.

For this activity, you will need the following:

- Spinach leaves
- Variegated leaves (examples include *Coleus*, *Hosta*, holly, clover)

- 70 percent ethanol solution
- 0.01 M iodine solution
- Starch
- Glass test tubes
- Forceps
- Water bath
- Hot plate
- Beakers
- Petri dish
- Paper towels
- Hot mitts
- Container for collecting ethanol waste

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Make sure students know that a colorimetric indicator is a substance that changes color in the presence of a certain chemical. Iodine is an indicator that turns purplish black in the presence of starch.
- Ask students why ethanol, but not water, removes chlorophyll from leaves. Response should indicate that chlorophyll, which is embedded in membranes, is hydrophobic and is therefore soluble in hydrophobic solvents but not water.
- Remind students that ethanol is flammable. Tell them that iodine can stain clothing.
- Remind students to use the hotplate with care.

Structured Inquiry

Step 1: Set up a large beaker of boiling water using a hot plate. While waiting for the water to boil, create a data table in which you can draw your spinach leaf four times and use the following four categories: (1) before any treatment, (2) after boiling in water, (3) after boiling in 70 percent ethanol, and (4) after staining with iodine. Obtain a spinach leaf and draw the appearance of the spinach leaf before treatment. Note the color.

Step 2: Hypothesize/Predict: Based upon your knowledge of photosynthesis and starch production in plants, predict where in the leaf you expect to find the most starch. Add your prediction to the data table you created in Step 1.

Step 3: Once the water is boiling, add the spinach leaf to the boiling water for 30 seconds using a pair of forceps. After 30 seconds, remove the spinach leaf from the water, place it on a Petri dish, and look at the leaf. Is it uniformly green? Are some parts darker than others? Draw, in your data table, the appearance of the spinach leaf after boiling in water.

Step 4: Student-led Planning: Obtain two test tubes. Add water to each, and add a spoonful of starch to one. Add a drop of iodine to each and note the color.

Step 5: Place the boiled spinach leaf in a glass test tube using forceps. Add enough 70 percent ethanol to the glass test tube to cover the leaf and immerse this tube in the boiling water bath for 10 minutes or until all color from the leaf has been removed. Use forceps to remove your leaf from the test tube and dip it in a test tube of water for one minute to rehydrate it. Place it in a Petri dish. Draw a picture of your spinach leaf after boiling in ethanol.

Step 6: Critical Analysis: Use a dropper to apply iodine solution to the leaf. Record the intensity of the bluish-black color formed as well as where in the leaf the color formed. Label the starch-containing areas on your leaf drawing. Is the prediction you made in Step 2 supported by your data?

Step 7: Collect any ethanol waste in the collection container.

Guided Inquiry

Step 1: Hypothesize/Predict: Look at the variegated leaves provided by your instructor and think about leaf color. Are all leaves as green as spinach? Are all leaves entirely green? Pick two different-colored areas from a variegated leaf and predict where starch is likely accumulated. Write your ideas in your notebook.

Step 2: Student-led Planning: With your lab partner, create a table to record your data and test your hypothesis from Step 1 using ethanol and iodine. Be sure to record the intensity of the bluish-black color formed for each leaf (and/or section of leaf).

Step 3: Critical Analysis: Are the predictions you made in Step 1 supported by your data? Discuss your answer with your lab partner and write it in your notebook.

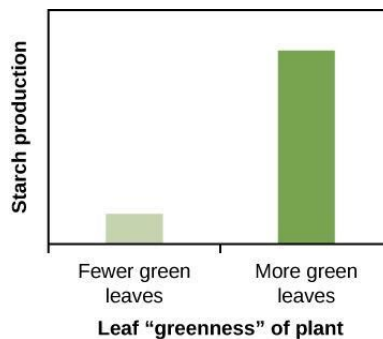
Step 4: Collect any ethanol waste in the collection container.

Assessments

1. Based on this experiment, what relationship might you expect between the leaf *greenness* of various plants and their relative abilities to convert sunlight into chemical energy in the form of starch? Make a graphical model of your prediction. How might the growth of a greener plant differ from that of a less green plant? Explain. [SP 1, Concept Explanation; SP 4, Describing and Representing Data; SP 6, Argumentation]
2. A student microscopically observed leaves from two different plants and noticed that one had twice as many chloroplasts as the other. How do you predict the rates of starch production will compare between these two plants? [SP 6, Argumentation]
3. In variegated leaves, explain how leaf cells lacking chlorophyll obtain free energy to sustain their growth. How does this compare to the way that consumers (like humans who eat plant products) obtain free energy to sustain their growth? [SP 1, Concept Explanation]

[Answer Key]

1. Since more green pigmentation is associated with having more chloroplasts, greener plants would be capable of more starch production during sun exposure. Thus, one might expect that plants with greener leaves would grow faster and/or bigger. Such a graph might look as follows:
- 2.



Having more chloroplasts would allow that plant to capture more free energy from the sun and photosynthesize at a faster rate, producing starch at twice the rate as the plant with half as many chloroplasts.

3. Plant cells that lack chlorophyll cannot photosynthesize to produce starch. However, those cells within a leaf that do photosynthesize can provide enough starch to share with and sustain non-photosynthetic cells. Consumers like humans and other organisms that eat plant materials and other photosynthetic organisms obtain chemical free energy from the starch within the ingested photosynthetic organisms to sustain their growth.

The strategy used by consumers is similar to that used by non-photosynthetic plant cells: use the starch produced by photosynthetic cells as a chemical free energy source.

Activity 2: Pre-Assessment

1. **Answer the following questions in your notebook:** Explain how the dark reactions got their name? Can they happen independently of exposure to the sun? Explain.
2. **Answer the following question in your notebook:** How may the amount of sun exposure affect the dark reactions?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 2: Determining the Effect of Light on Starch Accumulation

While the name of the dark reactions suggests that they can happen in the dark, can they? No, a better name for the dark reactions of the Calvin cycle is the light-independent reactions. By *light-independent* we mean that the Calvin cycle does not involve the free energy of the sun directly. However, for the Calvin cycle to occur, the light reactions—or more precisely, the light-dependent reactions—carried out by photosynthetic pigments within thylakoid membranes, must occur to convert the free energy from the sun into chemical free energy in the form of NADPH and ATP. The dark reactions must be continually supplied with NADPH and ATP from the light reactions in order to continue, even if the dark reactions do not directly involve the sun.

One problem that some plants living in hot climates encounter is balancing their need for CO₂ to carry out the dark reactions with their need to retain water. While opening stomata on leaf surfaces to get CO₂ for carbon fixation, plants living in these conditions risk a major loss of water, and become dehydrated. However, if they close their stomata to retain water, the O₂ produced as a by-product of the light reactions can accumulate inside plant cells and serve as an alternative substrate to RuBisCO when CO₂ levels are low. This process, called **photorespiration**, is harmful to the plants in that it significantly reduces carbohydrate production by the dark reactions, using up NADPH and ATP without fixing CO₂ (Figure 12.6), while also using O₂ that otherwise would be used as a terminal electron acceptor during respiration to make ATP. Photorespiration is problematic for **C₃ plants**, those lacking any mechanism to avoid this, which are named as such because the first fixed carbon product contains 3 carbons.

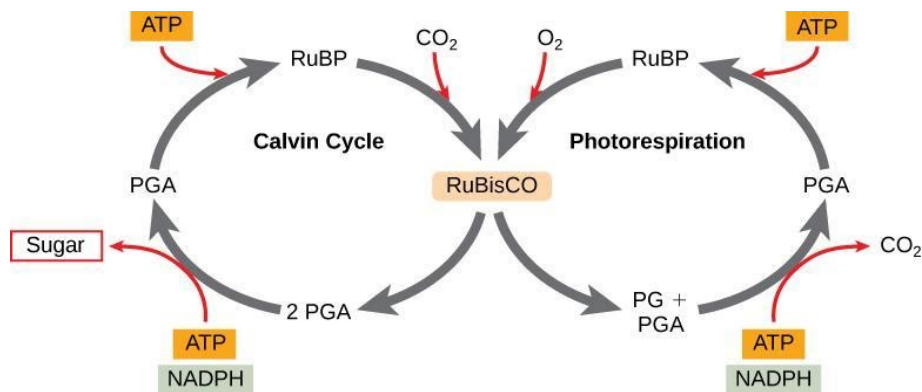


Figure 12.6: Photorespiration occurs when cellular O_2 levels are high and CO_2 levels are low. When this occurs, RuBisCO may bind to O_2 instead of its typical substrate CO_2 . This results in the use of NADPH and ATP without the production of carbohydrates.

Some plants have evolved mechanisms to overcome photorespiration (Figure 12.7). In some plants, called the **C_4 plants**, CO_2 is first added to a 3-carbon molecule to produce a 4-carbon molecule in a mesophyll cell on the plant leaf's exterior. This mesophyll cell then serves as a CO_2 supply for a more internal cell, called a bundle sheath cell. The mesophyll cell releases CO_2 from the 4-carbon molecule directly in the bundle sheath cell's vicinity. Interestingly, due to their distinct adaptive advantage, C_4 plants evolved independently multiple times in different plant lineages. One commercially significant C_4 plant is corn.

Other plants, called **CAM plants**, evolved a pathway known as crassulacean acid metabolism (CAM). CAM plants open their stomata at night, allowing them to retain their water but also allowing the flow of CO_2 into the plants. This CO_2 is then added to a 3-carbon molecule to produce a 4-carbon molecule, which serves as a CO_2 source during the day, when the plant's stomata are closed. While these mechanisms are similar, C_4 plants spatially separate the incorporation of CO_2 into the 4-carbon molecule in a cell different from the one carrying out the Calvin cycle, while these two activities occur in the same cell, but at different times, in CAM plants. Cacti and succulents are examples of CAM plants.

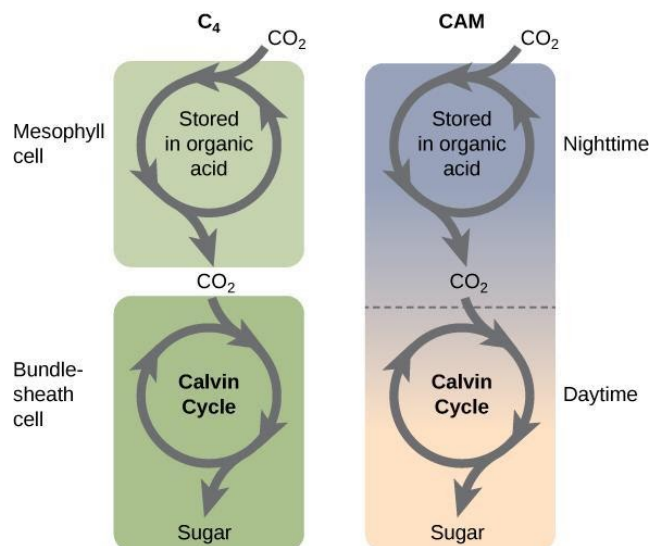


Figure 12.7: To avoid photorespiration, plants have evolved strategies to either spatially (C₄ plants) or temporally (CAM plants) separate the incorporation of CO₂ into a 4-carbon molecule, which subsequently serves as a CO₂ source, from the Calvin cycle.

Safety Precautions

- Ethanol is highly flammable and should not be exposed to open flames.
- Wear eye protection when working with solutions of 70 percent ethanol and iodine.
- Use caution when using iodine solution as it may stain skin and/or clothing.
- Use caution when handling hot liquids.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent people from slipping.

For this activity, you will need the following:

- Spinach leaves
- Potted house plants including
 - C₃ plants: peace lily, golden pothos, or philodendron
 - CAM plant: jade
- Thick black paper cut into strips
- Paper clips
- 70 percent ethanol solution
- 0.01 M iodine solution

- Glass test tubes
- Forceps
- Water bath
- Hot plate
- Beakers
- Petri dish
- Paper towels
- Hot mitts
- Container for collecting ethanol waste

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Notes

The following are recommendations for the Structured Inquiry for Activity 2:

- Show a diagram of the Calvin cycle and photorespiration. Explain that photorespiration serves no physiological function and wastes energy.
- Explain that C_4 and CAM plants have independently evolved the ability to reduce the occurrence of photorespiration. In C_4 plants photosynthesis takes place in the interior of the leaf where less oxygen is present. In CAM plants carbon dioxide is taken up at night and stored as organic acid. C_3 plants do not possess either of these adaptations and are more susceptible to photorespiration.
- Ask students what environmental conditions would make C_4 or CAM metabolism most advantageous. Response should indicate that these two types of metabolism are most advantageous in hot, dry conditions as the stoma must be kept closed to prevent water loss, which causes carbon dioxide levels to fall and oxygen levels to rise.

Structured Inquiry

Step 1: Student-led Planning: Take a C_3 plant and cover several of the leaves with black paper strips, as shown in Figure 12.8, securing the strips with paper clips. Be sure the strip goes around both the front and back of each leaf and that the black paper is secured tightly to the leaves. Expose the plant to sun and water as normal for two to seven days prior to continuing with the lab. Your teacher may have you move to the Guided Inquiry below at this time.

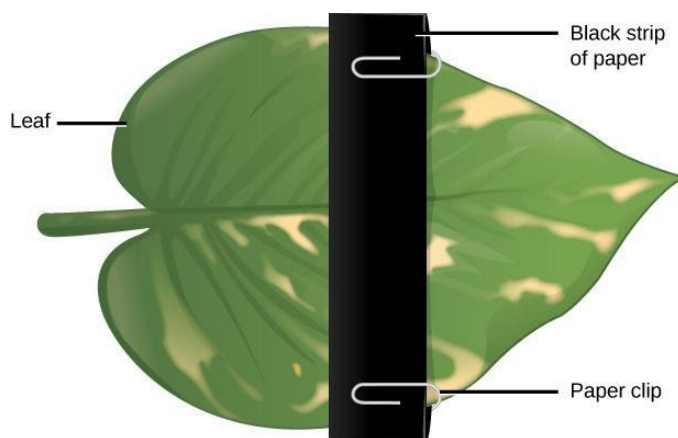


Figure 12.8: Cover several leaves of a potted plant with black strips, securing with paper clips.

Step 2: Hypothesize/Predict: You will now repeat the procedure you used previously to detect starch production in leaves. Based upon your knowledge of starch production due to photosynthesis, predict where you expect starch production to occur in a leaf that has been partially covered with a black strip of paper. How do you expect starch production to compare between the covered and uncovered sections of the leaf? Create a data table for drawing a leaf that has been covered with a black paper strip. In your data table, include space for drawing covered and uncovered sections of the leaf four times each and use the following four categories: (1) before any treatment, (2) after boiling in water, (3) after boiling in 70 percent ethanol and rehydration, and (4) after staining with iodine. Add your predictions regarding starch production in the covered versus uncovered sections of the leaf to this data table.

Step 3: Prepare a large beaker of boiling tap water on a hot plate. While waiting for the water to boil, draw the appearance of both the covered and uncovered sections of the leaf before treatment. Once the water is boiling, add the leaf to the boiling water using a pair of forceps. After 30 seconds, remove the leaf from the water and observe, drawing the appearance of each section (the covered and the uncovered sections) in your data table.

Step 4: Remove the chlorophyll content from the leaf by boiling in 70 percent ethanol and rehydrate it, drawing the appearance of both the covered and uncovered leaf sections in your data table.

Step 5: Critical Analysis: Stain the leaf with iodine and draw a picture of the leaf in your data table. Record the intensity of the bluish-black color formed in the leaf sections as well as the locations in the leaf where the color formed, indicating these using arrows on your leaf drawing. Are the predictions you made in Step 2 supported by your data?

Step 6: Collect any ethanol waste in the collection container.

Guided Inquiry

Step 1: Hypothesize/Predict: Think about the differences between C_3 , C_4 , and CAM plants. Predict how the differences in their CO_2 fixation strategies prior to the Calvin cycle may affect their production of starch in both the presence and absence of light. How might you test this? Write your ideas in your notebook.

Step 2: Student-led Planning: With your lab partner, design an experiment to examine the effect of CO_2 fixation strategy (C_3 , C_4 , or CAM) on starch accumulation in leaves in both the presence and absence of light. Once your teacher approves, create a table to record your data, prepare your plants with the black paper strips, and expose them to the sun and water as normal for two to seven days prior to your experiment, per your design.

Step 3: Treat your leaves to remove chlorophyll, rehydrate, and stain with iodine, recording data on the relative amounts of starch found within your various leaves and sections of leaves. Be sure to record the intensity of the bluish-black color formed for each leaf and each section of leaf.

Step 4: Critical Analysis: Are the predictions you made in Step 1 supported by your data? Discuss your answer with your lab partner and write it in your notebook.

Step 5: Collect any ethanol waste in the collection container.

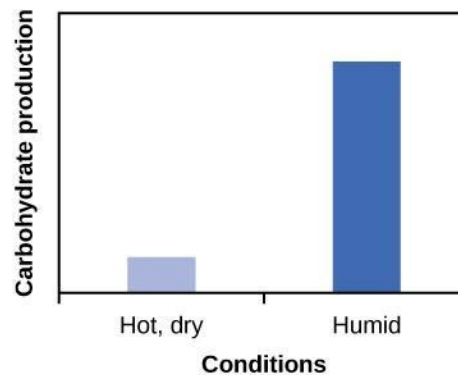
Assessments

1. How does sun exposure affect the dark reactions' conversion of the chemical free energy from NADPH and ATP to that of carbohydrates? Explain. [SP 1, Concept Explanation]
2. Consider a C_3 plant grown in hot, dry conditions compared to humid conditions. How do you predict the acquisition of chemical free energy in the form of carbohydrates will compare between these two conditions? Explain. Make a graphical model of your prediction. How would plant growth be affected? [SP 6, Argumentation; SP 4, Representing and Describing Data]
3. Compare how the acquisition of chemical free energy in the form of carbohydrates is affected by humidity in C_3 plants compared to C_4 plants? [SP 6, Argumentation]

[Answer Key]

1. Reducing sun exposure will reduce the amount of NADPH and ATP made during the light reactions. Thus, there will be less NADPH and ATP to fuel the dark reactions, and less starch can be made as a result of reduced sun exposure.

2. During hot, dry conditions, stomata are likely to be closed to prevent water loss from the plant. As such, the amount of CO_2 within the plant available for carbon fixation by RuBisCO will be reduced. Thus, one would expect reduced carbohydrate production under these conditions. By contrast, in humid conditions, water loss from the plant is no longer a problem so plants would have their stomata open. As such, the amount of CO_2 within the plant available for carbon fixation by RuBisCO would not be limiting and RuBisCO activity, and hence carbohydrate production, would be expected to be higher under these conditions. C_3 plants would be expected to grow more under humid conditions compared to hot, dry conditions. Additionally, rates of photorespiration would be lower under humid conditions since the open stomata would allow for escape of O_2 from plant cells. A chart of relative carbohydrate production might look like this:



3. Under hot, dry conditions, C_3 plants would be significantly hampered in their ability to produce carbohydrates by the dark reactions, since internal CO_2 levels would be low due to closed stomata to prevent dehydration. Additionally, photorespiration triggered by the inability of O_2 to escape plant cells would be increased. However, C_4 plants have evolved a strategy to sequester even the very low amounts of internal CO_2 into a 4-carbon molecule within mesophyll cells, which then serve to donate CO_2 to more internal bundle-sheath cells, which carry out the Calvin cycle to produce carbohydrates. Thus, C_4 plants avoid photorespiration and can fix CO_2 even in hot, dry climates. While one would still expect higher carbohydrate production by C_4 plants under humid conditions compared to dry conditions, the difference in carbohydrate production between these two conditions is much less than in C_3 plants. Because of the great advantage that C_4 plants have for growth in hot, dry conditions compared to C_3 plants, C_4 capabilities have evolved independently in multiple lineages of plants.

Lab 13: Mitosis and Meiosis

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
4.6 Cell Cycle	IST-1 Heritable information provides for continuity of life.	IST-1.B Describe the events that occur in the cell cycle. IST-1.B.C Explain how mitosis results in the transmission of chromosomes from one generation to the next.	IST-1.B.1 In eukaryotes, cells divide and transmit genetic information via two regulated processes. IST-1.B.2 The cell cycle is a regulated series of events for growth and cell reproduction and consists of interphase, mitosis, and cytokinesis. IST-1.C.1 Mitosis transfers a complete genome from a parent cell to two genetically identical daughter cells.
4.7 Regulation of Cell Cycle	IST-1 Heritable information provides for continuity of life.	IST-1.D Describe the role of checkpoints in regulating the cell cycle. IST-1.E Describe the effects of disruptions to the cell cycle on the cell or organism.	IST-1.D.1 A number of internal controls of checkpoints regulate progression through the cycle. IST-1.D.2 Interactions between cyclins and cyclin-dependent kinases control the cell cycle. IST-1.E.1 Disruptions to the cell cycle may result in cancer and/or apoptosis.
5.1 Meiosis	IST-1 Heritable information provides for continuity of life.	IST-1.F Explain how meiosis results in the transmission of chromosomes from one generation to the next.	IST-1.F.1 Meiosis ensures the formation of haploid gametes in sexually reproducing diploid organisms.

		IST-1.G Describe the similarities and/or differences between the phases and outcomes of mitosis and meiosis.	IST-1.G.1 Mitosis and meiosis are similar in the way chromosomes segregate but differ in the number and genetic content of daughter cells produced.
5.2 Meiosis and Genetic Diversity	IST-1 Heritable information provides for continuity of life.	IST-1.H Explain how the process of meiosis generates genetic diversity.	IST-1.H.1 Separation of homologous chromosomes in meiosis I ensures that each gamete received a haploid set of maternal and paternal chromosomes. IST-1.H.2 During meiosis I, homologous chromosomes can exchange genetic material via “crossing over.” IST-1.H.3 Sexual reproduction in eukaryotes increases variation.
5.6 Chromosomal Inheritance	SYI-3 Naturally occurring diversity among and between components within biological systems affects interactions with the environment.	SYI-3.C Explain how chromosomal inheritance generates genetic variation in sexual reproduction.	SYI-3.C.3 Certain human genetic disorders can be attributed to chromosomal changes, such as nondisjunction.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams or photographs.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A. Identify a testable question based on observation, data, or a model. 3.B. Predict the results of an experiment. 3.D Make observations, or collect data from representations of laboratory setups or results.
SP 4 Representing and Describing Data Represent and describe data.	4.A Describe data from a table.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including means and percentages.
SP 6 Argumentation Develop and justify scientific arguments with evidence.	5.A. Make a scientific claim. 5.B Support a claim with evidence. 6.C Provide reasoning to justify a claim by connecting evidence to biological theories. 6.E Predict the causes or effects of a change in one or more components of a biological system based on data and visual representations.

Cell theory states that all existing cells originated from preexisting cells. DNA is the genetic material of cells, and in eukaryotic cells, it is packaged into chromosomes. You have two copies of each chromosome: one from your mother (from the egg) and one from your father (from sperm).

During **mitosis**, a cell splits into two daughter cells, with two copies of each chromosome in each. In **meiosis**, four cells are produced—with only one copy of each chromosome. The processes of **mitosis** (non–sex cell division or cell division of somatic, or body cells) and **meiosis** (generation of sex cells or gametes) support the cell theory. The cell cycle illustrated in Figure 13.1 shows the life cycle of somatic cells. Mitosis and meiosis share similar

processes; however, the outcomes are different and serve different purposes. **Interphase** (a period between cell division and **cytokinesis**, or division of the cytoplasm into two separate cells) represents the portion of the cell cycle in which DNA is not visible under the light microscope. When a cell is signaled to replicate, the DNA is copied during the S-phase, or **DNA synthesis** phase, in the middle of interphase. In this lab, you will observe cells in different phases of mitosis and in interphase.

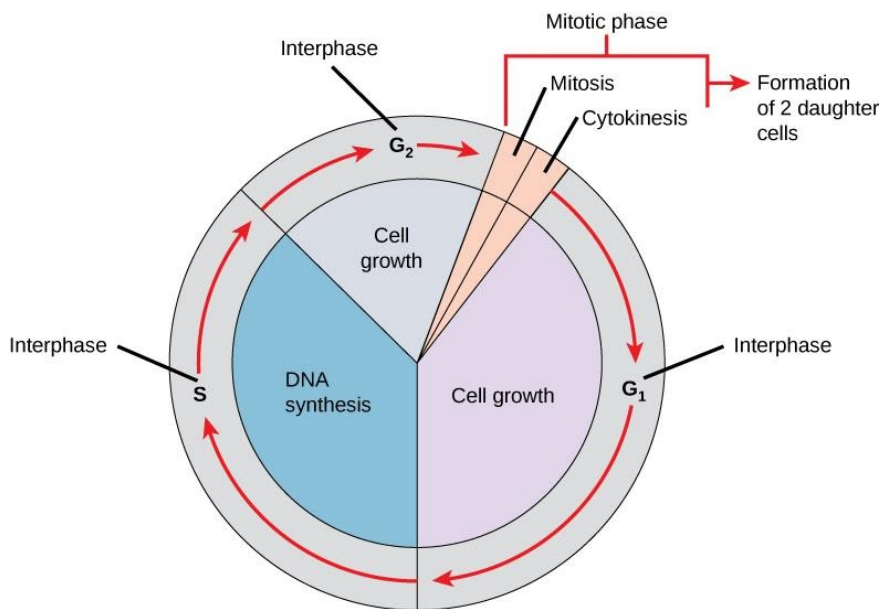


Figure 13.1: The cell cycle consists of interphase and the mitotic phase.

In this lab, you will learn

- how to observe the stages of mitosis in whitefish blastula cells
- how to identify and describe the stages of mitosis in whitefish blastula cells
- how to observe and describe the stages of mitosis in onion root tips
- how to read a karyotype
- how to determine karyotype abnormalities and identify an associated disorder or syndrome

Instructor Preparation and Teaching Tips

- Assemble slides necessary for the lab: whitefish blastula cells and onion root tips. Order slides (+10 percent additional) one month prior to lab.

- Introduce the lab with the properties of life: growth, reproduction, homeostasis, organization, metabolism, response to stimuli, and adaptation. Ask the students to identify these properties, and then begin to expand on growth and reproduction at the cellular level.
- Ask the students to think about how tall he or she was at five years old. Ask students to think about how the difference in size was achieved.
- Draw out the cell cycle. Use the proportions from Figure 13.1 to set the stage for students to understand the relative lengths of each stage of the cell cycle over a 24-hour period.

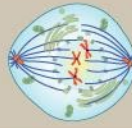
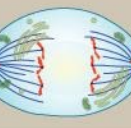
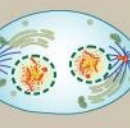
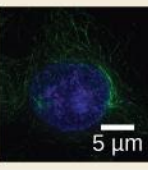
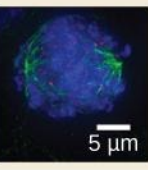
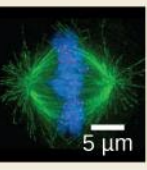
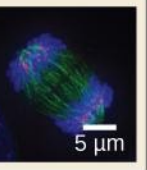
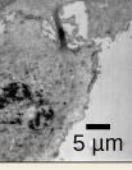
Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** List three reasons why organisms need to produce new cells.
2. **Answer the following question in your notebook:** What cellular structures must be replicated to ensure that new cells are functional after cell division?
3. **Discuss the answers to questions 1 and 2 with a partner (think, pair, share) and then the class.**

Activity 1: Observe the Stages of Mitosis in the Blastula of a Whitefish

All organisms have the need to stay alive and reproduce. Mitosis addresses the need for cell growth, maintenance, and repair. During interphase, the cell's DNA is replicated so that there are two copies of each chromosome, called the **sister chromatids**. In eukaryotes, all chromosomes must be duplicated prior to mitosis and cytokinesis to ensure each new daughter cell has the full complement of genetic information. Other structures, such as organelles, are replicated during the G_1 portion of the cell cycle (See Figure 13.1). As you can see in Figure 13.2, mitosis is divided into specific phases. During **prophase**, the chromosomes coil up, and sister chromatids become visible under a microscope. The nuclear membrane surrounding the chromosomes also disappears. In **metaphase**, the sister chromatids align in the center of the cell, attached to **spindle fibers**. During **anaphase**, the sister chromatids separate and move to opposite poles of the cell. In **telophase**, the chromosomes arrive at the poles and begin to decondense while the nucleus reforms. Figure 13.2 makes it look like the phases are very distinct. The phases, however, transition without stopping. Notice also other important structures, such as the spindle fibers and **kinetochores** on the **centromeres** of each chromosome. Each pair of sister chromatids has a protein structure, called a kinetochore, which becomes attached to spindle fibers. The spindle fibers pull the sister chromatids apart, toward opposite ends of the cell.

Cancer is the uncontrolled growth and mitotic division of cells. Some chemotherapy drugs, including taxanes such as Taxol from the yew tree and alkaloids from the vinca plant, interfere with mitosis by binding to microtubules and preventing spindle fibers from separating sister chromatids, thus leading to cell death.

Prophase	Prometaphase	Metaphase	Anaphase	Telophase	Cytokinesis
					
• Chromosomes condense and become visible. • Spindle fibers emerge from the centrosomes. • Nuclear envelope breaks down. • Nucleolus disappears.	• Chromosomes continue to condense. • Kinetochores appear at the centromeres. • Mitotic spindle microtubules attach to kinetochores. • Centrosomes move toward opposite poles.	• Mitotic spindle is fully developed; centrosomes are at opposite poles of the cell. • Chromosomes are lined up at the metaphase plate. • Each sister chromatid is attached to a spindle fiber originating from opposite poles.	• Cohesin proteins binding the sister chromatids together break down. • Sister chromatids (now called chromosomes) are pulled toward opposite poles. • Non-kinetochore spindle fibers lengthen, elongating the cell.	• Chromosomes arrive at opposite poles and begin to decondense. • Nuclear envelope material surrounds each set of chromosomes. • The mitotic spindle breaks down.	• Animal cells: a cleavage furrow separates the daughter cells. • Plant cells: a cell plate separates the daughter cells.
					

MITOSIS

Figure 13.2: Mitosis is divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase.

Safety Precautions

- Be careful handling glass slides, as the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.

For this activity, you will need the following:

- Prepared slide of whitefish blastula cells or online images

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Walk through the basics of using a compound light microscope. All students should begin by learning to carry the microscope to/from their lab sites with one hand below the base and the other hand on the arm. Before using the microscope, the objective lens should be placed under the lowest power; coarse and fine focus are next used to get the image into view. Objective lenses can be rotated into place to view the images at higher powers. Following the shift to higher objective lenses, emphasize to students that they should *use only the fine focus* knob to clarify the image view; never use the coarse focus after shifting from the lowest-power objective lens.
- Indicate to students that any images that are recorded in the lab notebook should be given a descriptive caption that includes the contents of the image (e.g., whitefish blastula cells) and the power used to view the image. Remind your students how to determine viewing power: objective lens power multiplied by ocular lens power.
- Review the method used to scan through a slide image (see Figure 13.4).
- Practice identifying the various stages of mitosis, by visiting http://www.biology.arizona.edu/cell_bio/activities/cell_cycle/cell_cycle.html. Students will get to see the onion root tip cells prior to the lab investigation and give them confidence in identifying the different mitotic stages.

Structured Inquiry

Step 1: Hypothesize/Predict: Using Figure 13.1, predict the percent of time that a cell would spend in each phase. Based on this prediction, how much time do you think cells will spend in interphase as opposed to mitosis? Write your prediction in your notebook.

Step 2: Student-Led Planning: Look at Figure 13.2. In your notebook, make a table defining the characteristics of the stages of mitosis, as well as interphase, that you can use to identify each stage under the microscope. Note: There are four major phases of mitosis, plus prometaphase, which is a transition phase between prophase and metaphase.

Step 3: Using the prepared slide, record the number of cells in each phase of the cell cycle. Use the method shown in Figure 13.3 to count the cells. Record your count in a data table like that shown in Table 13.1. Share your data with the class to create a group total count.

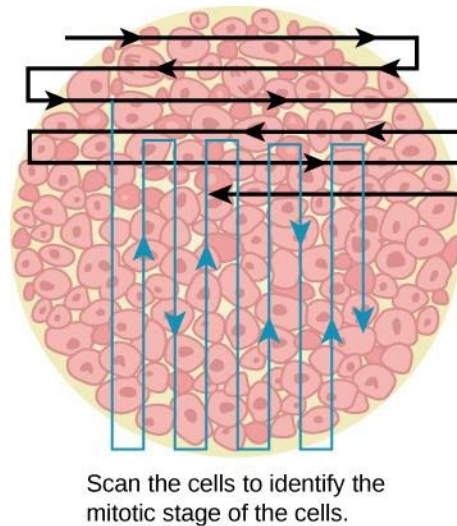


Figure 13.3: Scan and count cells by using either a horizontal (black line) or vertical (blue line) back and forth scanning technique with your eyes.

Table 13.1: Results of Cell Stage Identification

Phase or Stage	Individual Totals	Class or Group Totals	Percent
Interphase			
Prophase			
Metaphase			
Anaphase			
Telophase			
Cytokinesis			
Totals			100%

Step 4: In your data table, calculate the percentage of cells in each phase.

Step 5: Critical Analysis: Are the predictions you made supported by your data (observations and calculations)? Do your results match the diagram as presented in Figure 13.1? Why or why not?

Assessments

1. Explain why interphase could be the longest phase and mitosis and cytokinesis are generally much shorter phases of the cell cycle. [SP 1, Concept Explanation]
2. Explain the importance of spindle fibers in mitosis and why the use of antimitotic drugs that block spindle fiber formation are used to treat cancer. [SP 1, Concept Explanation]

[Answer Key]

1. Interphase is the functional period of a cell between divisions, representing a large percentage of time and the number of cells seen in the prepared slide or images. A smaller portion of the life cycle is devoted to mitosis and cytokinesis, in which two daughter cells, with copies of the original genome as well as necessary organelles, are formed.
2. Spindle fibers attach at the kinetochore on the centromeres of sister chromatids. The spindle fibers (microtubules) are disassembled by the kinetochores, drawing the sister chromatids apart and toward the opposite poles of the original cell during anaphase. Any disruption of the assembly of the microtubules (spindle fibers) prevents that separation of the sister chromatids. A tumor grows through unregulated mitosis, and antimitotic drugs block this process, rendering tumor cells unable to produce more genetically dysregulated cells.

Activity 2: Pre-Assessment

1. **Answer the following question in your notebook:** In a plant, where would you likely find cells that are actively undergoing mitosis? Where would you likely find cells that aren't actively undergoing mitosis? Explain your answers.
2. **Answer the following question in your notebook:** Think about how a plant grows. In which parts of the plant would many cells likely be undergoing mitosis?
3. **Discuss the answers to questions 1 and 2 with a partner (think, pair, share) and then the class.**

Activity 2: Observe the Stages of Mitosis in Onion Root Cells

Plant cells also use mitosis for growth, maintenance, and repair. The plant's cell wall, as well as the nuclear material, makes observing mitosis much easier. Figure 13.4 shows some of the similarities and differences between plant and animal mitosis. For example, during cytokinesis an animal cell pinches apart into two daughter cells, while a plant cell develops a new cell wall, called a **cell plate**, between the new daughter cells.

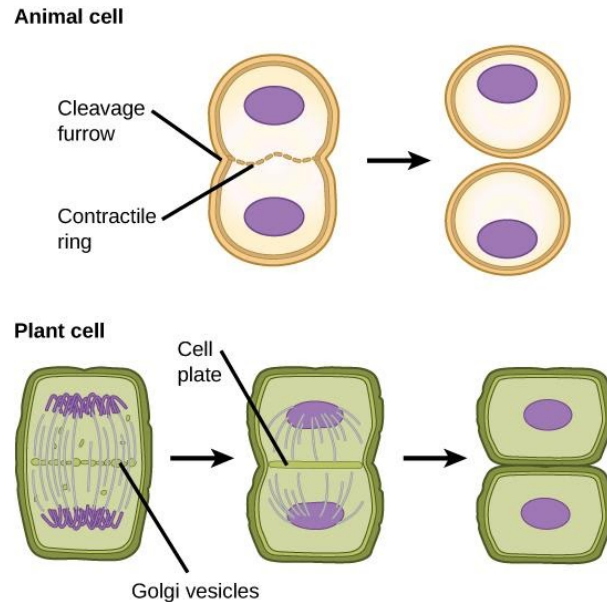


Figure 13.4: Cytokinesis differences between plant and animal cells

Safety Precautions

- Be careful handling glass slides, as the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.

For this activity, you will need the following:

- Prepared microscope slides of stained onion root tips or online images

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 2:

- Orient your students to the root tip and otherwise, to give them a sense of location of cells. Many differences exist between animal and plant cells; the most obvious in this case is the shape of the cells, particularly as one moves toward the tip of the root.

- Introduce the terms associated with the root tip:
 - **Root cap:** the tip of the root that protects the plant as it pushes into the soil
 - **Zone of division:** the site where cells are actively undergoing mitosis
 - **Zone of elongation:** the site where daughter cells are lengthening during growth
 - **Zone of maturation:** location where daughter cells are differentiating into cells that play a role in storage, protection, and conduction

Structured Inquiry

Step 1: Hypothesize/Predict: In your notebook, hypothesize what regions of the onion root tip might differ in the number of cells undergoing mitosis. What is your reasoning behind this hypothesis? Record your answers in your notebook.

Step 2: Student-Led Planning: In your notebook, make a drawing of each phase of mitosis, as well as interphase, in a plant cell. Label, in each drawing, the defining features that you will look for when identifying each stage under the microscope.

Step 3: Critical Analysis: Are the predictions you made in Step 1 supported by your observations? Why do you think cells undergoing mitosis in the onion root cell are distributed the way they are? What differences between plant and animal cells are visible under the microscope slide?

Assessments

1. Describe some similarities and some differences between animal and plant mitosis. [SP 1, Concept Explanation]
2. Based on your observations, explain how onion roots grow at the cellular level. [SP 1, Concept Explanation; SP 3, Questions and Methods]

[Answer Key]

1. Similarities include using the same phases of cell cycle. The phases of the cell cycle use similar mechanisms. Differences include the use of a cell plate during cytokinesis of a plant versus the use of a contractile ring in animals, causing the cell furrow between daughter cells. Asters and centrioles (centrosomes) are visible in animal cells not in plants.
2. The root cap pushes through the soil because the zone of elongation just behind the root cap continually provides new cells that push the root cap deeper into the soil. The zone of maturation above the zone of elongation contains cells that provide support and are not active in mitosis.

Activity 3: Pre-Assessment

1. **Answer the following question in your notebook:** Looking at Figure 13.2 and Figure 13.9, compare the outcomes of mitosis versus meiosis.
2. **Answer the following question in your notebook:** After meiosis, some daughter cells may not contain the correct number of chromosomes. What failure in the meiosis process could cause this to occur?
3. **Discuss the answers to questions 1 and 2 with a partner (think, pair, share) and then the class.**

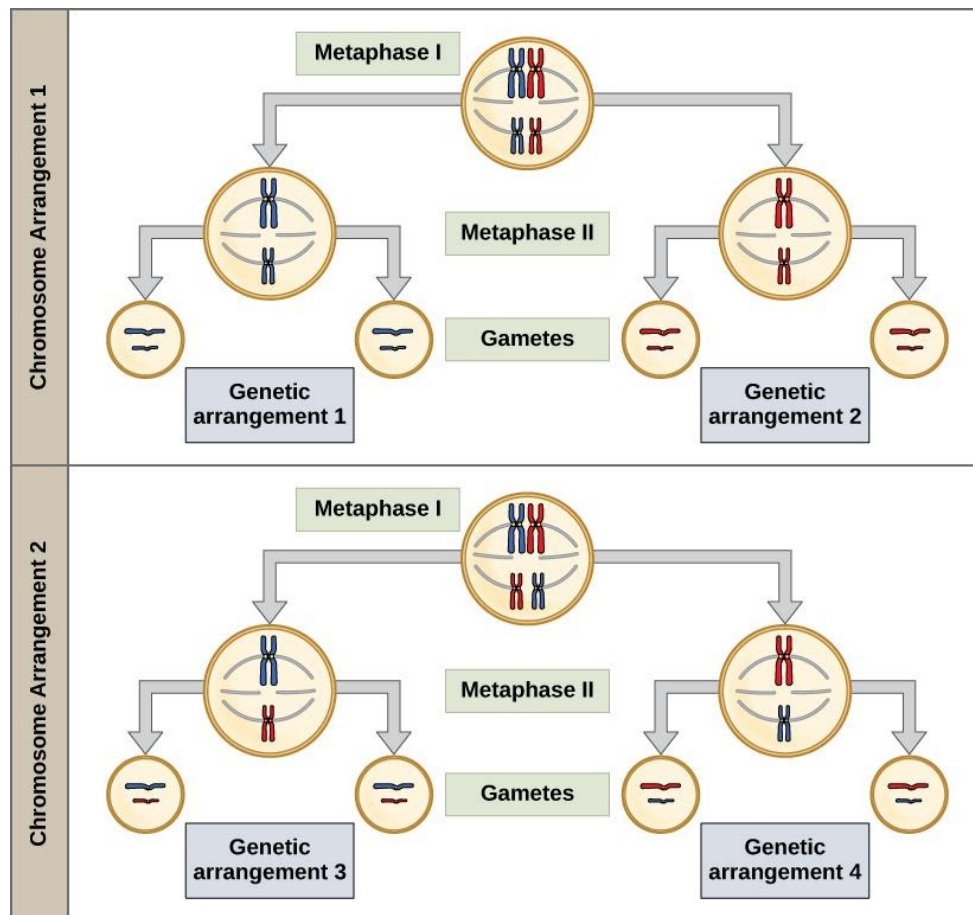


Figure 13.9: Overview of meiosis.

Activity 3: Meiosis and Fertilization

Meiosis is the process in sexually reproducing eukaryotes that forms sex cells or gametes, which include sperm and eggs (ova). To avoid doubling the number of chromosomes in each generation, **reduction division** (halving the number of chromosomes) in gamete production is necessary. Meiosis uses very similar mechanisms to mitosis. There are, however, several significant differences. The source cells for meiosis are found in the reproductive organs of animals (gonads: ovaries and testes) and plants (flowers: ovaries and anthers). Major differences between mitosis and meiosis include the association of **homologous chromosomes** (sister chromatids of the same chromosomes) attached as a **tetrad** group of four, as well as crossover of chromosome regions during prophase I of meiosis I. Crossover provides one source of genetic variation in offspring.

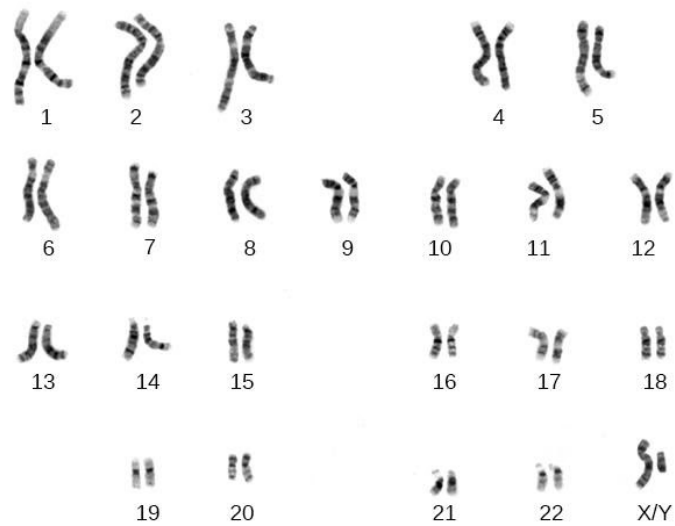
Humans have 23 different chromosomes. Note that we have a **diploid** (2N) set of chromosomes: a full set of 23 chromosomes received from the sperm and another set of 23 from the ovum (egg) during fertilization. See Figure 13.10. During metaphase I of meiosis I, homologous chromosomes are separated. The chromosome number is reduced from diploid (2N) to haploid (1N) by the end of meiosis I, with each cell retaining duplicate sister chromatids. Meiosis II results in four haploid (1N) cells in sperm, each with only one set of each of the 23 chromosomes. In female humans, meiosis results in uneven division of the cytoplasm and the formation of two **polar bodies** (nuclei only) that are not involved in **fertilization**. One note about human fertilization: meiosis II is completed only after the sperm has entered the ovum.

There are several malfunctions that can occur during meiosis. **Nondisjunction** occurs when the sister chromatids in tetrads separate unevenly. Nondisjunction results in one cell getting an extra chromosome while another cell is missing a chromosome. Nondisjunction is associated with certain human genetic disorders. For example, Down syndrome is usually caused by nondisjunction that results in three copies of chromosome 21. **Translocations** can occur when chromosomes exchange genetic information with nonhomologous chromosomes. For a more detailed list, see this web page:

<https://www.genome.gov/11508982/chromosome-abnormalities-fact-sheet/>.

Karyotypes (chromosome spreads) are made by stopping cells in mitosis with a chemical and then dyeing with Giemsa stain. A picture is taken through a microscope and then digitally enlarged to see the chromosomal banding, or G-bands. Dark and light banding patterns help identify chromosomes and alterations to normal chromosomes. The chromosome spreads can be seen in Figure 13.10. Human females have two X chromosomes, and males have one X and one Y.

Human Male



Human Female

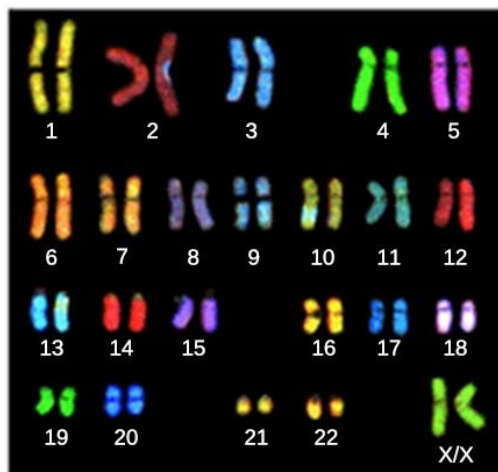


Figure 13.10 Sorted human male and female chromosomes.

Safety Precautions

None

For this activity, you will need the following:

- Images of karyotypes

For this activity, you will work *in pairs*.

Activity 3 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 3:

- Begin the teaching of meiosis with a discussion of the cells associated with sexual reproduction in animals: eggs and sperm. The combination of the egg and sperm during fertilization forms the foundation of genetic material found in the resulting offspring. Ask: Do these cells, the egg and sperm, have the same amount of genetic material as the cells discussed in Activities 2 and 3? Why or why not?
- Introduce terminology associated with meiosis (boldface words in the introduction to Activity 3), and highlight similarities between meiosis and mitosis. Direct the students' attention to Figure 13.9 and indicate that, similar to mitosis, the cell replicates its DNA, and that material is allocated to daughter cells. Further indicate that unlike mitosis, the division process occurs twice in meiosis, such that a) the steps of mitosis are repeated in meiosis; and b) the final cell product contains only half of the total number of chromosomes.
- Introduce the idea that the analysis of genetic material provides insight into an organism's phenotype. One method used to assess whether and where genetic abnormalities exist in human patients is to examine its chromosomal composition or karyotype. Karyotyping can be one of the first steps taken to understand the origin of developmental issues. It may not be immediately clear upon a person's symptoms/presentation what abnormalities exist. Irregularities in the centromere location (the 'pinched-in' site on each chromosome) or banding locations and patterns (also called 'G-bands') can inform doctors about the possible health conditions that exist in patients.

Structured Inquiry

Step 1: Hypothesize/Predict: A karyotype shows the number and appearance of chromosomes in the nucleus of a cell. Predict what you would look for in an abnormal karyotype. Record your predictions in your notebook.

Step 2: Examine the three karyotypes shown in Figure 13.12, Figure 13.13, and Figure 13.14. Compare these karyotypes to the normal karyotypes shown in Figure 13.10. Can you tell if the individual is female, male, or indeterminate (does not have a normal distribution of sex chromosomes)? Record any abnormalities in your notebook, and research the meaning of the changes in chromosome number or appearance.

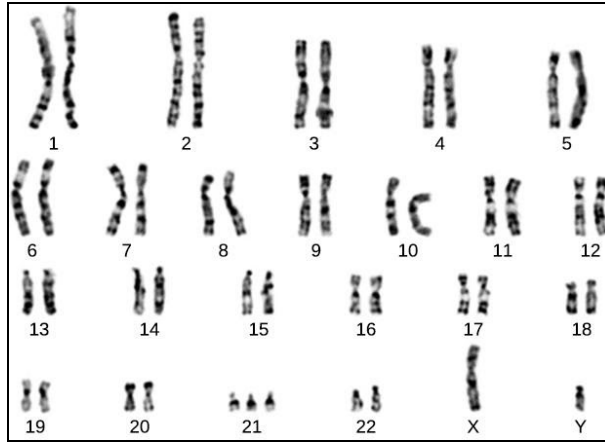


Figure 13.12: Karyotype 1.

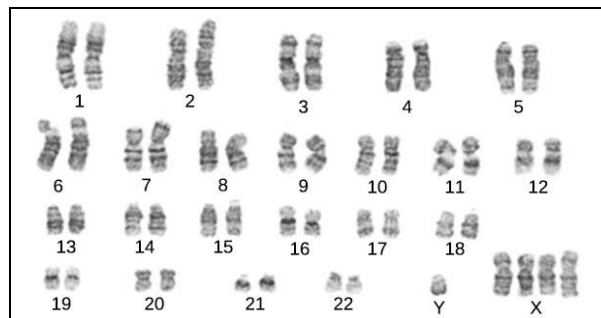


Figure 13.13: Karyotype 2.

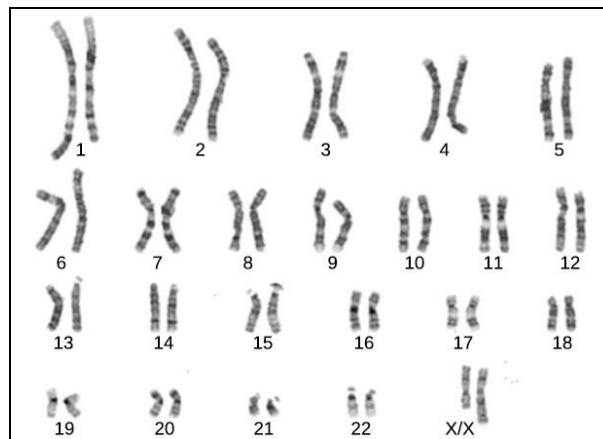
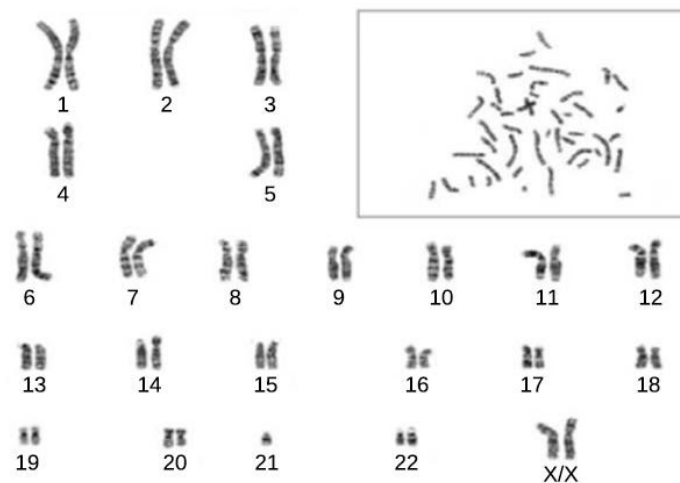


Figure 13.14: Karyotype 3.

Step 3: Critical Analysis: What are the implications of nondisjunction in a karyotype? Using Karyotype 1 (Figure 13.12) as an example, explain the implications of the nondisjunction malfunction during meiosis for the chromosome count of the daughter cells.

Assessments

1. This Robertsonian translocation involves a fusion of the long arms of two non-homologous chromosomes during meiosis. A patient possesses a Robertsonian translocation among chromosome 14 onto chromosome 13. No genetic material is missing from either chromosome. Explain why it is possible that there is no abnormality in the person but could be in the patient's offspring. [AP 1, Concept Explanation; SP 6, Argumentation]



2. Crossing over of chromosomes during prophase I is common. How does this process increase the genetic diversity of the daughter cells? [SP 1, Concept Explanation]

[Answer Key]

1. It is possible that, if the translocation doesn't disrupt a necessary gene or other important genetic region, the person with this karyotype would be phenotypically normal. Any sperm has a chance of receiving either an extra chromosome 14 or a missing chromosome 14. If the sperm was missing the chromosome, the resulting diploid genotype would be incomplete and the offspring would likely be nonviable. However, a sperm containing an extra chromosome 14 would likely be viable, as it is not missing any genetic information.
2. Crossing over allows genetic information from inherited chromosomes to mix. For example, if the person forming gametes has a chromosome from one parent with genes coding for brown hair and no freckles, and a homologous chromosome from the other parent with genes coding for red hair and freckles, crossover could produce a chromosome with genes coding for red hair but no freckles.

Lab 14: Mendelian Genetics

This investigation addresses the following Require Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
5.3 Mendelian Genetics	EVO-2 Organisms are linked by lines of descent from common ancestry. IST-1 Heritable information provides for continuity of life.	EVO-2.A Explain how shared, conserved, fundamental processes and features support common ancestry. IST-1.I Explain the inheritance of genes and traits as described by Mendel's laws.	EVO-2.A.1 DNA and RNA are carriers of genetic information. IST-1.I.1 Mendel's laws of segregation and independent assortment can be applied to genes that are on different chromosomes. IST-1.I.2 Fertilization involves the fusion of two haploid gametes, restoring the diploid number of chromosomes. Rules of probability can be applied to analyze passage of single gene traits, and patterns of inheritance often can be predicted from data, including pedigrees.
5.4 Non-Mendelian Genetics	IST-1 Heritable information provides for continuity of life.	IST-1.J Explain deviations from Mendel's model of the inheritance of traits.	IST-1.J.1 Patterns of inheritance of many traits do not follow ratios predicted by Mendel's laws, e.g., linked traits. IST-1.J.1 Sex-linked traits often can be predicted from pedigree analysis. IST-1.J.3 Many traits are often the product of multiple genes or results from physiological processes. IST-1.J.4 Some traits result from non-nuclear inheritance, such as mitochondrial DNA.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.A Describe biological concepts, and/or processes. 1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D. Represent relationships within biological models, including mathematical models and diagrams.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on observation, data, or a model. 3.B State the null and alternative hypotheses, or predict the results of an experiment. 3.C Identify experimental procedures that are aligned to the question. 3.D Make observations or collect data. 3.E Propose a new/next investigation based on evidence/data from previous experiments.
SP 4 Representing and Describing Data Represent and describe data.	4.A Describe data from a visual representation, including patterns.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including equations in the curriculum, ratios, and percentages. 5.C Perform chi-square hypothesis testing. 5.D Use data to evaluate a hypothesis or prediction.
SP 6 Argumentation	6.A Make a scientific claim. 6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. 6.C Provide reasoning to justify a claim by connecting evidence to biological theories. 6.D Explain the relationship between experimental results and larger concepts, processes, or theories. 6.E Predict the causes of effect of a change to a component(s) in a biological system based on a visual representation or data.

A **genome** is the entire genetic complement of a cell, which is composed of DNA. This DNA contains all the hereditary information of the cell, including the genes that code for proteins. These proteins are responsible for doing the work of the cell and are the building blocks of cell structure. The central dogma of genetics states that information flows from DNA, to RNA to protein. The genes within DNA contain the instructions on how to make proteins. Humans and other diploid organisms have two versions of most genes in every cell. These two versions are called **alleles**. One allele was provided by the organism's mother and the other by its father. The **genotype** consists of the information present in the DNA of the organism. It also denotes the alleles for a specific gene. The protein or trait expressed by the genotype is called its **phenotype**.

Gregor Mendel, a German monk, (shown in Figure 14.1) crossed garden peas to better understand the inheritance of traits. The traits he examined in peas followed what is now called a Mendelian pattern of inheritance. In **Mendelian inheritance**, one allele is dominant, and the other allele is recessive. The dominant allele is always expressed, even if it is paired with a recessive allele in a **heterozygous** genotype (one dominant and one recessive allele). The only way a recessive trait will be expressed in Mendelian inheritance is if a genotype is **homozygous** (both alleles are the same) for the recessive alleles. From his experiments, Mendel determined two principles. The first is the **principle of segregation**, which states that the two alleles separate from each other during the formation of sex cells (gametes). The second law is the **principle of independent assortment**. This principle states that genes for different traits are distributed from a parent to offspring independent of other genes. Therefore, no gene following Mendelian inheritance influences whether another gene will be inherited or not. Most genes and traits in humans and other organisms follow far more complicated inheritance patterns involving different dominance patterns, no dominance, or the linkage of genes that violates the principle of independent assortment. However, studying Mendelian genetics provides an excellent introduction to the basic principles of inheritance.

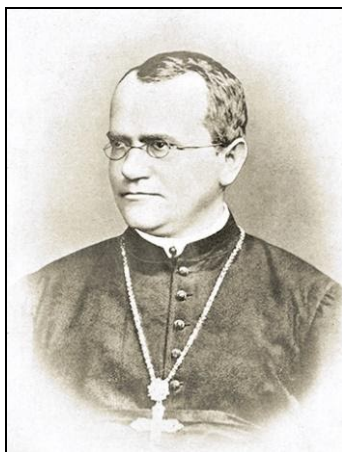


Figure 14.1: Photograph of Gregor Mendel.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Ask students to define, in their own words, the terms defined in the introduction to clarify any confusion.
- Ask students to give examples of the principles of Mendelian inheritance in class.
- Review an example of a polymorphic trait, such as eye color, to emphasize that many traits do not follow Mendelian genetics. Point out that examples of single-gene inheritance in humans are rare. One example is the ability to taste the bitter compound PTC (phenylthiocarbamide). Another example is an ordinary form of sickle-cell anemia.

Students' Misconceptions

- Address the following common misconceptions:
 - The dominant allele is also the most frequent in the population. Give the blood group O as an example or the dominant achondroplasia (dwarfism) in the human population.
 - The dominant allele is always normal or beneficial, and the recessive allele is always harmful. In Huntington's disease, for example, the dominant allele is lethal.
- Stress that the so-called "good" or "bad" genes do not have any moral connotation and refer simply to the ability of the individual to lead a healthy life. With proper care and suitable medication, many harmful genes can be controlled. Give as an example phenylketonuria (PKU), a rare condition in which the amino acid phenylalanine is not metabolized. It is treatable by following a strict diet low in phenylalanine.

In this lab, you will learn

- the basic principles of inheritance, the definition of genotype and phenotype, and the assumptions of Mendelian inheritance
- how to handle, identify, and cross Mendelian traits in the fruit fly *Drosophila melanogaster* and predict offspring genotypes using a Punnett square
- how to statistically test the probability that the difference between an observed and an expected result is due to chance

Activity 1: Pre-Assessment

1. **Answer the following questions in your notebook:** How would you know if a trait follows a Mendelian inheritance pattern, assuming you know and can track the genotypes and phenotypes of an organism as it produces offspring?

2. **Answer the following questions in your notebook:** What is a Punnett square, and what does it show? Using a Punnett square, predict the offspring of a cross between two heterozygous parents for gene A ($Aa \times Aa$).
3. **Discuss the answers to questions 1–3 with the class.**

Activity 1: Single Trait Inheritance

Based on the principles set forth by Mendel, we can predict what genotypes and phenotypes offspring will have based on the genotypes and phenotypes of their parents. One efficient way to do this involves using a Punnett square. A Punnett square is a grid where all the alleles of one parent are provided as the column headers while all of the alleles from the second parent are provided as the row headers (Figure 14.2). When the alleles from the two parents are combined in the grid, the internal squares predict the genotypes of their offspring. In addition, multiple Punnett squares can predict offspring genotypes across several generations. The **first filial generation (F_1)** is the offspring that results from crossing the original, **parental generation**. The **second filial generation (F_2)** is the offspring that results from crossing F_1 individuals.

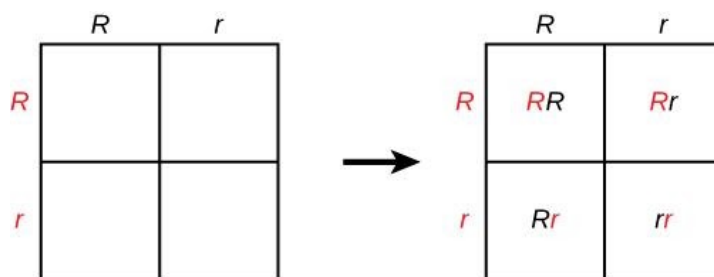


Figure 14.2: A Punnett square is used as a visual representation of crossed traits and the results of the crosses. Capital R represents the dominant trait, and lowercase r represents the recessive trait. The first square is a cross between the two dominant traits R and R. RR is the result.

Safety Precautions

- Do not let the flies fly away.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Fruit flies
- Glass vials
- Fly food

- Paint brushes for manipulating flies
- Foam plugs
- Stereomicroscope
- Fly anaesthetizing substance

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- The fly anaesthetizing substance could be a commercial anaesthetizing substance, carbon dioxide or simply placing flies in a freezer for a short period of time
- Inspect a few flies before class to make sure that they carry the phenotypes that they are supposed to carry and that there was no confusion in the order.
- Have students sort a large number of flies in a limited amount of time. Most students have no experience identifying phenotypes or assigning sex to flies. Knowing in advance what they are supposed to see will help them work faster.
 - Select and store a few specimens that will be preserved for prelab display. Store them in the freezer.
 - Set up a demonstration stereomicroscope showing at least one male and one female for comparison as well as samples of the phenotypes if possible.
 - Ask students to practice focusing stereomicroscopes before proceeding with flies, because they will depend on rapid and accurate visualization of traits and sexes.
 - Have color photographs of a male and a female fly ready as well as the phenotypes that will be scored. Although looking at actual specimens is better for the students, showing them pictures will help them recognize the phenotypes that they follow through the crosses. Many photographs are available online.
- Show a picture of the life cycle of a fruit fly to explain which stages the students will be using in their experiments. The life cycle of a fruit fly from egg to adult is about 11 days at 72 °F (22 °C). Many diagrams of the fruit fly life cycle are available online. (See http://flymove.uni-muenster.de/Genetics/Flies/LifeCycle/LifeCyclePict/life_cycle.jpg)
- Review the entire procedure for immobilizing flies with an anesthetic, sorting with a paintbrush, and transferring the flies to a vial before class. Assume that students will take longer to sort flies, and adjust your timing accordingly.
- Determine the sex of the fruit fly. The accuracy of the entire experiment depends on the ability to determine the sex of the flies and distinguish virgin females.
- Tell students to gently turn flies on their backs with a brush. They should notice that males tend to be smaller and have a darker and more rounded tip of the abdomen. A second trait of males is the presence of the tarsal sex combs on the first pair of legs, which most students will find difficult to identify. Consider having a practice exercise in which students are asked to reliably identify the sex of a few flies, and check their work.

- Ask students to collect virgin females by one of the following methods:
 - The visual method, which students can easily follow without coming to class at odd hours. Virgin females are larger and lighter in color than their older counterparts that have been fertilized. Virgin males are also light in color but have distinctive genital traits.
 - The removal method, which requires waiting until mature pupae are present in the culture in the vial. At that point, dispose of all adult flies by tapping them into a clean, dry vial with a foam plug and discard them in the morgue. After all adults have been removed from the vial, wait about 10 hours and collect the females, which are in all likelihood virgins.
- Summarize the manipulation necessary to cross flies for the experiments:
 - Remind students to work quickly.
 - Prepare the vials with media and label with the date and parental genotypes. Tell students to mark the vials with their initials.
 - Select about four to six virgin females with the first genotype tested and the same number of males of the second genotype.
 - When transferring flies, advise students to position the vial on its side and to delicately place the flies along the plastic side of the tube, not directly on the medium at the bottom where they could get stuck. Close the vial with the foam plug. Let the vial remain on its side until the flies start to move around again.
- Tell students that if a fly wakes up and flies away, it means that the whole sample was not anaesthetized long enough, and all the flies will wake up soon. They must apply more anesthetic to the whole sample and ignore the one fly that got away rather than chase the lone escaped fly and lose the whole sample.
- Have ready a fly morgue. A simple homemade disposal bottle can be prepared by filling bottles with a mixture of water, ethanol, apple cider vinegar, and a drop of dish detergent to break the surface tension. See <https://blogs.brandeis.edu/flyonthewall/fly-life-a-day-in-the-life-of-a-fly-scientist/>.

Tips

- Remind students that they must work quickly, because flies will wake up and fly away. Several methods of anesthetizing flies are possible. Ether is not advisable for use in a classroom because it is highly flammable, volatile, and toxic to flies if applied too long. Commercial anesthetic compounds are available. The use of CO₂ is convenient but expensive because CO₂ pads must be purchased. Leaving flies for 8 to 12 minutes in a freezer should immobilize them long enough for scoring.
- Plan to check the cultures every few days for possible fungal infections, which would invalidate the results.

The following are recommendations for the Guided Inquiry for Activity 1:

- Emphasize that students should use Punnett squares and not try to guess genotypes and phenotypes.
- Review backcrosses if necessary.

Structured Inquiry

Step 1: Hypothesize/Predict: In this activity, you will perform crosses using the fruit fly *Drosophila melanogaster*. Your teacher will indicate the phenotypes that you will monitor in your crosses and will show you photographs of the traits (e.g., eye color vermilion/sepia, body color gray/ebony, normal wings/curly wings). You will begin by crossing a wild-type parent with a recessive parent. Knowing the dominant and recessive traits of the parent, predict the genotype and phenotype of the first-generation offspring using Punnett squares (see Figure 14.2). Draw the two Punnett squares in your notebook. Add your predictions to the first data table. You will fill the second one with your actual data later.

Step 2: Student-Led Planning: Listen carefully to your teacher's instructions on how to handle, anesthetize, determine the sex of, and count your flies. Practice, with your partner, how best to use the stereomicroscope. After you understand the procedure, draw a table in your notebook to record your data (sex and phenotype of parent, first generation, and second generation.)



Figure 14.3: A typical vial of *Drosophila melanogaster*. The food medium is at the bottom of the vial, with fly larvae (maggots) on its surface. The fruit fly pupae can be seen adhered to the inner wall of the vial.

Step 3: Cross the wild-type parent and recessive parents as described by your teacher in Step 1. Then incubate your vials. After the flies have laid eggs, anaesthetize and re-collect the parent generation. Remove all the adult flies.

Step 4: After seven days, anesthetize the adult flies from the vials prepared in Step 3. Separate the male and female flies and count the phenotypes present, for each sex. Record your counts in your notebook.

Step 5: Repeat Steps 3 and 4 for the F_2 generation. Record your counts in your notebook.

Step 6: Critical Analysis: Are the predictions you made in Step 1 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your partner and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Predict the original cross and the phenotypic frequencies of an already-crossed group of flies. The phenotypic frequency can be found by dividing the number of flies of a particular trait by the total number of flies. Using flies you already have available, examine the flies and record the sex and traits. Prepare data tables (to record sex and dominant and recessive traits) and Punnett squares.

Step 2: Student-Led Planning: Plan how you will cross your flies. After your teacher has approved, make your crosses for at least two generations. Record your data in your lab notebook.

Step 3: Critical Analysis: Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your partner and then write your answers in your notebook.

Assessments

1. You are analyzing the inheritance of a new gene and find that it does not have a clear dominant and recessive allele. Instead, both alleles seem to be expressed when both are present. Does this gene follow a Mendelian inheritance pattern? Why or why not? [SP 1, Concept Explanation]
2. Answer the following about the assumptions of Mendelian inheritance: [SP 1, Concept Explanation; SP 3, Questions and Methods]
 - a. Do most traits in humans follow a Mendelian inheritance pattern? Why or why not?
 - b. How could you determine that a trait does or does not follow the assumptions of Mendelian inheritance?

[Answer Key]

1. This does not follow a Mendelian inheritance pattern, because Mendelian inheritance assumes that there is only one dominant allele and that, when present, the dominant allele prevents the expression of the second allele (the recessive allele).

2.
 - a. Most traits in humans do *not* follow Mendelian inheritance, because many follow different patterns of dominance or could involve linked genes that violate the principle of independent assortment.
 - b. Answers will vary. Sample answer: One would know that a trait does not follow the assumptions of Mendelian inheritance by performing crosses. If the ratio of observed offspring genotypes differs from that expected from Mendelian inheritance, then that provides evidence that the trait does not follow the assumptions of Mendelian inheritance. Typically, the ratios are compared statistically, such as using a Chi-squared test, to determine the probability that they are different.

Activity 2: Pre-Assessment

1. **Answer the following question in your notebook:** What does the chi-square test tell you?
2. **Answer the following question in your notebook:** Explain the difference between expected and observed data.
3. **Answer the following question in your notebook:** What is a null hypothesis?
4. **Discuss the answers to questions 1, 2, and 3 with the class.**

Activity 2: Test for Independence (Chi-Square Test)

The **chi-square** test is an independence test for the likelihood that an observed distribution is due to chance. Chi-square is calculated using the following equation

$$X_c^2 = \sum \frac{(O_i - E_i)^2}{E_i},$$

where X is the chi-square test statistic, c is the significant level of the test (we will use 0.05), O is the observed value for variable *i*, and E is the expected value for variable *i*.

To interpret the data, use a standard chi-square table, as provided by your teacher. On the left side of the table is the **degree of freedom** (*df*) which is calculated by subtracting 1 from the number of categories in the data. Across the top is the **probability** (*p*-value) or the probability that the observed value matches the expected value. It is used to determine whether the **null hypothesis** should be accepted or rejected. The null hypothesis states that there is no significant difference between the groups being measured.

Safety Precautions

- None

For this activity, you will need the following:

- Standard chi-square test table
- Calculator

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 2:

- Review with students the rules of statistics applied to genetics. The probability of two independent events is equal to the product of the probabilities of each. The probability of events that exclude each other is equal to the sum of probabilities.
- Ask students to provide examples, and have some of your own ready.
- Ask students if the number of flies of a certain phenotype after each cross should be identical or close to the ones they predicted.
- Ask students how they would decide that the results do not support their hypotheses.
- Ask students to define null hypothesis and interval of confidence. Draw on the board a diagram of the bell curve showing the distribution of results and outliers.
- Ask students what could be the possible reasons for divergence between predicted and measured outcomes. Let the students discuss the answers among themselves, and write reasons on the board.

The following are recommendations for the Guided Inquiry for Activity 2:

- Have students practice the chi-square test on two sets of data, one from a dihybrid cross for independently segregated alleles and one for linked alleles. The null hypothesis in each case is that the traits follow a Mendelian inheritance pattern. This exercise should build confidence in students that they can recognize whether to accept or reject a null hypothesis.
- Emphasize the need for working with many flies. At least 100+ flies should be scored for each cross.

Structured Inquiry

Step 1: Use chi-square analysis to determine if the data from the Activity 1 Structured Inquiry is independently assorted. Watch the video (<https://www.youtube.com/watch?v=kckn5CMHNsE>) to learn how to perform a chi-square test. Create a data table for your analysis, and show your calculations in your notebook.

Step 2: Hypothesize/Predict: Predict whether you will accept or reject your null hypothesis (i.e., if they are independently assorted). What is your alternative hypothesis? Add your predictions to the data table you created in Step 1.

Step 3: Student-Led Planning: Using the data you collected in the previous activity, perform a chi-square test. Work with your partner.

Step 4: Critical Analysis: Based on your chi-square test, will you accept or reject your hypothesis? Why or why not? What methods could you use to improve your results? Discuss with your partner, and then write your answers in your notebook.

Guided Inquiry

Step 1: Now, use chi-square analysis to determine if the data from the Activity 1 Guided Inquiry is independently assorted. Create a data table for your analysis, and show your calculations in your notebook.

Step 2: Hypothesize/Predict: Predict whether you will accept or reject your null hypothesis (i.e., are your observed results significantly different from your expected results?). What is your alternative hypothesis? Add your hypotheses to the data table you created in Step 1.

Step 3: Student-Led Planning: Using the data you collected in the previous activity, perform a chi-square test. Work with your partner. Your expected data would be the number of flies that should possess the different phenotypes based on the ratios predicted by Mendelian inheritance for each cross.

Step 4: Critical Analysis: Based on your chi-square test, will you accept or reject your hypothesis? Why or why not? What does this tell you about the validity of your data? Discuss with your partner, and then write your answers in your notebook.

Assessments

1. What does the chi-square test tell you about a set of observed versus expected results? [SP 3, Statistical Tests and Data Analysis]
2. Describe another situation, outside the realm of science, where a chi-square test would be useful. [SP 3, Statistical Tests and Data Analysis]
3. What would change the degree of freedom in a chi-square test? [SP 3, Statistical Tests and Data Analysis]

[Answer Key]

1. The chi-square test is a statistical test used to determine the probability that the difference between an observed and expected result is real or due to chance.
2. Answers will vary. One example is in business, where an accountant could examine if the difference between observed and expected profits are significant or not.

3. The degree of freedom is determined by the number of data categories. Therefore, adding a category to an experiment would increase the degree of freedom by one.

Lab 15: DNA Restriction Analysis

This investigation addresses the following Required Course Content and Science Practices from the AP CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
Topic 6.1 DNA and RNA Structure	IST-1 Heritable information provides for continuity of life.	IST-1.K Describe the structures involved in passing hereditary information from one generation to the next. IST-1.L Describe the characteristics of DNA that allow it to be used as the hereditary materials.	IST-1.K.1 DNA, and in some cases RNA, is the primary source of heritable information. Prokaryotes and eukaryotes can contain plasmids. IST-1.L.1 DNA exhibits specific nucleotide base pairing: A pairs with U, and C pairs with G.
Topic 6.8 Biotechnology	IST-1 Heritable information provides for continuity of life.	IST-1.P Explain the use of genetic engineering techniques in analyzing or manipulating DNA.	IST-1.P.1 Genetic engineering techniques, including electrophoresis, can be used to analyze and manipulate DNA and RNA.

SCIENCE PRACTICES

Science Practices	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.A Describe biological concepts and/or processes. 1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on an observation, data, or model. 3.C Identify experimental procedures that are aligned to the question, including variables and controls. 3.D Make observations, or collect data from representations or laboratory setups or results. 3.E Propose a new/next investigation based on evidence/evaluation from a previous experiment.
SP 4 Representing and Describing Data Represent and Describe Data	4.B Describe data from a table, including trends and/or patterns and relationships between variables.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculating, including equations in the curriculum.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.A Make a scientific claim. 6.A Support a claim with evidence. 6.B Provide reasoning to justify a claim.

Bacteria and archaea are unicellular organisms and do not possess organized immune systems like multicellular organisms. However, they do possess **restriction endonucleases**, which are enzymes that defend against viral invasion by cutting through viral DNA. By cutting genes, these DNA *scissor enzymes* destroy viral function inside the bacteria. The bacteria protect against auto-digestion of their own genetic information by adding methyl ($-CH_3$) groups to their DNA sequences that would otherwise be targeted by endonucleases. These sequences of nucleotides are called **restriction sites**. Scientists have developed ways to use restriction endonucleases to select certain strands of DNA and cut them away. This is incredibly useful for isolating a specific gene on a DNA strand that contains thousands of genes!

Figure 15.1 shows an example of a restriction endonuclease called EcoRI. EcoRI recognizes a specific palindrome on the DNA strand. A **palindrome** is a section that reads the same way frontward and backward. For example, the restriction enzyme, EcoRI, recognizes the sequence GAATTC on both sides of the double helix. Each type of restriction enzyme recognizes a different set of palindromes. The restriction enzymes cut through the sugar-phosphate background, leaving **sticky ends**, where complementary DNA strands can be attached (a process known as annealing). This allows different DNA strands to be joined together, so DNA can be recombined.

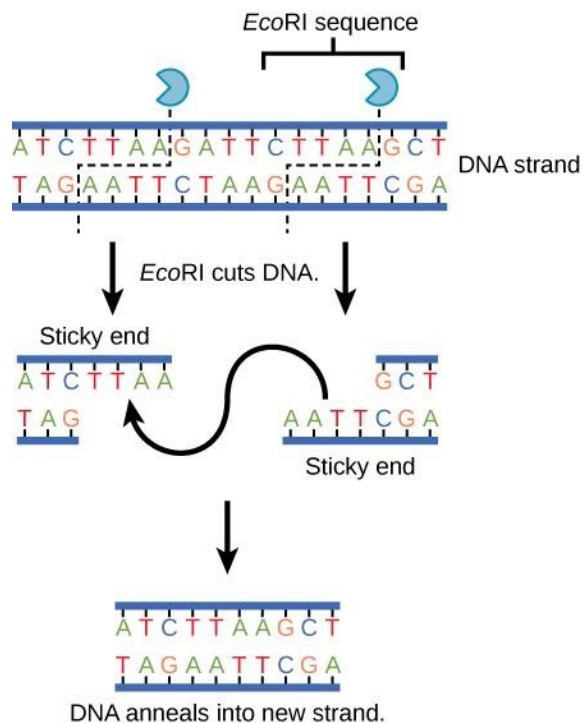


Figure 15.1: Restriction enzymes cut the sugar-phosphate backbone of DNA at specific restriction sites. The cut DNA can then be recombined with a different DNA strand at the restriction sites.

Restriction enzymes are often used in research and forensics. Restriction fragment length polymorphism (RFLP) was the original procedure used in DNA fingerprinting. Crime scene DNA was isolated and *restricted* into fragments using restriction enzymes. The fragments were then separated into bands using **electrophoresis**, a method where DNA fragments are separated out by weight using an electric field. The separated bands of DNA were then compared to determine if different samples of DNA showed the same banding pattern. If the banding patterns were similar, it suggested that the DNA came from the same person. Therefore, RFLP can be used to match the DNA found at crime scenes with those of victims or suspects. Another use of restriction enzymes is in **recombinant DNA technology**, where DNA from one organism can be removed and inserted into the DNA of another organism.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Note that New England Biolabs (<https://www.neb.com/>) sometimes provides free restriction enzymes to educators. Ask for the education outreach department
- Explain that bacteria use restriction enzymes to cut up viral DNA. Show a diagram of a restriction site. Explain that a restriction enzyme nicks only one strand of the DNA, but because the sequence is palindromic, two nicks are made. Show the sticky ends that are produced after two cuts are made.
- Show the structures of cytosine and methylated cytosine. Explain that bacteria methylate their own DNA so it is not cut apart by restriction enzymes.
- Show a diagram of the use of restriction enzymes in recombinant DNA technology. Explain that two different fragments cut by the same restriction enzyme can be annealed together because they have complementary sticky ends.
- Show a diagram that explains how restriction fragment length polymorphism (RFLP), or DNA fingerprinting, is used in forensic analysis. Explain that restriction enzymes cut DNA into small fragments, but the pattern of cutting varies from person to person. RFLP analysis can be used to determine who was present at a crime scene, and to assess genetic relationships.
- Explain that electrophoresis is a technique that can be used to separate fragments of DNA. In electrophoresis DNA is loaded into a gel and an electric field is applied. The DNA, which has a negative charge, moves through the field, but small pieces move through the gel faster than large pieces. Thus, electrophoresis separates DNA on the basis of size.

In this lab, you will learn

- how to predict where restriction enzymes will digest a sample of DNA into fragments
- how to digest samples of DNA with restriction enzymes
- how to separate DNA using electrophoresis

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** How could we measure the size of an unknown fragment of DNA?
2. **Answer the following question in your notebook.** What would be suitable controls for an experiment involving restriction enzymes? (Hint—Think about how you would know the sizes of your fragments also.)
3. **Discuss the answers to questions 1 and 2 with a partner and then share with the class.**

Activity 1: Digestion of DNA

In this activity, you will determine the size of pre-digested DNA (pre-cut with restriction enzymes). As mentioned above, DNA can be separated out by size using gel electrophoresis. In gel electrophoresis, DNA fragments are inserted into a gel medium called **agarose**. The DNA will be loaded onto a gel using **loading dye**, which helps visualize the movement of the charged molecules through the agarose gel. The loading dye also contains a viscous sugar solution to ensure that the DNA sample sinks into the small depressions in the gel, called **wells**. The loading dyes do not actually stain the DNA during electrophoresis. To judge the size of a DNA fragment, **molecular size markers**, or just *markers*, are inserted into the gel next to the DNA fragments. The marker contains DNA fragments of several known sizes. They are used to estimate the length of an unknown DNA fragment. Visualizing DNA in the gel is problematic since it is transparent to the eye. Therefore, after the DNA has run through the gel, you will use a **DNA stain** to help visual the DNA.

Safety Precautions

- Wash your hands before and after these activities.
- Never eat or drink in the lab. This lab involves the use of poisonous buffers, DNA stains, and other chemicals
- Wearing aprons, gloves, and goggles are highly recommended. The DNA stain can stain hands and clothing, and can harm the skin.
- Use caution with hot agarose.
- If any solutions get into your eyes, flush with water.

- Use caution with electrical connections:
 - Set gel in a box and load samples with the electrodes disconnected.
 - Turn off the power supply before connecting or disconnecting the gel box.
 - Turn off power supply and unplug all leads before handling the gel box and removing the gel.
- Dispose of used materials as indicated by your instructor.

For this activity, you will need the following:

- Pre-digested DNA samples and control sample
- Two 20 μ L micropipette with μ L pipette tips
- Waste container for used pipette tips
- Practice gels with practice loading dye (50 percent glycerol with methylene blue)
- 1 percent agarose in TAE buffer gel or premade agarose gel
- Masking tape (1-inch wide) (Not needed if gel trays have adjustable walls)
- Loading dye
- 1X TAE buffer
- Micro-centrifuge (optional)
- Electrophoresis gel box and power supply
- Staining trays
- DNA stain
- Lambda DNA
- Various restriction enzymes (availability determined by instructor)

For this activity, you will work *in pairs or groups*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Inform students that the DNA stain is poisonous and must only be handled with gloves.
- Model use of the micropipette and explain how to read it. Show students where the stops are for filling and ejecting, and explain that liquid should be drawn in slowly to avoid bubbles. If students have not used micropipettes before, give them plenty of time to practice with water.
- Show how to inject dye into a practice well. Explain that the dye must be ejected slowly or it will shoot out of the well. Give students plenty of opportunity to practice.

- Model how to prepare and pour the gel, and how to set up the electrophoresis chamber. Tell students to watch the direction of movement of dye after they turn on the power. If the dye moves in the wrong direction, they will need to cut the power and reverse the leads. Tell students not to touch the apparatus when a current is running through it.
- Experiment using a microfuge and explain how to use it, and emphasize that the microfuge must be balanced. A loud noise indicates that it is not balanced and should be shut off immediately.
- Explain that DNA size is measured in base pairs. A base pair is a nucleotide and its complementary partner on the other DNA strand.
- Explain that the molecular size markers, which are sometimes referred to as a ladder as they have the appearance of rungs of a ladder, are DNA fragments of known size. A plot of the size of each fragment in base pairs versus distance traveled in the gel yields a standard curve that can be used to determine the size of an unknown fragment. However, a DNA fragment travels at a rate that is inversely proportional to the logarithm of its size, and for this reason a linear plot will produce a curved line that is hard to read. Instead, students will plot their data on semi-log paper, which has a logarithmic scale on one axis and a linear scale on the other axis. On a logarithmic scale, each line represents a 10-fold increase in value. A plot of DNA size on the semi-log scale (the y-axis) versus migration distance (the x-axis), yields a straight line, which is much easier to read. Provide students with a sample plot so they know what the data should look like.
- Show students a lambda phage restriction map, such as the one shown here: https://www.neb.com/~media/NebUs/Page%20Images/Tools%20and%20Resources/Interactive%20Tools/DNA%20Sequences%20and%20Maps/Lambda_map.pdf. Explain that lambda phage DNA is 48,502 base pairs long and the number next to each restriction site is the distance from base pair one. Explain that some restriction enzymes only cut the DNA once; the site that is cut is called a unique site. Show students how to calculate the fragment sizes that will result when DNA is cut at one or more sites.

Structured Inquiry

Creating the Agarose Gel

Step 1: Pay close attention to your teacher, who will demonstrate how to use the micropipette using the process below:

1. Determine the volume required by twisting the thumbwheel dial to the required volume. Note that numbers are read from top to bottom. There may be a red line indicating a decimal point in the display. For example, 015 with a red line between the 1 and 5 would read 1.5 μL .
2. Attach (seat) a clean pipette tip by gently pushing the micropipette into the top of the tip. Be gentle removing the tip to avoid spilling the whole box.

3. Press the push button (plunger) on top to the first (soft) stop. Insert the disposable pipette tip (only the tip) into the liquid to be transferred. Slowly release the push button to suck up the liquid. Note—If you have pressed the push button too hard and gone to the harder *blow-out* setting you will pull up more than the desired volume.
4. Insert the pipette tip into the microfuge tube or other area where you need to move the material.
5. Press the push button past the first stop to the second (hard) stop to transfer (blow out) the liquid. Do not release the push button until you have removed the tip from the tube.
6. Eject the disposable tip into the appropriate waste container. Do not reuse tips, unless instructed, to avoid cross contamination.

Step 2: Your teacher has prepared 1 percent agarose gel solution that he or she will pour into your gel tray. Prepare your gel tray. Some trays have casting gates that can be locked before pouring the molten agarose. Other trays have open sides that must be taped with masking tape (Figure 15.2). Make sure the masking tape is tightly applied to the open edges of the gel tray. Place the comb across the tray as demonstrated by your instructor.

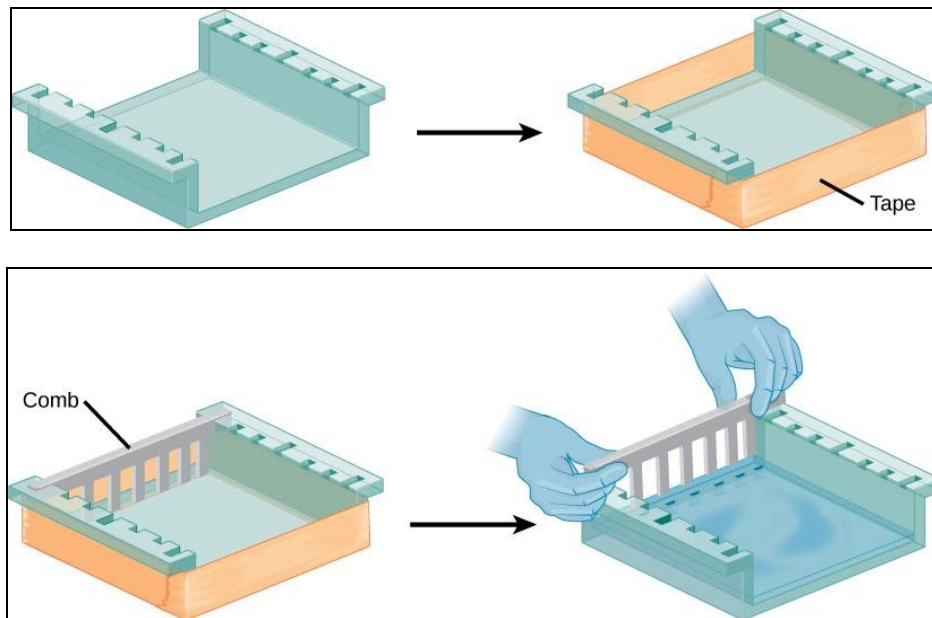


Figure 15.2: If your gel trays need to be taped, apply the tape straight to form a wall that will hold the gel. Be sure to insert the comb straight into the gel. All the comb teeth should penetrate an equal distance into the gel. Once the gel is fully set, remove the comb slowly by pulling straight up on both ends of the comb.

Step 3: Your teacher will now pour the warm 1 percent agarose into your tray. If there are bubbles, use a clean micropipette tip to move the bubbles away from the comb. Wait until the gel has become opaque to ensure the agarose has solidified and is ready for use. This usually takes about 20 minutes at a room temperature of 21 °C. Once the gel is fully set, remove the comb slowly by pulling straight up on both ends of the comb (see Figure 15.2).

Loading, Running and Staining the Agarose Gel:

Step 1: Your instructor will supply you with a practice gel in a Petri dish. Fill the dish with tap water.

Step 2: Obtain a microfuge tube of practice loading dye. Draw up 5–10 μL of dye. Hover the tip over the well, just touching the surface of the liquid. The tip should make a slight dimple in the water surface. Slowly depress the plunger of the micropipette, releasing the loading dye into the well. The dye should fill the well. Pull the tip out of the liquid before releasing the push button.

Step 3: (Note—amounts and procedures might differ based on DNA supplies.) Obtain the pre-digested DNA samples and controls in microfuge tubes from your instructor. Add 2 μL of loading dye to your samples. Make sure you use a new pipette tip for each. Cap each tube and pulse spin in the micro-centrifuge (alternately spin and then stop). To avoid damage and injury, let the rotor come to a complete stop. Do not stop the rotor with your fingers. When you remove your sample from the centrifuge, tap it on the table 3 times to help the heavier constituents settle to the bottom. Alternately you can tap the microfuge tube with your finger, as shown in Figure 15.3 to coax the DNA and loading dye together into the bottom of the microfuge tube.

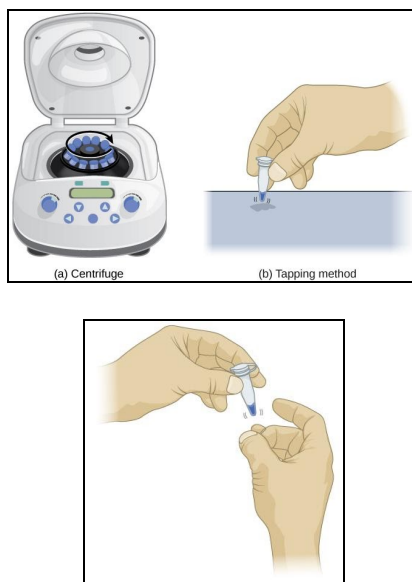


Figure 15.3: Centrifuge and tapping method to move small amounts to the bottom of microfuge tubes. If no centrifuge is available, flick the bottom of the microfuge tube with your finger at least 20 times to thoroughly mix its contents.

Step 4: Student-led planning. Place your gel in the gel box as shown by your teacher (see Figure 15.6). The wells should be oriented at the black terminal end (negative side). DNA is negatively charged. Based on this, which way will the DNA travel across the gel? Create a diagram in your notebook showing the expected direction of travel.

Step 5: Add enough 1X TAE buffer to top your gel by 2 mm. Depending on the size of your gel box, this may be approximately 275 mL.

Step 6: Student-led planning. Load your gel, as practiced in Step 2, with the pre-digested DNA samples you prepared in Step 3, and control DNA, in the order indicated by your instructor. The samples contain the loading dye that you practiced with in Step 2. Generally, the standard, which you will use to determine the size of your DNA samples, are placed into the left-most well. The other samples are loaded to the right of the standard, as shown in Figure 15.4. How can you ensure that the DNA will not run off the gel? Write your answer in your notebook.

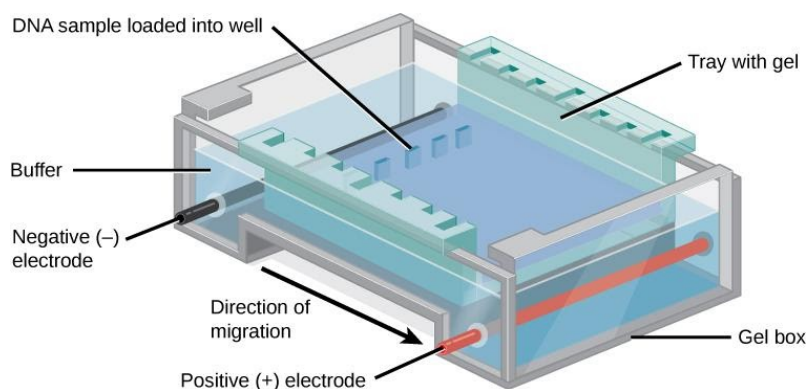


Figure 15.4: Place your sample tray so that the wells are located closest to the negative electrode of the gel box.

Step 7: Place the top on the gel box with the red terminal aligned with the red side and the black terminal aligned with the black side (Figure 15.7). Plug the cords into the power supply. Again, double check: red with red, black with black. If the terminals are misaligned, your gel will run backwards and off the gel, yielding no results.

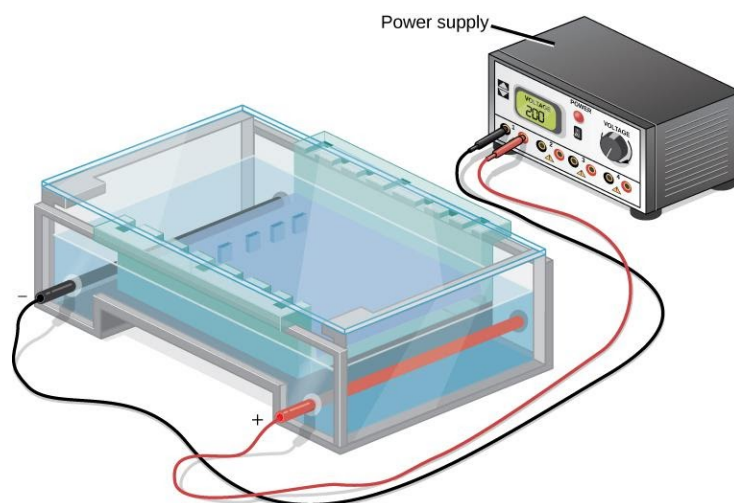


Figure 15.5: Be sure that the red and black wires are plugged into the corresponding colors on both the gel box top and the power supply (red to red and black to black).

Step 8: Turn on the power supply and *run* your loaded gel for the time described by your teacher. Generally, gels run for 20 minutes at 200 V using 0.25X TAE or 30 minutes at 100V using 1X TAE buffer.

Step 9: After the proper amount of time, the bands of loading dye on your gels should be approximately 2–3 cm from the opposite edge of the gel. Remove the top of the gel box. Then remove the gel tray, draining extra liquid back into the box. Carefully slide the gel off the tray into a staining tray. Your teacher will stain your gel, which allows you to visualize the bands of DNA fragments, and instruct you on when you can observe the results. Clean up your lab table.

Step 10. Once your gel is ready, your teacher will place it on a light table for better viewing. Place a millimeter ruler next to the gel and take a picture of the gel using your phone camera. Be sure to take a picture from directly above the gel, as you will use your photo to take measurements in the next step.

Step 11: Using the photograph of the gel and ruler you took in the previous step, measure (in millimeters) the distance migrated by all bands in each lane from the front of the well to the forward edge of each band, as shown in Figure 15.6 and Figure 15.7. Create a data table for your data in your notebook.

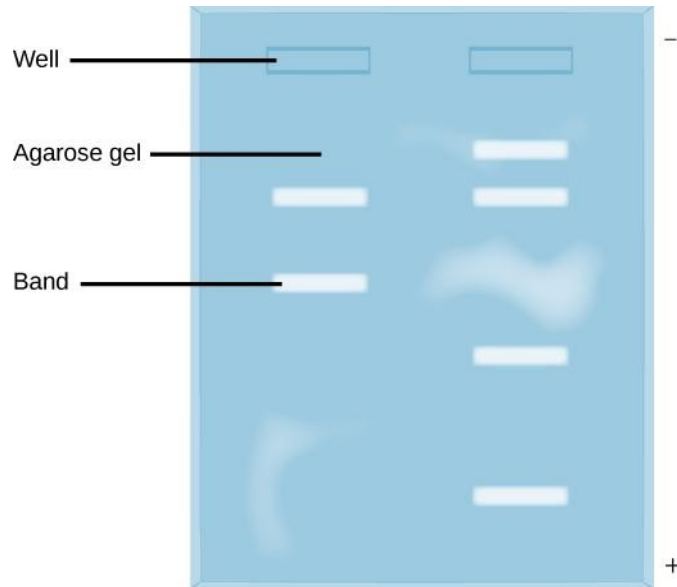


Figure 15.6: Diagram of a gel that has been run.



Figure 15.7: Example of a DNA Gel with Ruler. The standard lane is on the far left. This lane is used to determine the size of the DNA fragment, in base pairs.

Step 12: Using the standard in the leftmost row of samples, estimate the size, in base pairs, of the DNA bands in each well. Add your data to the table you created in Step 11.

Step 13: Your teacher will provide you with semilog graph paper with DNA size, in base pairs, on the y-axis and distance travelled, in mm, on the x-axis. Plot the data from the table you created in the previous two steps on the semilog graph paper.

Step 14: Critical Analysis: Was there a control in this activity? If so, what does the control reveal about how well the gel worked? What evidence does the gel provide to support the action of restriction enzymes? How did the fragments sort in the gel, size-wise? Why did they sort this way? Discuss with your partner and then write your answers in your notebook.

Guided Inquiry

Step 1: Figure 15.8 shows the structure of the bacteriophage Lambda, a virus that invades the bacteria *Escherichia coli*. The genome of Lambda is 48,502 base pairs long. **Figure 15.25** shows the genes located on Lambda that provide the information on making new viruses.

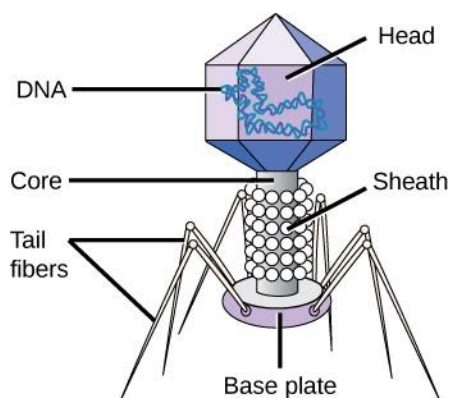


Figure 15.8: The Lambda phage is a virus that invades, and reproduces in, the cells of bacteria.

Step 2: Hypothesize/Predict: Examine this link to find the complete list of restriction sites for the Lambda bacteriophage:
https://www.neb.com/~media/NebUs/Page%20Images/Tools%20and%20Resources/Interactive%20Tools/DNA%20Sequences%20and%20Maps/Lambda_sites.pdf. In addition, your instructor will provide a list of available restriction enzymes. Figure 15.9 shows a partial restriction map for Lambda. Based on the introduction to this activity, your pre-assessment questions and the restriction sites for the Lambda bacteriophage, predict where the restriction enzymes available in your lab will cut the Lambda DNA. How large will each fragment likely be, in base pairs? Draw and record the predicted DNA fragment sizes in your notebook.

Step 3: Student-led planning: Test the hypothesis you made in Step 2 by setting up a restriction digest as you did in the Structured Inquiry. Your instructor will guide you in how to set up the solutions that you will analyze in a second agarose gel. Example amounts may include, 4 μL of Lambda DNA, 6 μL of buffer, and 1 μL of specific restriction enzyme in a microfuge tube. Don't forget to provide controls for your experiment. Also, be sure to spin or tap down liquids to the bottom of the micro tube.

Step 4: Incubate your samples at 37 °C for 30 minutes in a water bath. Then, analyze your samples using gel electrophoresis and the same procedure you used in the Structured Inquiry. You may need to store your digested samples in the refrigerator if you cannot immediately run the gel. Record all your procedures in your notebook

Step 5: Critical Analysis: Was there a control in this activity? If so, what does the control show? What evidence does the gel provide to support the action of the restriction enzymes you used? How well did your estimation of the fragment sizes on the map match your results? Explain. Discuss with your partner and then write your answers in your notebook.

Assessments

1. Look at the plasmid (an extrachromosomal piece of DNA) in **Figure 15.11**. If you digested this plasmid with restriction enzymes BamHI, HindII, and Scal, how many fragments would appear in a gel? How long (# of base pairs) would each fragment be? Draw a gel showing the digestion. [SP 2, Visual Representations; SP 5, Statistical Tests and Data Analysis]

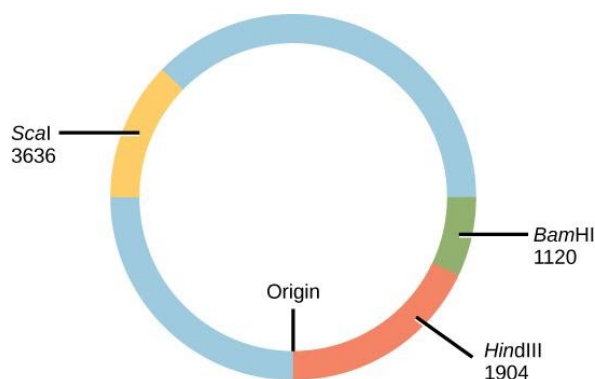
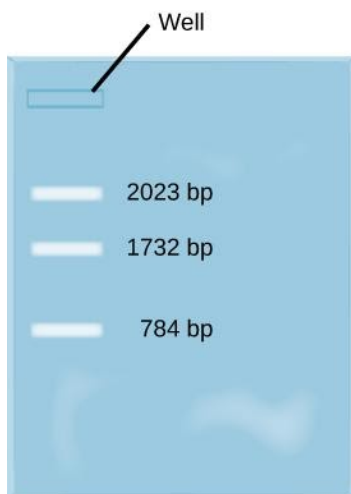


Figure 15.9: Partial restriction map for the Lambda phage.

2. Explain how electrophoresis can separate out molecules, such as DNA, by size using electrical charge and gravity. Would electrophoresis work on an electrically neutral molecule? Explain your answer. [SP 1, Concept Explanation]

[Answer Key]

1. 2023, 1732, 784



2. Electrophoresis separates out molecules of different size by applying a constant electrical charge. The molecules have an electrical charge and are attracted to the opposite electrical charge generated by the electrophoresis chamber. However, due to gravity, the larger fragments will not travel as far across the gel as the smaller fragments if the electrical field is applied for the same amount of time to all fragments.

References/Further Investigation

Using Restriction Enzymes: <https://www.youtube.com/watch?v=THmE61QNOFA>

DNA Fingerprinting: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3831584/>

Why Do you Have to Wear Gloves when Handling DNA:

<http://www.biohit.com/resource/files/media/articles/liquid-handling/science/biohit-certified-pipette-tip-article.pdf>

Agarose Sieving:

<https://www.promega.com/resources/pubhub/enotes/what-percentage-agarose-is-needed-to-sufficiently-resolve-my-dna-sample/>

Practice Loading Gel Video: <https://www.youtube.com/watch?v=KXniytDZKYQ>

How to Load a Gel: <https://www.youtube.com/watch?v=zDjjg7wbx0s>

Blank Semi-Log Graph Paper:

<http://www.mathnstuff.com/math/spoken/here/2class/340/gif/semilog.gif>

How to do a Semi-Log Graph and Trendline in Excel:

<https://www.youtube.com/watch?v=y55VmSIMBjE>

Lab 16: Bacterial Transformation

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
Topic 6.1 DNA and RNA Structure	IST-1 Heritable information provides for continuity of life.	IST-1.K Describe the structures involved in passing hereditary information from one generation to the next. IST-1.L Describe the characteristics of DNA that allow it to be used as the hereditary materials.	IST-1.K.1 DNA, and in some cases RNA, is the primary source of heritable information. Prokaryotes and eukaryotes can contain plasmids. IST-1.L.1 DNA exhibits specific nucleotide base pairing: A pairs with U, and C pairs with G.
Topic 6.8 Biotechnology	IST-1 Heritable information provides for continuity of life.	IST-1.P Explain the use of genetic engineering techniques in analyzing or manipulating DNA.	IST-1.P.1 Genetic engineering techniques, including bacterial transformation, can be used to analyze and manipulate DNA and RNA.
Topic 7.8 Continuing Evolution	EVO-3 Life continues to evolve within a changing environment.	EVO-3.A Explain how evolution is an ongoing process in all living organisms.	EVO-3.A.1 Populations of organisms continue to evolve. EVO-3.A.2 All species of organisms have evolved and continue to evolve; evidence includes the development of resistance to antibiotics in bacteria.

SCIENCE PRACTICES

Science Practices	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.A Describe biological concepts and/or processes. 1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe characteristics of a biological concept, process, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on an observation, data, or model. 3.C Identify experimental procedures that are aligned to the question, including variables and controls. 3.D Make observations, or collect data from representations or laboratory setups or results. 3.E Propose a new/next investigation based on evidence/evaluation from a previous experiment.
SP 4 Representing and Describing Data Represent and Describe Data	4.B Describe data from a table, including trends and/or patterns and relationships between variables.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculating, including equations in the curriculum.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.A Make a scientific claim. 6.A Support a claim with evidence. 6.B Provide reasoning to justify a claim.

Biologists view the role of deoxyribonucleic acid (DNA) in an organism the same way a chef views a recipe. Like a recipe, DNA contains the information for a cell's *ingredients*, as well as *instructions* for how cell components should function. The *ingredients* of a cell, or its molecular structure, are encoded in the DNA's genes. Proteins are particularly important ingredients because they form many structural components of an organism. Some proteins are also enzymes, which speed up chemical reactions within the cell. A cell's *instructions* are the regulatory genes that coordinate the functions of other genes. Unlike a recipe, DNA is written in a universal language called the genetic code that all cells can interpret.

The discovery of the genetic code led to the hypothesis that the DNA of one organism could possibly be transferred into another organism. Therefore, one organism could use, or express, the genes of another organism. This hypothesis was successfully tested by Herbert Boyer and Stanley Cohen in 1973. They modelled a natural process called **transformation**, in which bacterial cells take up DNA molecules from the environment and incorporate them into their genomes. Boyer and Cohen first removed bacterial genes for antibiotic resistance from one type of bacterium by cutting up the bacterial DNA using restriction enzymes. They then inserted the antibiotic resistance genes into the DNA of a plasmid. A **plasmid** is a small ring of DNA, some of which can be integrated into the DNA of a bacterium. Bacteria that can take up plasmids and incorporate their DNA are called **competent bacteria**. In the Boyer and Cohen's experiment, the bacteria were made competent by stressing them using a heat shock treatment. The bacteria then incorporated the plasmid, including the antibiotic resistance gene, into their own DNA, allowing the bacteria to become resistant to antibiotics. Furthermore, whenever a transformed bacterium reproduced, it passed on the resistance gene to all of its offspring, eventually forming an entire colony of antibiotic-resistant bacteria. The experimental protocol is summarized in Figure 16.1. Using this technique, the researchers created the first recombinant DNA organism. In fact, Boyer and Cohen learned how to modify the DNA information to produce unique characteristics in organisms.

Molecular Cloning

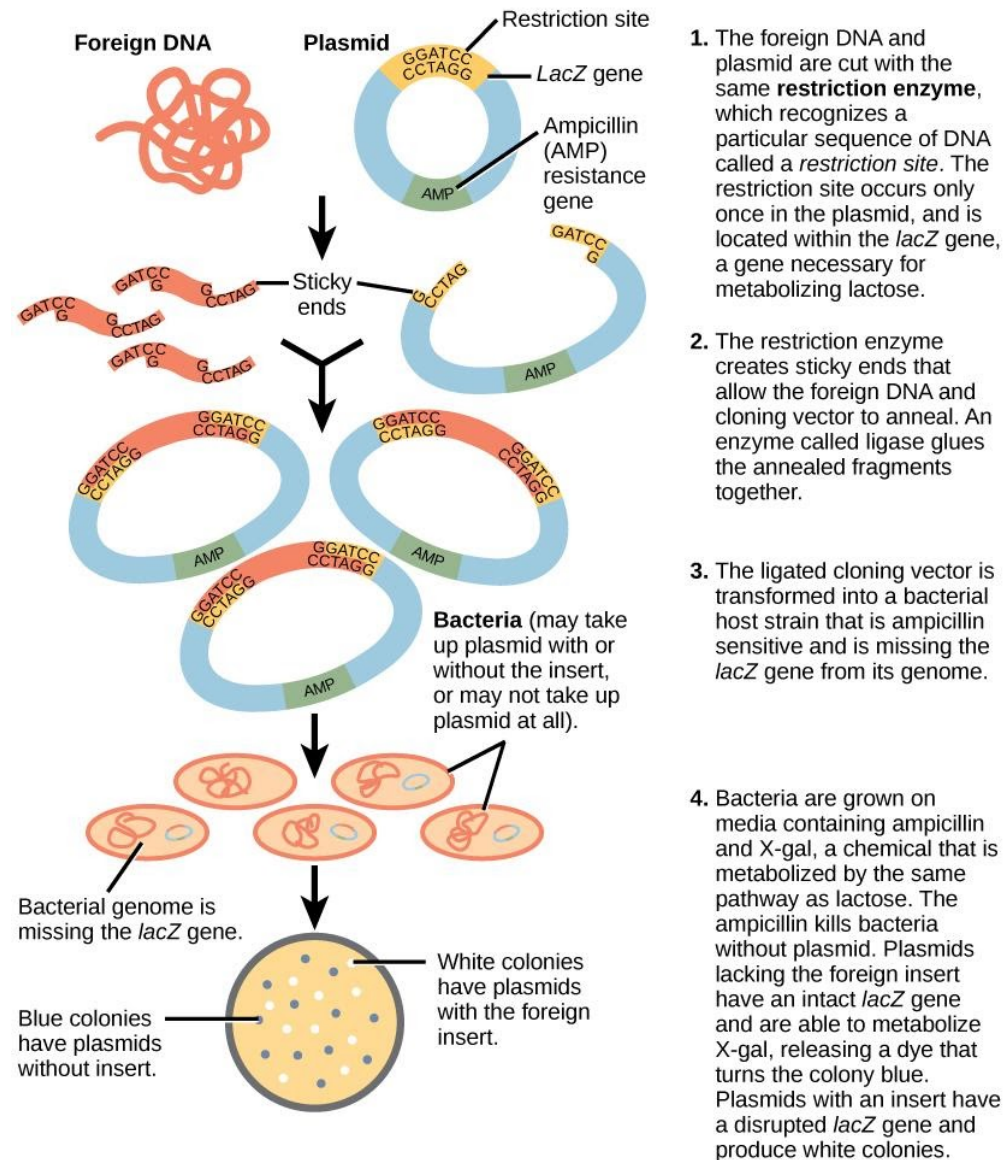


Figure 16.1: In Boyer and Cohen's bacterial transformation, the bacteria were made competent by stressing them using a heat shock treatment. The plasmid then becomes part of the bacterium's genome, including the antibiotic resistance gene. This allows the bacteria to become resistant to antibiotics.

In this lab you will learn

- how to perform a gene transfer in bacteria using a plasmid
- how to inoculate and grow transformed cell cultures on microbial media
- how to measure gene expression of transformed cells by detecting the presence of active reporter genes

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** What techniques are used to transfer a gene from one bacterium into another?
2. **Answer the following question in your notebook:** Explain one way that scientists can control the expression of genes in a bacterial cell.
3. **Be prepared to discuss the answers to questions 1 and 2 with the class.**

Activity 1: Transformation of *E. coli* Cells

In this lab, you will carry out a version of the classical experiment conducted by Boyer and Cohen. You will insert a plasmid into a strain of bacteria that is receptive to taking up and expressing the plasmid. Just like in the Boyer and Cohen experiment, this plasmid contains a gene for antibiotic resistance. However, it is linked to a green fluorescent protein gene and a regulatory gene that is activated by the expression of antibiotic resistance. Bacteria that are not transformed typically die from being exposed to ampicillin. Your procedure involves two stages (Figure 16.2). Stage 1 represents the transformation stage, where you will induce the cells to take up the green-fluorescent and antibiotic-resistance genes. Stage 2 represents the analysis stage, where you will grow the bacteria to ensure you achieved transformation.

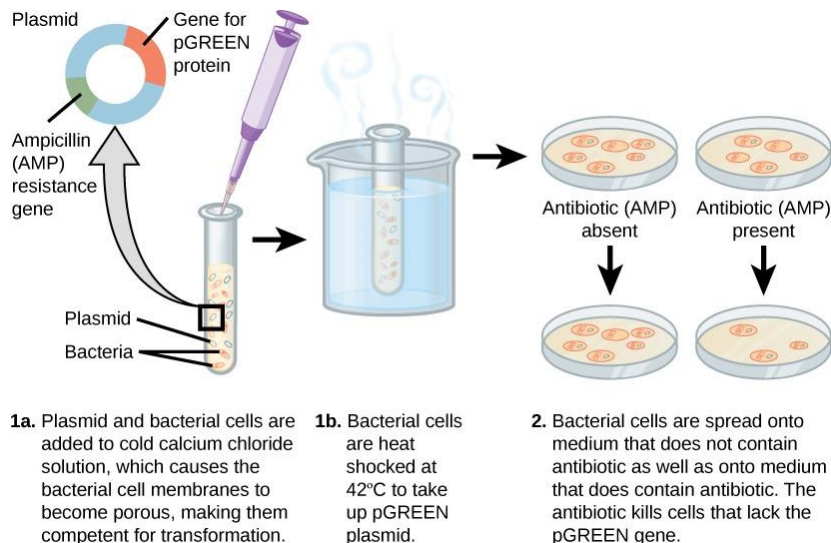


Figure 16.2: The transformation process, in Stage 1.

The transformation being performed in this activity requires that cells be in a competent state. Competence is a condition in which the bacterial cells can pass DNA easily through their cell walls and cell membranes. This is achieved by placing the cells in a calcium chloride solution (CaCl_2) and exposing them to a heat shock. Heat shock is the process of subjecting a cell to a higher temperature than ideal environmental temperature for a short period. The role of calcium chloride in the competence process is to counteract the electrostatic repulsion between the plasmid DNA and the bacterial cellular membrane. This allows the plasmid to come in contact with the cell membrane. The heat shock produces pores in bacterial cell membranes and permits the plasmid DNA to pass into the cell as shown in Figure 16.3. In this stage of transformation, you will make competent cells and then store them in the refrigerator until you complete the transformation process.

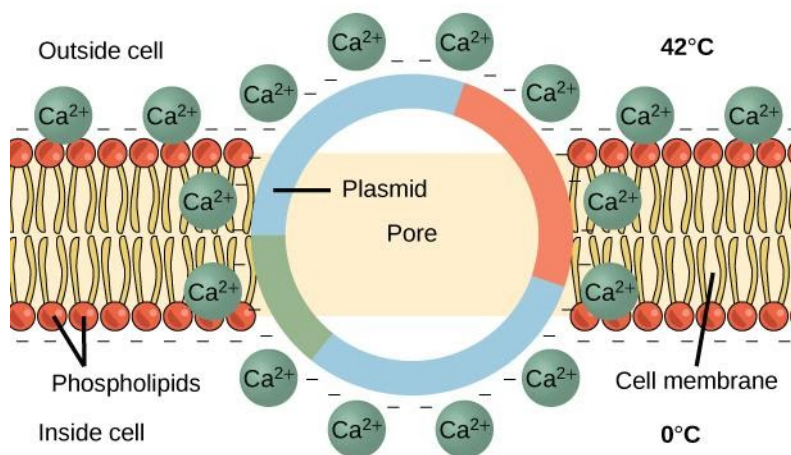


Figure 16.3: Heat shock transformation of *E. coli*.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Review the three major mechanisms of horizontal gene transfer (HGT) in bacteria, transformation, transduction, and conjugation.
- Ask students what are the main evolutionary consequences of horizontal gene transfer. They should conclude that bacteria can diversify much faster than can be predicted by mutations alone.
- Ask students what would be the consequence of HGT if many plasmids carried antibiotic resistance genes.
- Ask students for examples of recombinant DNA technology which may be familiar. For example, recombinant human insulin is used by diabetics. Factor VIII is used to treat hemophilia. Be prepared to answer questions on genetically modified organisms in food, but don't allow controversy to take over class time.

- Remind students that the transformation lab requires organizational skills because all the steps are crucial and must be performed as directed and following timing of steps matters.
- Ask students to prepare a flow chart of the procedure and go over it with the class.
- Advise students to divide the tasks before starting the lab. Good coordination of effort is essential for a successful lab.

Safety Precautions

- Use caution when handling biological specimens.
- Wear safety goggles throughout the duration of the laboratory activity.
- It is highly recommended that you wear rubber gloves and a lab apron when handling any chemicals. In addition, use aseptic techniques in handling bacterial cultures to avoid infection.
- Immediately inform your teacher of spills.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent people from slipping.
- Immediately wash your hands if they directly contact any chemicals or cultures. It is important to wash your hands after the laboratory activity to avoid any possible contamination of other surfaces with chemical residues or biological materials that might be on your hands.

For this activity, you will need the following:

Materials:

- Surgical gloves
- Safety goggles
- Waterproof marker
- Micro tube rack
- Eight disposable 1 mL calibrated plastic needle-point pipettes (or adjustable micropipettes, if available)
- Eight disposable plastic inoculating loops
- 1 polystyrene warm-beverage cup filled with crushed ice
- 20 μ l micropipette (one per class)
- Micropipette tips for class (for 20 μ l micropipette)

- 42 °C water bath (one per class)
- Floating test tube holder (one per class)
- 37 °C incubator
- Parafilm
- Blue LED light box (one per class)
- Refrigerator
- Biological hazardous waste container

Chemicals and Media:

- One Luria broth media Petri plate
- One Luria broth + Amp media Petri plate
- One bottle of sterile Luria broth (one per class)
- Squirt bottle with 10 percent bleach solution
- Squirt bottle with 70% ethanol solution
- Crushed ice
- Micro tube filled with 50 mM CaCl_2
- Micro tube filled with Luria broth (two per group)

Living materials:

- One streak plate of *E. coli* per group (Strain MM294 or competent *E. coli* cells)
- One pGREEN or pGLO plasmid solution (0.005 $\mu\text{g}/\mu\text{l}$; one per class)

Note—These materials may be provided for the class as separate materials or as components in a transformation kit. Your instructor will tell you how these materials will be distributed to your group.

For this activity, you will work in *groups of 3–4*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Preparing competent cells is a complicated procedure even for experienced molecular biologists. Consider ordering competent cells or, at least, prepare ahead of the lab some competent cells to test and transform them to test the procedure.
- To streak cells for students, follow procedures from a standard microbiology manual.

- Troubleshoot:
 - Nothing grew: the loop was not cooled enough before touching bacterial samples.
 - No single colonies: the loop was not flamed between contacts with bacterial samples or not streaked over an area large enough to dilute the culture.
- If pouring your own plates, follow a standard protocol remembering that ampicillin is added aseptically after the LB agar solution has been autoclaved and cooled to 50 °C.
- If an autoclave is not available, prepare LB agar broth in a flask and boil for about 10 minutes mixing well the content and being careful not to allow the solution to boil over. Cool to about 50 °C, add ampicillin if required, and pour agar in Petri plates. Let the Petri plates solidify, and, then, turn them upside down for storing. Leaving plates at room temperature for two to three days decreases the amount of condensation.
- Demonstrate all aseptic techniques.
- Consider running a lab practice session to give students an opportunity to handle pipettes, aseptic techniques, handling of loops and Petri plates if enough material is available. Students should be reminded to look at the loop to make sure that they picked a sample, and the loop is not empty.
- Remind students that plates must be labeled along the edge of the bottoms with their initials, date, and experimental conditions. Lids should not be labeled. Labeling plates in the center will interfere with the reading of results.
- Remind students to keep plates covered unless they are actually spreading cultures. Ask them what would happen if they left the plates open. Students forget that microorganisms are ubiquitous. Ask them if something would grow on the LB+amp plates. This is an opportunity to remind students that not all microorganisms are bacteria and that mold will contaminate plates.
- Emphasize that speed and accuracy are paramount to the success of the transformation lab. If students are not familiar with experiments that last over several days, they may not realize that the consequences of mistakes made on day 1 will not be apparent until day 3.
- Give students the following advice to spread cultures evenly on the surface of the Petri plate:
 - Spread culture with a loop using a back and forth motion and turning the plate 90° twice.
 - Incubate plates inverted to allow condensation to drip on the lid, but do not invert the plates immediately after spreading the culture. It is best for the instructor to invert all the plates after the activity is completed by the students.
- If an incubator at 37 °C is not available, plates can be left at room temperature for about 72 hours until colonies reach optimal size.
- Turn off the lights in the room to observe the GFP glow and let eyes adapt to darkness first. Pictures will require a long exposure time.
- Remind students that gloves are considered biohazard material after they are used with cultures. They should be disposed of accordingly.

- If an autoclave is not available, use 10 percent freshly diluted bleach to disinfect Petri plates, loops, and other items that came into contact with bacteria.
 - Bleach should be used in the hood or, if not possible, containers/pans should be covered when not in use.
 - Students should wear goggles, gloves and aprons to protect their clothes.
 - Once loops and pipets have been allowed to soak in bleach for 15 minutes, they can be disposed of as regular trash. Cover the surface of Petri plates with bleach and let it sit with the disinfectant for about 1 hour. Always check local regulations before proceeding.
 - If there is exposure, take first aid measures at once
 - If eyes and face are exposed, and eyes should have been protected with goggles, rinse using eyewash station or faucet for at least 15 minutes or as directed by safety protocol.
 - If skin is exposed, rinse with faucet water for 15 minutes.

The following are recommendations for the Guided Inquiry for Activity 1:

- Help students recognize that there is a lawn of bacteria. Students may have difficulty recognizing the difference between an empty plate and a lawn where no individual colonies are visible.
- Ask the students why an experiment is only as good as its controls. Ask students why three control plates are necessary. It is important to show that the cells are viable, that the LB/amp plate does inhibit growth if the resistance genes are not transformed in the cells, and that plasmid transformation is a low efficiency process. Without ampicillin in the medium, transformed cells would be outnumbered and barely detectable.

Structured Inquiry

Stage 1: Inducing Competency and Transforming *E. coli*:

Step 1: Hypothesize/Predict: You will grow your bacteria on media containing ampicillin. Describe what you would expect if your transformation is successful. Write your ideas in your notebook.

Step 2: Use the waterproof marker to label two micro tubes with a short group name. Also label one micro tube – and one +. Place the labeled micro tubes in the micro tube rack. Have a Styrofoam cup filled with crushed ice ready.

Step 3: With the plastic needle-point pipette, transfer 250 µl of the 50 Mm CaCl₂ solution to each micro tube. Dispose of the needle-point pipette.

Step 4: Remove one sterile plastic loop from the package and scrape up one large colony from the *E. coli* streak plate. Transfer the *E. coli* to the – micro tube and swirl the loop in the CaCl_2 solution. Make sure you see no residual bacteria on the loop. Dispose of the loop in the biological hazardous waste container. Close the cap on the – micro tube and place it in the cup containing the crushed ice.

Step 5: Repeat Step 3, this time placing the *E. coli* into the + micro tube and returning the micro tube to the rack instead of placing it in ice.

Step 6: Remove one sterile plastic loop from the package and dip the loop part only into the pGREEN solution. Swirl the loop in the solution in the + micro tube. Close the cap on the + micro tube and place it in the cup with the ice. Dispose of the loop in the biological hazardous waste container.

Step 7: After 15 minutes on ice, carry the ice bath containing the – and + micro tubes to the 42 °C water bath. Place both micro tubes in the floating micro tube holder and let them sit for 90 seconds. Remove both micro tubes from the 42 °C water bath and immediately return them to the ice bath.

Step 8: If instructed by your teacher, place your – micro tube and + micro tube in the micro tube rack and store the racks in a designated refrigerator. The competent bacteria can be stored for no more than two days.

Step 9: Clean up:

- Return the *E. coli* plate to the teacher for storage or disposal.
- Empty the ice from the insulated beverage cup and return the cup.
- Return the micro tube filled with 50 mM CaCl_2 to your teacher.
- Return any other materials to the teacher.
- Obtain the 70% ethanol solution bottle and squeeze some ethanol onto your workspace. Wipe down the table with paper towels and place the used towels in the trash. Then wipe down the tables again with paper towels wetted with tap water. Dry the table with a fresh set of paper towels.

Step 10: Critical Analysis: Discuss with your team the reason for adding the pGREEN plasmid solution to only one micro tube. How would your results likely be affected by leaving the bacteria in the 42 °C water bath for more than 180 seconds or less than 90 seconds? Record your answers in your lab book. Explain what likely happened during your experiment if you see no bacterial growth.

Stage 2: Culturing the Bacteria

Step 1: Retrieve your micro tube rack from the freezer. Let your rack sit until it reaches room temperature.

Step 2: Using a disposable needle-point pipette or other pipette, add 250 μL of Luria broth solution to the micro tube. Close the lid on the micro tube. Dispose of the needle-point pipette in the biohazard waste container. Let the micro tube incubate in the rack for 10 minutes at room temperature. Be sure to follow all teacher instructions.

Step 3: While the micro tube is incubating, use the waterproof marker to label the lower half of the two Luria broth + Amp media Petri plates with your group name and the date. Then label one plate – *plasmid* and the other plate + *plasmid*.

Note— Look at Figure 16.5 before continuing to the remaining steps. The figure shows you how to add the contents of your micro tubes to the Luria broth media plates. You will need to use a separate needle-point pipette and separate plastic sterile loop for each plate. It is important to use the following steps to inoculate each Luria broth media plate with the bacteria in the micro tubes:

1. Place the Luria broth + Amp media plates on the table with the lower plate down.
2. Remove the upper plate.
3. Inoculate the plate with the *E. coli*.
4. Immediately replace the upper plate.
5. Do not move the Luria broth media plates until it is time to place them in the incubator.

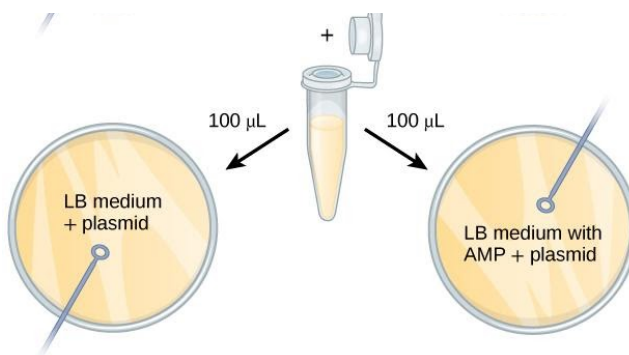


Figure 16.5: Illustration of process for steps 7–10.

Step 4: After the 10-minute incubation period, use a pipette to transfer 100 μ L of your micro tube solution to the media in the lower half of the – plasmid Luria broth+Amp plate. Use a plastic sterile loop to spread the liquid evenly across the surface of the media. Dispose of both the needle-point pipette and the plastic sterile loop in the biohazardous waste container.

Step 5: Use a new disposable needle-point pipette to transfer 100 μ L of your + micro tube solution to the media in the lower half of the + plasmid Luria broth + Amp plate. Use a plastic sterile loop to spread the liquid evenly across the surface of the media. Dispose of both the needle-point pipette and the plastic sterile loop in the biohazardous waste container.

Step 6: Let the plates sit at room temperature for 10 minutes before moving.

Step 7: Carefully carry your four plates for placement in a 37 °C incubator. Leave the plates in the incubator for 24 hours. If an incubator is not available, store the plates at room temperature for at least 72 hours.

Step 8: Clean up:

- Return all remaining materials to the teacher.
- Place your micro tubes containing the bacteria into the biohazardous materials container or in an area designated by your teacher.
- Obtain a squirt bottle with 70% ethanol solution and squeeze some ethanol onto your workspace. Wipe down the table with paper towels and place the used towels in the trash. Then wipe down the tables again with paper towels wetted with tap water. Dry the table with a fresh set of paper towels.

Step 9: The *E. coli* you placed on the Luria broth media plates have now had time to multiply. As they multiplied, the *E. coli* with the pGREEN plasmid replicated the plasmid as well as their own DNA. Create a drawing of the bacterial growth on each plate in your notebook, noting which growth is spotty versus extensive. Your goal in this inquiry is to identify which plate shows the presence of transformed *E. coli* that are expressing the green fluorescent protein reporter gene. Remember, the green fluorescent protein reporter gene is telling you that the ampicillin resistance gene is also being expressed.

Step 10: Collect your four incubated Luria broth media plates and carefully carry them to your workspace. You should have the plates labeled as followed from Activity 1:

- Luria broth (LB) media Petri plate – *plasmid*
- Luria broth with ampicillin (LB + Amp) media Petri plate – *plasmid*
- Luria broth (LB) media Petri plate + *plasmid*
- Luria broth with ampicillin (LB + Amp) media Petri plate + *plasmid*

Step 11: Carefully remove the Parafilm from each plate and dispose of the Parafilm in the biohazard container. (The Parafilm may have some bacterial contamination.)

Step 12: Record your findings in your notebook. Draw the growth patterns of each plate. You may also want to take a photograph of each plate.

Step 13: One at a time, remove the lid from each plate and illuminate the bacterial plates with the blue LED light boxes. Record your findings in your notebook.

Step 14: Clean up:

- Dispose of the four Petri plates in the biohazardous materials container or place in an area designated by your teacher.
- Return the LED light boxes to the teacher.
- Obtain the squirt bottle with 70% ethanol solution and squeeze some ethanol onto your workspace. Wipe down the table with paper towels and place the used towels in the trash. Then wipe down the tables again with paper towels wetted with tap water. Dry the table with a fresh set of paper towels.

Step 15: Critical Analysis: Based on your results, which plate or plates contained bacteria that were expressing the ampicillin resistance gene on the pGREEN plasmid? Compare your data to the data from the other students in class. Did any of the groups have data different from yours? If there was a difference, explain what could have caused this variation. Record your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: In your initial experiment, you tested for transformation by growing your transformed and untreated bacteria on media containing the antibiotic ampicillin. What was the role of the ampicillin in determining whether the bacteria were transformed? Explain how you were able to distinguish between the control bacteria that you didn't transform and the transformed bacteria. What would occur if you placed your non-transformed and transformed bacteria on LB plates that did not contain ampicillin? Write your hypothesis in your notebook.

Step 2: Student-led Planning: Write out the steps to test your hypothesis about culturing non-transformed *E. coli* and transformed *E. coli* having the pGREEN vector on plain LB plates. Remember to keep in mind how you will safely dispose of your lab waste. Submit your experimental procedure to your teacher. Once your teacher approves of your procedure, perform your experiment and record your data in an appropriate format. Write your results in your notebook.

Step 3: Carry out the procedure you devised in Step 2. Your setup will be as shown in Figure 16.6.

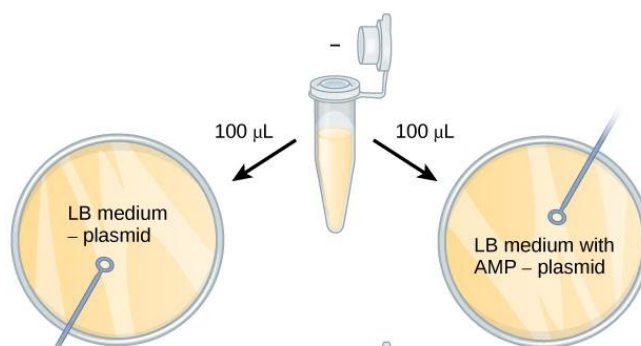


Figure 16.6: Experimental treatments for Guided Inquiry.

Step 4: Critical Analysis: Compared to the growth on the LB + Amp plates, what additional information did you discover by growing the non-transformed and transformed bacteria on the plain LB plates? How would this information be useful to scientists who are conducting transformation experiments? What is the value of using the GFP reporter gene in assessing the success of cell transformation? Write your results in your notebook.

Assessments

1. Explain why ampicillin must be placed in the Luria broth medium used to grow the transformed cells? [SP 1, Concept Explanation; SP 3, Questions and Methods]
2. Explain why the heat shock step is essential for transformation to occur? [SP 1, Concept Explanation; SP 3, Questions and Methods]
3. In this activity, you induced competence in *E. coli* cells. However, certain environmental conditions that stress bacteria can induce a natural competence. What is the value of competence to survival under stressful conditions? [SP 1, Concept Explanation; SP 6, Argumentation]
4. Describe how your results would most likely change if the ampicillin-resistance gene underwent a severe mutation within the bacteria? [SP 6, Argumentation]
5. Draw the gene sequence that shows why expression of the ampicillin-resistance gene also causes the expression of the green fluorescent protein. [SP 1, Concept Explanation; SP 2, Visual Representation]
6. Heat shock transformation does not guarantee that all bacteria will take up plasmids or that all bacteria will have the same number of plasmids. How could you detect this in your results? [SP 2, Concept Explanation; SP 3, Questions and Methods]

[Answer Key]

1. Ampicillin is the antibiotic that will induce expression of the ampicillin-resistance gene that was inserted in the pGREEN plasmid.
2. The heat shock process permits the cell membrane of *E. coli* to take in the plasmid by making pores in the cell membrane. Otherwise, the plasmid will remain outside the *E. coli* cell.
3. Competence encourages *E. coli* to take in DNA that is present in the environment. This process could provide novel genes that improve survival during stressful conditions.
4. If the ampicillin resistant gene was severely mutated, it likely will not be expressed and the bacteria will die from the ampicillin before being able to reproduce. Therefore, the plates with ampicillin will have no bacterial growth.
5. A drawing should show the ampicillin-resistance gene and green fluorescent protein gene in line with the regulatory gene that induces the ampicillin-resistance gene. For example: ampicillin-resistance regulatory gene followed by ampicillin-resistance gene followed by green fluorescent protein gene.
6. The intensity of the fluorescent protein gene is an indicator of transformation efficiency. So, *E. coli* on the LB+Amp + plasmid plate would not glow under blue light if the bacteria died from the ampicillin and cells with fewer plasmids would have fainter colonies than cells with more plasmids.

Extension Activities 3:

1. Carry out a study to determine whether exposure of the pGREEN plasmid to ultraviolet light affects the expression of the plasmid.
2. Perform a DNA extraction on *E. coli* cells to determine whether you can isolate the pGREEN plasmid from the transformed cells on the LB plate + *plasmid* and the LB+Amp plate + *plasmid*. Then use the polymerase chain reaction and electrophoresis to confirm the results.

Lab 17: Hardy-Weinberg Equation

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRE COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
7.1 Introduction to Natural Selection	EVO-1 Evolution is characterized by a change in the genetic makeup of a population over time and is supported by multiple lines of evidence.	EVO-1.C Describe the causes of natural selection.	EVO-1.C.1 Natural selection is a major mechanism of evolution.
7.2 Natural Selection	EVO-1 Evolution is characterized by a change in the genetic makeup of a population over time and is supported by multiple lines of evidence.	EVO-1.E Describe the importance of phenotypic variation in a population.	EVO-1.E.1 Natural selection acts on phenotypic variations in populations.
7.4 Population Genetics	EVO-1 Evolution is characterized by a change in the genetic makeup of a population over time and is supported by multiple lines of evidence.	EVO-1.H Explain how random occurrences affect the genetic makeup of a population. EVO-1.J Describe the change in the genetic makeup of a population over time.	EVI-1.H.1 Evolution is also driven by random occurrences, including mutation, genetic drift, and migration/gene flow. EVO-1.J.1 Mutation results in genetic variation, which provides phenotypes on which natural selection acts.
7.5 Hardy-Weinberg Equilibrium	EVO-1 Evolution is characterized by a change in the genetic makeup of a population over time and is supported by multiple lines of evidence.	EVO-1.K Describe the condition under which allele and genotype frequencies will change in populations.	EVO-1.K.1 Hardy-Weinberg is a model for describing and predicting allele frequencies in a non-evolving population under certain conditions, such as a large population. EVO-1.K.2 Allele frequencies in a population can be calculated from genotype frequencies.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.C Explain how biological concepts or processes are represented visually related to larger biological principles, concepts, processes, or theories. 2.D Represent relationships within biological models, including mathematical models.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on an observation, data, or a model. 3.B State the null and alternative hypotheses, or predict the results of an experiment.
SP 4 Representing and Describing Data Represent and describe data.	4.B Describe trends and/or patterns in data.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including mathematical equations from the curriculum. 5.D Use data to evaluate a hypothesis, including rejecting or failing to reject the null hypothesis.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.B Support a claim with evidence from biological principles, concepts, processes, and/or data.

Population genetics is the field of biology that studies genetic variation in populations over time. Change that occurs in a population over time is known as **evolution**. It involves changes in the proportion or frequency of specific alleles in a given population from one generation to the next. These changes can be due to factors such as recombination, selection, and mutations. An example of evolution in a population due to selection might involve alleles that code for traits that convey survival benefits that are favored over other alleles that code for traits that are less beneficial. The favorable allele would gradually increase in frequency over time while the unfavorable allele would diminish and potentially disappear.

Allele frequency is defined as the proportion of alleles in the population that are the particular type in question. It is represented as a fraction of 1. For example, if an allele makes up 65 percent of the total population and another makes up 35 percent, the frequencies will be 0.65 and 0.35, respectively. The frequencies of all the different alleles for one particular gene will always add up to 1.0. One allele is usually designated as p (typically the more common or dominant allele) and the other is usually designated as q (typically the less common or recessive allele). Therefore, $p + q = 1.0$. According to the **Hardy-Weinberg model**, there will be no change in allele frequency in a sexually reproducing population (genetic equilibrium) if:

- There is no mutation.
- No migration occurs that alters the population.
- Random mating between individuals in the population occurs.
- The population is large enough so that changes in allele frequencies will not be due to chance.
- No selection occurs.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- Brainstorm the definition of evolution with the students. What does it mean to them? At what scale do we measure evolution? Can it happen quickly, or does it take a long time to happen?
- Define population genetics. Why do we need to know how populations change over time? What are some practical applications of understanding the changes in genetic structure over time?
- Define allele, recombination, selection, and mutations. How do these things relate? Discuss the definition of allele frequency and why the students think $p+q=1$. Make sure they can make sense of the equation.
- Discuss the Hardy-Weinberg Model and how it applies to determining allele frequency. Ask the students to brainstorm as to why each of the requirements need to be met in order to have genetic equilibrium. What would happen if something happened to change one of the requirements?

In this lab, you will learn

- how genetics affects the sense of taste
- about the components of the Hardy-Weinberg equation
- how to use the Hardy-Weinberg equation to calculate allele frequencies of dominant and recessive alleles

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Have the students think about sample size and small populations. How would the class compare to the entire United States? North America? The whole human population? Have them think about how certain allele frequencies might vary from region to region.
- Have the students discuss why it's unlikely for Hardy-Weinberg Equilibrium to ever be reached with most alleles. Have them try to think of ways that it could be reached.
- Discuss how the equation is derived from $p+q=1$. Why do we need to think about homozygous and heterozygous individuals? Are there cases where you will never have a heterozygous individual? How about a homozygous individual?
- Have the students discuss the evolutionary advantages and disadvantages of having the ability to taste bitter. What regions of the world might have more people with the ability to taste bitter? What places might not?

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** Why might the allele frequencies from a sample population not match those from the general population?
2. **Answer the following questions in your notebook:** Would you expect allele frequencies of a given allele to remain constant among humans in the United States? Would you expect the conditions of Hardy-Weinberg to still be met in the next 50 years within the United States? Why or why not?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 1: Hardy-Weinberg Equation: Determining PROP Tasters vs. Non-tasters

If the Hardy-Weinberg model applies, then the frequencies of genotypes designated as either pp , pq , or qq will not change over time. Hardy Weinberg equilibrium can be written as an equation and can be used to estimate the proportions of these alleles. The **Hardy-Weinberg equation** is

$$p^2 + 2pq + q^2 = 1.0,$$

where p^2 represents the homozygous individual for the dominant allele, pq represents the heterozygous individual, and q^2 represents the homozygous individual for the recessive allele.

The ability to taste certain bitter compounds is genetically determined. In this lab, we will conduct a simple experiment to study gene proportions using the class as a sample population. We will be using a chemical called PROP (6-n-propylthiouracil), shown in Figure 17.1 and found in bitter tasting foods, like the Brussels sprouts shown in Figure 17.2. Individuals that can taste this compound are homozygous dominant (TT; highly sensitive- **supertaster**) or heterozygous (Tt; moderately sensitive- **medium-taster**). Those individuals unable to taste are homozygous recessive (tt; **non-taster**).

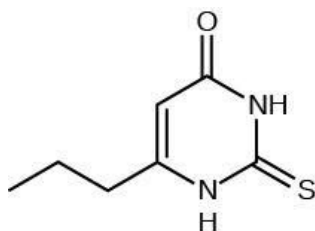


Figure 17.1: Chemical structure of PROP (6-n-propylthiouracil).



Figure 17.2: Supertasters have less preference for and tend to avoid bitter-tasting foods like Brussels sprouts.

Safety Precautions

- PROP is used to treat hyperthyroidism. You should not be exposed to high amounts of it. The taste strips only have very small amounts.

For this activity, you will need:

- Control taste strip
- PROP taste strip

For this activity, you will work *in groups of 4*.

Structured Inquiry

Step 1: Hypothesize/Predict: Based on your previous experience with bitter tasting foods, hypothesize about whether you are a supertaster, medium-taster, or a non-taster. Predict your reaction to PROP. Write your prediction in your notebook.

Step 2: Student-led Planning: In your notebook, create a data table to enter your group's reactions to the control and PROP taste strips. Create another table to show the reactions of the entire class. You should indicate whether the individual believes that the strips are extremely bitter (supertaster), bitter (medium-taster), or cannot be tasted (non-taster).

Step 3: Place a control taste strip on your tongue. Allow the saliva to saturate the strip. Discuss with your partner what the strip tasted like. Record your reaction. Classify the taste as extremely bitter, bitter, or not bitter.

Step 4: Repeat Step 3 and place the PROP taste strip on your tongue.

Step 5: Critical Analysis: Record the reactions of all group members in your data table. Are the predictions you made in Step 1 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Guided Inquiry 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Guided Inquiry 1:

- Revisit the discussion on sample size. Have them discuss how their group might compare to the whole class. Then have them discuss how it would compare to other regions.
- Have them think about why there is variation across populations. Why is this variation important? What other traits can they think of that might vary across different groups of people? Why are these variations important? What are some applications of understanding the variation?

Guided Inquiry

Step 1: Hypothesize/Predict: Predict the gene frequencies of the dominant and recessive PROP tasting genotypes based on the proportion observed in your group. How do you think it compares to the entire class? Write your ideas in your notebook.

Step 2: Student-led planning: Obtain data from the other groups in the class. Create a table in your notebook to place allele frequencies for the class.

Step 3: Use the class data and the Hardy-Weinberg equations, $p + q = 1$ and $p^2 + 2pq + q^2 = 1.0$, to calculate allele frequencies for the supertasters, medium-tasters, and non-tasters.

Step 4: Critical Analysis: Determine whether your prediction is supported. Are there more individuals with the dominant phenotype? With the recessive phenotype? How does this compare to your group's data? Is either population (group or class) in Hardy-Weinberg equilibrium? Discuss your answer with your group members and write your responses in your notebook.

Assessments

1. What are some reasons that the values for p and q for your class might differ from the frequencies reported for the entire North American population of humans, in terms of PROP tasting? [SP 1, Concept Explanation; SP 6, Argumentation]
2. What are the limitations of Hardy-Weinberg equilibrium? [SP 1, Concept Explanation]
3. A population of bears has individuals with black fur (dominant) or brown fur (recessive). Black bears have the genotype BB or Bb. Brown bears have the genotype bb. The frequency of the BB genotype is 0.59. [SP 5, Statistical Tests and Data Analysis]
 - a. What is the frequency of heterozygous bears?
 - b. What is the frequency of the B allele?
 - c. What is the frequency of the b allele?

[Answer Key]

1. The population within the class is much smaller than the entire North American population. Therefore, the class population would likely differ in its proportion of the various tasters from a larger population due to random chance.
2. The Hardy-Weinberg equilibrium is an idealized model that has the following assumptions:
 - There is no mutation.
 - No migration occurs that alters the population.
 - Random mating.
 - The population must be large enough so that changes in allele frequencies will not be due to chance.
 - No selection occurs.
3. a. $2pq = 0.356$, b. $p = 0.768$, c. $q = 0.232$

Lab 18: Natural Selection Simulation

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
7.1 Introduction to Natural Selection	EVO-1 Evolution is characterized by a change in the genetic makeup of a population over time and is supported by multiple lines of evidence.	EVO-1.C Describe the causes of natural selection. EVO-1.D Explain how natural selection affects populations.	EVO-1.C.1 Natural selection is a major mechanism of evolution. EVO-1.C.2 According to Darwin's theory, individuals with more favorable phenotypes can better compete for limited resources and survive, thus passing traits to subsequent generations. EVO-1.D.1 Evolutionary fitness is measured by reproductive success. EVO-1.D.2 Fluctuating biotic and abiotic environments can affect the rate of evolution.
7.2 Natural Selection	EVO-1 Evolution is characterized by a change in the genetic makeup of a population over time and is supported by multiple lines of evidence.	EVO-1.E Describe the importance of phenotypic variation in a population.	EVO-1.E.1 Natural selection acts on phenotypic variations in populations. EVO-1.E.2 Environments change and apply selective pressure to populations. EVO-1.E.3 Some phenotypic variations significantly increase or decrease fitness of the organism in particular environments.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.A Describe the characteristics of a biological concept, processes, or model represented visually. 2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.C Explain how biological concepts represented visually relate to larger biological principles, concepts, processes, or theories. 2.D Represent relationships within biological models, including diagrams.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on an observation, data, or a model.
SP 4 Representing and Describing Data Represent and describe data.	4.B Describe trends and/or patterns in data.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.A Make a scientific claim. 6.E Predict the effects of a change in one or more concepts in a biological system based on data.

Evolution is the change in a population over time. One of the major driving forces of evolution is the process of **natural selection**, in which organisms that are better adapted to a particular environment tend to survive and reproduce. The concept of natural selection was first brought to light by Charles Darwin and Alfred Wallace in the 1850s. Their observations illuminated several requirements or conditions that must be met for natural selection to occur:

- Organisms must have **variation**, or differences in their physical and behavioral characteristics.
- Organisms must be in competition for resources.
- Some organisms must be better **adapted**, or suited, to their environment than others.
- Some organisms must have greater reproductive success.

These concepts lead to what is known as the *survival of the fittest*. This means that some organisms have adaptations that allow them to better survive in a particular environment. These organisms are able to successfully compete for resources and then produce offspring that are viable and able to pass their genes onto the next generation. These are said to be *selected for*. Those individuals that disappear over time have been *selected against*.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- More than a million live species on Earth. An explanation for the diversity in living things itself is a useful way to introduce this topic.
- Understanding evolution is the key to understanding diversity. The mechanism of evolution is natural selection, and though students may have a general understanding of how evolutionary change works, they need to understand the range of evolutionary changes, from individuals with certain traits to how the traits in a whole population can change.
- Ask the students to discuss the relationship between Darwin and Wallace's work. How did they come to their conclusions? Were their ideas accepted right away? What more modern data help support their ideas?

In this lab, you will learn

- how one organism is naturally selected over another
- how natural selection occurs over multiple generations
- how environmental factors affect natural selection

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for Activity 1:

- Encourage the students to think like Darwin or Wallace. What were their observations that helped them come to their conclusions?
- Have them think about all the influences we have around us—climate, weather, geography, and even human-caused issues. What traits might be an advantage for certain scenarios? What traits might be a disadvantage? Are there any traits you can think of that might help in one scenario but be harmful in another?
- Students may struggle with the concept of time, as it can take generations, even after a large catastrophic event, for natural selection pressures to become evident. You might want to discuss catastrophic events versus slower-acting pressures, such as niche partitioning in an island system.
- Throughout the activity, ask students to think about different traits that modern plants and animals have that are perfectly adapted to our current climate but might be a disadvantage when the climate changes significantly in the future. How might these species adapt, or will they not be able to survive the change?

Activity 1: Pre-Assessment

1. **Answer the following questions in your notebook:** Variations in physical characteristics can either be advantageous or harmful for organisms. Give an example of when an organism's physical traits might be harmful. Give an example of when they might be beneficial. Describe how the frequency of harmful and beneficial traits changes over generations in a population.
2. **Answer the following question in your notebook:** Why would the frequency of physical characteristics in a population change over several generations?
3. **Answer the following question in your notebook:** If a flood occurred, affecting a population of raccoons, describe how you think some members of that population might be able to survive better than others. (Take being rescued out of the equation.)
4. **Answer the following question in your notebook:** Name at least two environmental changes that could occur that might affect the survival of organisms within a particular ecosystem.
5. **Discuss the answers to questions 1–4 with your class.**

Activity 1: Survival of the Fittest

In natural selection, any inherited trait that makes survival likely for an individual is an **adaptation**. Those individuals that are best adapted to a given environment are more likely to survive and reproduce. In this activity, you will simulate natural selection occurring in a population over time. You will begin with a random number of pipe cleaners representing individual organisms with a particular phenotype. Brown or green pipe cleaners will represent brown or green caterpillars, like the one shown in Figure 18.1.



Figure 18.1: Green caterpillars can easily blend in with their environment, increasing their chances of survival.

Natural selection depends on the environment, as environmental factors drive natural selection. For example, a pond suddenly becomes a river as a result of flooding within a region. The flooding results in the loss of many animals, and only a few of the strongest survive. Natural disasters, climate change, chemicals that trigger mutation, and pH levels are all examples of environmental changes that might affect diversity or variation within a population. In this activity, you will examine how survival of an organism, as determined by a variety of selective forces, affects the evolution of a simulated population.

Safety Precautions

- None

For this activity, you will need the following:

- 30–50 pipe cleaners or chenille sticks (green and brown assortment)
- Paper plates

For this activity, you will work *in groups of four*.

Structured Inquiry 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry 1:

- Discuss and define selective advantage. Selective advantages are characteristics of a particular organism that enable it to survive better than others in the population.
- What might be some selective advantages of a brown caterpillar over a green one in grass? On dirt? What about on pavement? Think of a number of scenarios that could provide an advantage to an organism.
- How might you test this concept in nature? Ask students how they might measure selective advantage when studying organisms in their natural habitats.

Guided Inquiry 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Guided Inquiry 2:

- Review what a phenotype is and why certain phenotypes can have an advantage in one scenario over another. Can the students think of any organism that may have one phenotype in one habitat and another in a different habitat?
- How might selecting for certain phenotypes lead to speciation? How might phenotypic variation guide adaptation to a catastrophic event?

Structured Inquiry

Step 1: Collect an assortment of 30–50 pipe cleaners, roughly half of each color. Place five of each color on a plate that will represent the habitat. Set the others aside. These will be offspring.

Step 2: Hypothesize/Predict: Hypothesize about which caterpillars might survive better. Make a prediction that describes what will happen if a predator takes caterpillars at random, regardless of color, and then the remaining caterpillars only mate with caterpillars of the same color. Predict how the population will change over multiple generations if neither color confers an advantage.

Step 3: Student-Led Planning: You will record the number of individuals displaying each phenotype for 10 generations (i.e., the number of brown and green caterpillars). Create a table that will allow you to monitor each generation. Then, for each generation, first, determine the initial percentage of each color and record this information in your notebook. Remove three caterpillars of random colors from the habitat plate. To make this random, close your eyes and randomly remove three caterpillars. (These are the ones that died.) Set them aside in a dead caterpillar pile. Close your eyes again and randomly select three caterpillars to mate. Temporarily remove them from the habitat plate to make recording data easier. Then pair them with their same-colored mates from the habitat plate. The offspring will be the same color as the parents and will be chosen from the extra caterpillar plate (not the dead caterpillar pile). For example, if you randomly selected two green caterpillars and one brown caterpillar, and those

were able to pair and mate, select two greens and one brown from the extra caterpillar pile as offspring for the pairs. Record your results in your data table. Put the parents and offspring on the habitat plate. Repeat for nine more generations, or until you run out of caterpillars.

Step 4: Critical Analysis: Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and write your answers in your notebook.

Guided Inquiry 1

Step 1: Using 30–50 green and brown pipe cleaner caterpillars, repeat the steps in the Structured Inquiry. This time, you will choose a selective advantage. Discuss with your group what the selective advantages might be and record your responses in your notebook.

Step 2: Hypothesize/Predict: In green grass, which color would have a selective advantage? Why? Hypothesize about which caterpillar might survive better, then make a prediction that describes the future population of the caterpillars. How do you think the population will change over 10 generations?

Step 3: Student-Led Planning: With your group, decide on one trait that will confer a selective advantage to one color or the other. For example, if you decide that brown caterpillars are more visible to predators, choose two brown caterpillars to die. Then, choose the caterpillars that will mate. Color will not affect the ability to mate, so close your eyes to choose two caterpillars that will mate. Pair them with mates. Then add two additional caterpillars, of the same color that mated, to represent the offspring of the mating. This signifies the end of this generation. Repeat for nine more generations and record the number of green versus brown caterpillars at the start and end of each generation.

Step 4: Critical Analysis: Record the number of individuals displaying each phenotype for five more generations (i.e., the number of brown and green caterpillars). Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group, and then write your answers in your notebook.

Guided Inquiry 2

Step 1: Student-Led Planning: Pick another phenotype that could apply a selective advantage (e.g., reproductive success or protection against predators). Describe your phenotype to your teacher, including what advantage it gives to individuals that possess that phenotype and how that advantage will be simulated with the pipe cleaners. After your teacher approves, describe your phenotype in your notebook.

Step 2: Hypothesize/Predict: Predict how natural selection would occur with the new phenotype and hypothesize how it will affect the phenotypic frequencies of the population after 10 generations. Use the same simulation procedure from Guided Inquiry 1. How would the frequency of brown and green change after a natural disaster? Write your hypothesis in your notebook.

Step 3: Think about how you will simulate change in selection that occurs after a natural disaster. For example, you might decide that damage from a fire is brief, so for one or two generations, only green caterpillars die. Then things go back to normal. Or maybe the damage is severe and lasts for five generations. Change your selection based on your decision and continue. Write your plan in your notebook.

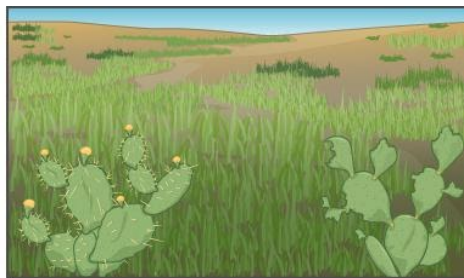
Step 4: Critical Analysis: Make a graph that depicts changes over the 10 generations. How does the percentage of each phenotype change over time? Discuss your answer with your group, and write it in your notebook.

Assessments

1. What factors might contribute to changes in a population? [SP 1, Concept Explanation; SP 6, Argumentation]
2. In the Arctic, there are white rabbits and brown rabbits. Which would be more likely to survive if there is extensive snow cover? As the climate changes (i.e., the temperature increases), what would you expect to happen? [SP 1, Concept Explanation; SP 6, Argumentation]
3. In a population of caterpillars, a mutation has suddenly arisen that makes green caterpillars less “tasty” to their predators. What would you expect to occur in the population over time? [SP 1, Concept Explanation; SP 6, Argumentation]
4. Observe what happened in Figure 18.2 and explain how it represents an example of natural selection. [SP 3, Visual Representations; SP 1, Concept Explanation]



1. A white-tailed deer prefers to eat from the spineless prickly pear cactus.



2. The prickly pear cactus with long spines grows flowers a few weeks later.

Figure 18.2: Describe the effect of natural selection that occurs in the two photographs.

[Answer Key]

1. Some factors that contribute to changes in a population are natural selection due to competition for resources, beneficial and harmful mutations, environmental changes, and greater reproductive success within populations.
2. The white rabbit would have a better chance at survival than the brown rabbit, as the white rabbit can more easily blend in with the white snow. The brown rabbit would be more subject to predation in the snow. If temperatures increase and it does not snow as much, brown fur would blend in with the bare soil, making the rabbit less obvious prey. A white rabbit would be more noticeable to a predator.
3. Predators would begin to avoid the green caterpillars because of their distaste for the newly mutated caterpillar, and they will find another source of food. As a result, over time, a greater percentage of the caterpillars would be green.
4. This image shows how organisms interact in an herbivore-plant relationship. Over time, the cactus with the spines will have greater reproductive success than the spineless cactus. Therefore, the frequency of cactus with spines will increase over time.

Lab 19: Plant Transpiration

This investigation addresses the following Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
1.1 Structure of Water and Hydrogen Bonding	SYI-1 Living systems are organized in a hierarchy of structural levels that interact.	SYI-1.A Explain how the properties of water that results from its polarity and hydrogen bonding affects its biological function.	SYI-1.A.3 The hydrogen bonds between water molecules result in cohesion, adhesion, and surface tension.
2.3 Cell Size	ENE-1 The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	ENE-1.C Explain how specialized structures and strategies are used for the efficient exchange of molecules to the environment.	ENE-1.C.1 Organisms have evolved highly efficient strategies to obtain nutrients and eliminate wastes to and from the environment.
2.8 Tonicity and Osmoregulation	ENE-2 Cells have membranes that allow them to establish and maintain internal environments that are different from their external environments.	ENE-2.I Explain how osmoregulatory mechanisms contribute to the health and survival of organisms.	ENE-2.I.1 Growth and homeostasis are maintained by the constant movement of molecules across membranes. ENE-2.I.2 Osmoregulation maintains water balance and allows organisms to control their internal solute composition and water potential, with water moving from areas of high water potential to areas of low water potential.
8.1. Responses to the Environment	ENE-3 Timing and coordination of biological mechanisms involved in growth, reproduction, and homeostasis depend on organisms responding to environmental cues.	ENE-3.D Explain how the behavioral and/or physiological response of an organism relates to changes in internal or external environment.	ENE-3.D.1 Organisms respond to changes in their environment through behavioral and physiological mechanisms.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.A Explain biological concepts and/or processes. 1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representations of biological concepts and processes.	2.B Explain relationships between different characteristics of biological concepts, processes, or models represented visually in applied contexts. 2.D Represent relationships within biological models, including diagrams.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or pose a testable question based on an observation, data, or a model. 3.B State the null and alternative hypotheses that are aligned to the questions, including variables and controls. 3.D Make observations, or collect data from representations of laboratory setups or results. 3.E Propose a new/next investigation based on evaluating evidence from a previous experiment.
SP 4 Representing and Describing Data Represent and Describe Data	4.A Construct a graph, plot, or chart. 4.B Describe data from a table or graph, including describing trends and/or patterns.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including applying equations in the curriculum, means, and rates. 5.C Use data to evaluate a hypothesis/prediction.
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.A Make a scientific claim. 6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. 6.E Predict the effects of change in one of more components in a biological system based on data.

Plants take in water from their roots. The water travels upward to all the plant's other parts, ending up in the leaves. Excess water evaporates from small openings on the undersides of the leaves called **stomata**. Stomata can open and close to regulate the loss or conservation of water, depending on the needs of the plant or in response to environmental conditions. The process of water evaporating from stomata is called **transpiration**.

Have you ever wondered how water moves upward against gravity without some kind of pump? The simple answer is that water moves from areas of high water potential to areas of low water potential. **Water potential** is the tendency of water to move from one area to another. In plants, the leaves tend to have lower water potential than the roots, so the movement is upward from under the ground toward the leaves. Different factors can affect whether water potential is high or low, including negative pressure (suction) caused by transpiration and the concentrations of solutes in the water and/or the surrounding soil.

When a plant does not have sufficient water moving through its structures and into its leaves, it can experience **water stress**, which can affect all processes within the plant. Water is the solvent through which plant materials move. Various things can cause water stress, including drought, high rates of transpiration, and high salinity in the soil.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- It may be helpful to begin the lab by giving the students a brief overview on the topic of plant transpiration. You can use real-life plant samples or a model of a plant to make the process more understandable to the students.
- Since the students will be working with a potometer, it would be useful to also demonstrate how to properly set up and use this instrument for the experiment.
- Upon finishing the overview of the topic, be sure to instruct students on the proper way to dispose of their plant samples and how to disassemble their potometer.
- Fresh, pre-cut plants should be available for this experiment. Have the proper quantity of plants you will need for your class size. Proper storage of plant samples should also be considered prior to this exercise to minimize premature decay of the plant material prior to the experiment.

In this lab, you will learn

- how to measure the rate of transpiration
- how to test and predict the way in which an environmental change affects the rate of transpiration
- how to observe patterns of and predict percentages of open versus closed stomata under different conditions

Activity 1: Pre-Assessment

1. **Answer the following questions in your notebook:** What environmental conditions do you think will most likely cause stomata to open? Which will most likely cause stomata to close? Explain your answer.

2. **Answer the following question in your notebook:** Why do you think the suction caused by transpiration is considered a negative-feedback mechanism?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 1: Measurement of Transpiration as Water Loss under Selected Conditions

You will conduct this first activity in three parts. First, you will assemble an apparatus called a potometer, illustrated in Figure 19.1. A potometer consists of a plant cutting stuck into one end of a U-shaped tube and a graduated cylinder stuck into the other end. This device creates water potential, which will allow you to measure transpiration. In the second part, you will collect data for transpiration under a set of control conditions. In the third part, you will choose from a list of variables to change the conditions for the plant and then you will collect transpiration data again using the modified apparatus.

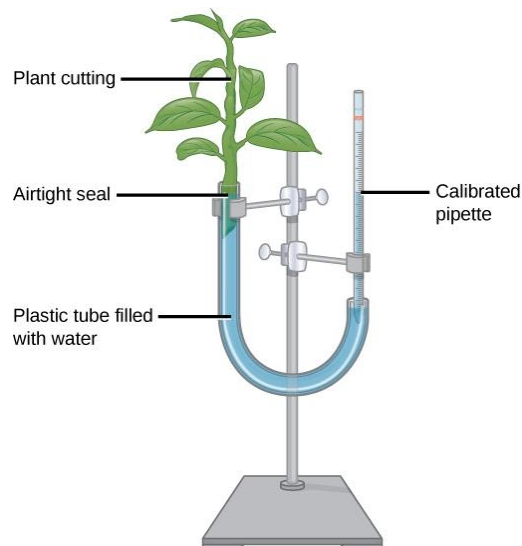


Figure 19.1: When transpiration takes place, water evaporates from open stomata. As the water potential difference increases between the bottom of the stem and the leaves, the stem draws in water from the tube, and the water level drops in the pipette.

Safety Precautions

- Use care with all glassware.
- Clean up any spilled water immediately to avoid slippage.
- Insert the pipette carefully to avoid breaking the tip.
- Do not allow electric cords to get wet.
- Be careful when handling hot lamps.

For this activity, you will need the following:

- Ring stand
- Plant stalk with leaves
- Incandescent lamp
- Clamps
- Plastic tubing
- Basin half-filled with water
- Calibrated 1.0-mL pipette
- Basin
- Syringe
- Petroleum jelly
- Fluorescent light or water tank with incandescent light
- Fan
- Water sprayer
- Plastic bag
- Timer

For this activity, you will work *in pairs*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Since this may be the first-time students have worked with a potometer, it may be beneficial to briefly lecture on the use of this instrument as well as to demonstrate how to properly use the instrument and take measurements prior to conducting the experiments. Since calibrated pipettes will also be used, remind the students of the importance of lab safety (e.g., students should *never* pipet anything by mouth).
- Since students will be measuring the level of water every five minutes, it will be helpful to have timers on hand to assist them in taking their measurements accurately.
- After introducing students to the topic, have them create their data tables prior to beginning the lab exercise to ensure that they are including the appropriate criteria in their lab reports for later analysis.

The following are recommendations for the Structured Inquiry for the Guided Inquiry for Activity 1:

- Students could remain in groups of two while doing the Guided Inquiry, but you may wish to have each student write their own hypotheses regarding the proposed outcomes of the experiment. This is to ensure that each student contributes equally and submits their own original content.

- You will want to check the students' hypotheses and results from the initial experimental design during the Structured Inquiry before allowing them to proceed with performing their experiment to ensure that they are on the right track and will not waste unnecessary materials.
- Since multiple variables will be tested in the Guided Inquiry portion of the exercise, have an ample supply of materials (e.g., fans, syringes, water sprayers, plastic bags, etc.) that they will need to conduct this portion of the exercise. They will also likely need advice on how to determine what controls to use for their experiment.
- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.
- In Step 3, clarify to students how to analyze their results. Additionally, providing a grading rubric for this activity with criteria describing each of the mandatory parts of this activity can help guide students to include the proper components in their experiment.

Structured Inquiry

Step 1: Set up the potometer, using Figure 19.1 as a guide. To reduce the formation of bubbles, cut the twig and insert it into the water-filled plastic tubing while underwater. Add the pipette and adjust water level as necessary so the starting point is high enough to allow for readable decreases during the experiment. If there are any air bubbles, you can use the syringe to remove them. Use clamps to attach the potometer to the ring stand, making sure the heights of the plant relative to the pipette are similar to those in Figure 19.1.

Step 2: Hypothesize/Predict: After you insert the stem into the tube, how much water do you think will move out of the pipette? Write your predictions in your notebook.

Step 3: Student-Led Planning: You will start with measuring changes in the water level for the plant under the typical light conditions of the room. Before you begin, make a data table to collect information about the water level and the change in water level as a function of time. You will take data every five minutes, for a total of 20 minutes.

Step 4: When you are ready, insert the stem, making sure it is submerged, and take an initial reading of the water level at time zero and subsequent readings every five minutes. Then, record the water loss in your data table every five minutes, for a total of 20 minutes. After the 20-minute period, remove the leaves from the photometer and set them aside, as you will need to measure the area of the leaf measured in the next step.

Step 5: Critical Analysis: Discuss with your partner how the first trial worked and if any adjustments need to be made to the setup to ensure more accurate results. Are the predictions made in Step 2 supported by the data? Can your results be improved? After the data collection, you will need to calculate a rate of transpiration per minute. To do this, you will take a water level reading every five minutes for 20 minutes for each part of the experiment. Then, you can graph the water measurements by time to estimate the rate of transpiration per minute. After this, you will need to determine the area of the leaf surface measured. Since there are multiple leaves of different sizes, you will trace each leaf on graph paper and add up the total area. The

final result of the experiments should show the rate of transpiration. Discuss with your partner and write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Choose from the following list of variables to alter the conditions for the plant:

- Use fluorescent light (if only an incandescent light is available, place a tank of water between the plant and the light source to absorb heat).
- Apply wind using a fan.
- Apply high moisture by spraying the plant with water and then enclosing it in a plastic bag.

How do you think your chosen variable will affect the rate of transpiration? Does the adjustment accurately mimic conditions in a natural environment? Why or why not?

Step 2: Student-Led Planning: How will you adjust your setup to incorporate your chosen variable? Create a plan with your partner. Then, have your teacher approve your plan before altering the setup. Once approved, alter your setup and create data tables for your experiment in your notebook.

Step 3: Collect the transpiration data using the potometer as in the Structured Inquiry. Record the measurements in your data table.

Step 4: Use the information in the Structured Inquiry to find the total surface leaf area of your plant sample and use it to calculate a final rate of transpiration for both trials. Graph your data in your notebook.

Step 5: Critical Analysis: How did the typical conditions compare with the altered conditions in terms of the rate of transpiration? Was the difference significant? Discuss your answers with your partner and write them in your notebook.

Assessments

1. One cutting has more leaves than another. Given this information, can you predict how the rate of transpiration would compare between the cuttings? [SP 3, Questions and Methods; SP 6, Argumentation]
2. Explain whether your prediction in the Guided Inquiry was accurate. Include a reason based on how the variable affected transpiration. [SP 1, Concept Explanation]
3. How might drought or flooding induced by climate change affect transpiration of plants, and how, in turn, would this affect the ecosystem? Give an example. [SP 6, Argumentation]

[Answer Key]

1. No, without knowing the surface area of the leaves on each plant, the estimated rate using only time and water changes is not reflective of how much transpiration would actually take place for a given plant.
2. Student answers will vary but should include accurate descriptions of how transpiration would be affected by the variable. For example, wind should cause an increase in transpiration because it would increase evaporation from the leaf.
3. Climate change can cause drought in areas, which would lead to water stress in a plant and cause transpiration to slow down, further reducing the amount of moisture in the ecosystem. Climate change can also cause flooding, which would lead to water saturation for plants, increasing transpiration significantly. Additionally, flooding may drown the plant entirely, which it could not survive. Reduction of plants in an ecosystem would have many detrimental effects, including loss of food sources and habitats for many other species.

Activity 2: Pre-Assessment

1. **Answer the following question in your notebook:** What environmental conditions could be applied to a plant that would cause it to open more of its stomata?
2. **Answer the following question in your notebook:** Do you think there is a condition that would result in mostly closed stomata?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 2: Comparison of Opening or Closing of Stomata under Selected Conditions

Plants will open or close stomata based on many factors. Stomata help regulate the water needs of the plant and exchange gases with the environment. If there is too much water inside the cells, the stomata will open to release the pressure. If water pressure is low, the stomata will remain closed to retain moisture. However, to keep water moving up from the roots and into the leaves, some stomata do need to open to build the negative pressure that allows roots to draw up water. Special cells, called **guard cells**, act like doors to open or close stomata.

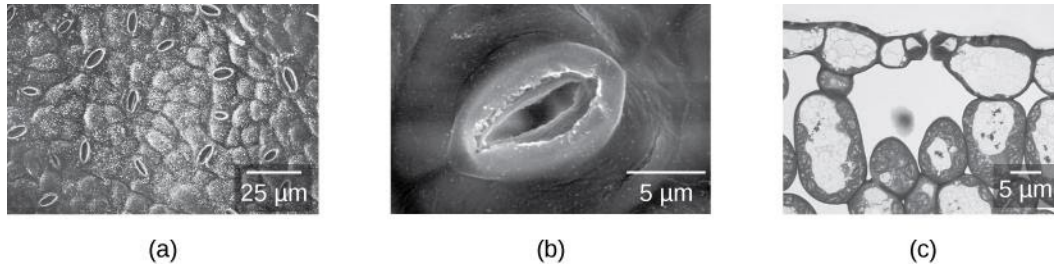


Figure 19.2: Transpiration takes place through open stomata, (a) generally scattered across the underside of leaves. (b) A single stoma. (c) A cross section of a stoma shows the two small guard cells above the large open air space within the leaf tissue. The guard cells contain darkened areas.

Safety Precautions

- Avoid directly inhaling nail polish fumes.
- Handle the microscope carefully and always carry it with two hands.

For this activity, you will need the following:

- Scissors
- Clear nail polish
- Empty microscope slides
- Microscope
- Leaves
- Clear cellophane tape
- Forceps

For this activity, you will work *in pairs*.

Activity 2 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 2:

- It may be useful to include a brief review on the different parts of a plant that are involved in plant transpiration. This can be given as part of the initial lecture before Activity 1, or you can introduce this topic prior to beginning Activity 2.
- Since students will be using a microscope for this exercise, it is recommended to describe to the students how the structure of stomata will look to assist them in their observations. Additionally, remind students of the proper use and care of a microscope (e.g., how to properly carry a microscope as well as how to clean and store it upon completion of the experiment).

- Since scissors will be used for this exercise, remind students of the tenets of lab safety. Also, you may want to consider having masks on hand to minimize the inhalation of the nail polish.
- Since slides will be used, it will be beneficial to demonstrate for students how to prepare slides of their samples. Additionally, have a sharps biohazard container on hand for proper disposal of broken slides.
- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.

The following are recommendations for the Structured Inquiry for the Guided Inquiry for Activity 2:

- Students could remain in groups of two while doing the Guided Inquiry, but you may wish to have each student write their own hypotheses regarding the proposed outcomes of the experiment. This is to ensure that each student contributes equally and submit their own original content.
- You will want to check the students' hypotheses and results from the initial experiment during the Structured Inquiry before allowing them to proceed with performing their new experiment, to ensure that they are on the right track and will not waste unnecessary materials.
- Since the students will be placing the leaves under different environmental/test conditions, multiple variables will need to be provided to assist them in this portion of the Guided Inquiry. Therefore, be sure to have an ample supply of the additional materials that they will need to conduct this portion of their exercises. Remind them that they will also need controls to use for this experiment.
- Instruct students on how to set up their data table prior to conducting their experiment so that it can be already prepared when they need to record their results.
- In Step 3, clarify to students how to analyze their results. Additionally, providing a grading rubric for this activity with criteria describing each of the mandatory parts of this activity can help guide students to include the proper components in their experiment.

Structured Inquiry

Step 1: Prepare slides of leaf samples at normal room conditions. To do this, you will cut a sample of a leaf and coat both sides of the leaf with clear nail polish. Allow it to dry completely by holding the sample and waving it gently in the air. Then, create wet mounts of the leaf samples and observe them under the microscope.

Step 2: Hypothesize/Predict: What percentage of open to closed stomata do you expect to find? Record your hypothesis in your notebook.

Step 3: Student-Led Planning: Prepare a data table to count the stomata. Then, place the slide under the microscope and count the number of stomata. Record your data in your table. If there is time, consider making more than one sample. Draw sketches of the samples.

Step 4: Critical Analysis: Is it simple or complicated to count the stomata? Is it possible to count the same ones more than once? How did you ensure this did not happen? What did the results show when the leaves from the two different trials were compared?

Guided Inquiry

Step 1: Hypothesize/Predict: Choose a condition to which you will expose a leaf sample for 20 minutes. How do you think the altered condition will affect the stomata? Record your ideas in your notebook.

Step 2: Student-Led Planning: Prepare a slide of the leaf sample by cutting a leaf and coating both sides with clear nail polish. Allow the leaf sample to fully dry before placing it on the microscope slide. Prepare a data table and count the number of closed and open stomata. Draw sketches of the samples.

Step 3: Critical Analysis: Compare your results to the results from the Structured Inquiry. What conclusion can you draw from the data? Write your answer in your notebook.

Assessments

1. In a certain habitat, the plants can keep their stomata open for most of the day. What does this suggest about the characteristics of the habitat? [SP 1, Concept Explanation]
2. Could a researcher estimate how many plants in an enclosed area that have their stomata open using the concentration of carbon dioxide in the air? Explain your answer. [SP 3, Questions and Methods; SP 1, Concept Explanation]
3. How could a research scientist use the methods in this lab to determine how to care for a given species of plant? [SP 3, Questions and Methods]

[Answer Key]

1. If plants can keep their stomata open for most of the day, this suggests that they live in a very humid environment, such as a cloud forest, where the air isn't dry enough to cause the interior of the plant to desiccate.
2. Open stomata would take in more carbon dioxide from the environment than closed stomata. Therefore, the researcher should find a lower concentration of carbon dioxide in the air if most of the plants had their stomata open and a lower concentration if most of the plants had their stomata closed.

3. A scientist could observe the average number of stomata open or closed under different environmental conditions to determine how much water the plant needs and the temperatures at which it can keep its stomata open and perform gas exchange.

Lab 20: Biodiversity in Leaf Litter

This investigation addresses the Required Course Content and Science Practices from the AP Biology CED:

REQUIRED COURSE CONTENT

Topic	Enduring Understanding	Learning Objective	Essential Knowledge
8.1 Responses to the Environment	ENE-3 Timing and coordination of biological mechanisms involved in growth reproduction, and homeostasis depend on organisms responding to environmental cues.	ENE-3.D Explain how the behavioral and/or physiological response of an organism is related to changes in the internal or external environment.	ENE-3.D.1 Organisms respond to changes in their environment through behavioral and physiological mechanisms (such as phototaxis).
8.5 Community Ecology	ENE-4 Communities and ecosystems change on the basis of interactions among populations and disruptions to the environment.	ENE-4.A Describe the structure of a community according to its species composition and diversity. ENE-4.B Explain how interactions within and among populations influence community structure. ENE-4.C Explain how community structure relates to energy availability in the environment.	ENE-4.A.1 The structure of a community is measured and described in terms of species composition and species diversity, including the application of Simpson's Diversity index. ENE-4.B.2 Interactions among populations determine how they access energy and matter within a community. ENE-4.B.4 Competition, predation, and symbioses, including parasitism, mutualism, and commensalism can drive population dynamics. ENE-4.C.1 Cooperation or coordination between organisms, populations, and species can result in enhanced movement or access to matter and energy.

8.6 Biodiversity	SYI-3 Naturally occurring diversity among and between components within biological systems affects interactions with the environment.	SYI-3.G Explain how the addition or removal of any component of an ecosystem will affect its overall short-term and long-term structure.	SYI-3.G.1 The diversity of species within an ecosystem may influence the organization of the ecosystem.
8.7 Disruptions to Ecosystems	EVO-1 Evolution is characterized by change in the genetic make-up of a population over time and is supported by multiple lines of evidence.	SYI-2.B Describe human activities that lead to changes in ecosystem structure and/or dynamics.	SYI-2.B.1 The distribution of local and global ecosystems changes over time. SYI-2.B.2 Human impact accelerates change at local and global levels.

SCIENCE PRACTICES

Science Practice	Skills
SP 1 Concept Explanation Explain biological concepts, processes, and models presented in written format.	1.C Explain biological concepts, processes, and/or models in applied contexts.
SP 2 Visual Representations Analyze visual representation of biological concepts and processes.	2.D Represent relationships within biological models including mathematical models, diagrams, and flow charts.
SP 3 Questions and Methods Determine scientific questions and methods.	3.A Identify or post a testable question based on an observation, data, or model. 3.D Make observations, or collect data from representations of laboratory setups or results. 3.E Propose a new/next investigation based on evaluation of a previous experiment.
SP 4 Representing and Describing Data Represent and describe data.	4.A Construct a graph, plot, or chart.
SP 5 Statistical Tests and Data Analysis Perform statistical tests and mathematical calculations to analyze and interpret data.	5.A Perform mathematical calculations, including mathematical equations in the curriculum (such as the Simpson Diversity index).
SP 6 Argumentation Develop and justify scientific arguments using evidence.	6.A Make a scientific claim. 6.B Support a claim with evidence from biological principles, concepts, processes, and/or data. 6.E Predict the causes or effects of a change in, or disruption to, one or more components in a biological system based on data.

According to recent records, Earth is inhabited by an estimated 8.7 million different species of organisms. All of these organisms live within a region of Earth called the biosphere. The **biosphere** is defined as the regions of Earth's surface, atmosphere, and hydrosphere that can sustain living organisms. A variety of ecosystems make up the biosphere. **Ecosystems** are composed of abiotic and biotic components that influence the biodiversity of the ecosystem. **Biodiversity** commonly refers to the variety and variability of life in a given ecosystem or on Earth as a whole.

Biodiversity is typically measured by species richness and species evenness. **Species richness** represents the number of different species in an ecosystem. **Species evenness** represents how close in number each species is in an ecosystem. The **diversity index** is calculated from species richness and species evenness.

In effect, the diversity index describes the biodiversity of an ecosystem. In simple terms, an ecosystem, such as the Antarctic ice sheet that is dominated by one or two species, is less diverse than an ecosystem, such as a tropical rain forest in which several different species have a similar abundance. However, two ecosystems with identical types of species may not have the same biodiversity. This is possible if one environment has a higher proportion of Species A and a lower proportion of Species B compared to another environment. Another complication of measuring biodiversity is that the biodiversity of a particular environment can vary over time, depending on seasonal changes in weather and other factors.

Leaf litter brings life to the uppermost layer of soil known as topsoil. Leaf litter contributes to the soil's humus component and provides energy, moisture, nutrients, and shelter to animals, bacteria, fungi, and protists. As leaves and other dying plant parts fall to the ground, they undergo the process of decomposition. **Decomposition** involves the recycling of energy and nutrients present in the cells and tissues of all organisms. During this process, dead cells and tissues are broken down by both the environment and organisms into molecules. Organisms that break down leaf litter and other dead matter are called **decomposers** or **detritivores**.

The amount and composition of leaf litter vary greatly between different types of ecosystems. For example, the leaf litter found in a wet area of a forest would likely contain more ash and cypress leaves than a dry area. In contrast, a drier section of the forest may have more oaks and linden leaves. Differences can even arise within an ecosystem, depending on the time of year and local variations in the terrain.

In this activity, you will be collecting samples of leaf litter from different locations. Your teacher will instruct you on where to collect the leaf litter samples. Based on what you learned about leaf litter variation, it is important to take notes on the area from which you collect the leaf litter. In addition, describe the climate of each area as well as the environmental conditions at the time you collect the leaf litter. You will then be asked to construct a Berlese funnel to isolate any organisms living in the leaf litter.

The Berlese funnel was designed specifically for trapping and separating insects from ground litter. It was developed by Italian entomologist Antonio Berlese in 1927 and works by taking advantage of the negative phototactic responses of leaf litter organisms. A diagram of a Berlese funnel is shown in Figure 21.1. It consists of a funnel containing a mesh and a lamp above the funnel. Leaf litter is placed in the Berlese funnel on top of the mesh screen. A container filled with a preservative (or water) is placed under the lower opening of the funnel. When the lamp is turned on, the heat and light produced by the lamp dry out and warm up the leaf litter. This in turn produces an unfavorable environment for the leaf litter organisms and drives them to the bottom of the funnel. The organisms then fall through the mesh into the container where they can be collected.

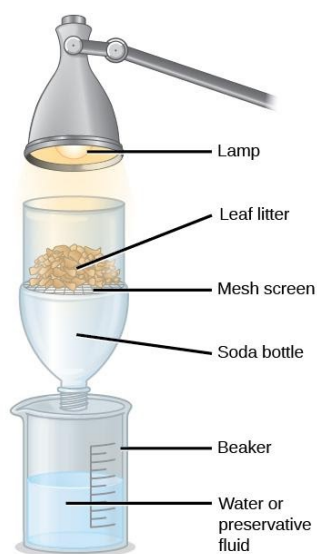


Figure 21.1: Design of a Berlese funnel.

In this activity, you will build a Berlese funnel to remove organisms from your leaf litter samples. Then, you will use your data to mathematically calculate the biodiversity of the leaf litter using a diversity index.

Instructor Introduction Notes

The following are recommendations for introducing this lab's background content to your students:

- If your students have not yet had an ecology introduction, it may be helpful to show a diagram and explain the different levels of ecological organization (organism, population, community, ecosystem, biome, and biosphere).

- Species richness and evenness can be introduced using a hypothetical data set. For example, a table containing four species can be set up, with the species listed as the row headers. Then, two columns could be set up, labelled Area 1 and Area 2 (or similar). Fill in the population sizes of the different species so that the numbers are more similar (greater species evenness) than the second area. You can then state that the two areas have similar species richness (because they have the same number of species) but differ in species evenness. If you drop one species to zero population in one of the areas, then the two areas will have different species richness.
- A diagram of a typical soil surface community may be shown to help students visualize the habitat they are about to study, as well as soil cores from different types of soil to show how the soil layers differ among different ecosystems. Point out the layers students are sampling (leaf litter plus part of the uppermost layer of soil).
- A drawn diagram, on the board, of how the Berlese funnel works is beneficial. You can explain the different parts of the funnel, and students could draw and label the diagram in their own notes as you explain each component.

In this lab you will learn

- how to collect and separate organisms from leaf litter
- how to use images or a key to identify leaf litter and soil organisms
- how to use a mathematical formula to calculate the diversity index as a means of explaining the biodiversity of leaf litter environments

Activity 1: Pre-Assessment

1. **Answer the following question in your notebook:** Explain why organisms living in leaf litter display negative phototaxis.
2. **Answer the following question in your notebook.** Leaf litter typically contains organisms that live in the upper layers of the topsoil. Explain the environmental factors (both of the physical environment and of other species) that would determine the number and types of organisms living in the leaf litter.
3. **Be prepared to discuss the answers to questions 1 and 2 with the class.**

Activity 1: Collect Leaf Litter and Set-up Berlese Funnel Traps

Safety Precautions

- Use caution when collecting leaf litter and other environmental samples. Watch out for sharp objects, harmful plants, and dangerous animals that may sting or bite. Wash your hands as soon as possible after collecting the samples.
- Do not touch the light bulbs on the Berlese funnel apparatus, as they are on for long periods during this lab and will likely become hot enough to cause burns.
- Do not ingest the alcohol in the collection beaker, as it is not edible and will cause extreme sickness if ingested.

- Wear safety goggles throughout the laboratory activity.
- It is highly recommended that you wear rubber gloves and a lab apron when handling any chemicals.
- Immediately inform your teachers of spills.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent slippage.
- Immediately wash your hands if they directly come in contact with any chemicals. It is important to wash your hands after the laboratory activity to avoid any possible contamination of other surfaces with chemical residues that might be on your hands.

For this activity, you will need the following:

- Plastic sandwich bags
- Notebook or index cards
- Pencil
- Ruler (cm)
- Waterproof marker
- Thermometer
- The Leaf Litter Sample Information Form
- Two-liter plastic soda bottle
- One-quart jar or 500-mL beaker
- Mesh screen
- Moveable neck lamp with a 60- or 100-watt incandescent bulb
- Scissors
- Tape
- Ethanol (70–95 percent) or isopropanol (70 percent)

For this activity, you will work in *pairs or groups*.

Activity 1 Instructor Preparation and Teaching Tips

The following are recommendations for the Structured Inquiry for Activity 1:

- Remind students days in advance that they are going outside for this lab and should wear appropriate clothing as well as shoes that they don't mind getting muddy.
- Students working in pairs may require a bucket or other carrying container for their equipment. If taking additional environmental data (see next bullet), then groups of at least three students may be best, given the greater amount of time required to take additional environmental data.

- The environmental data that students take at their sites can be expanded to include (1) soil moisture using a simple soil moisture meter sold at garden supply stores, (2) relative humidity taken with sling psychrometer (much teach how to calculate humidity from this as well), as well as soil nitrogen and phosphorus levels using simple test kits available at garden stores, and (3) precipitation levels using a rain gauge that students set up during a previous lab.
- Select naturally forested sites for student sampling, if possible. Disturbed soil or those near development or walkways may have a depressed leaf litter community.
- In constructing the Berlese funnel, use a mesh that has no greater than 1-cm² openings. This will prevent excessive debris from falling into the collection beaker. Water can be substituted for alcohol in the beaker; however, some organisms may be able to escape from the surface of the water if left for too long. Avoid filling the container more than halfway to the top if using water.

Structured Inquiry

Part 1: Collecting the Leaf Litter Samples:

Note—You may be instructed to collect two litter samples for this activity. It is very important to pay attention to the environmental conditions of each area where you collect a sample. You will be recording this information in your notebook. It is also important to get a complete representation of the leaf litter. The sample bags should include the full depth of leaf litter.

Step 1: Hypothesize/Predict: Describe the environmental conditions for the leaf sites that you sampled. Explain how the differences in the two sites may influence the number of species in each sample. How would the biodiversity of predators in the leaf litter community affect their prey and vice versa? Write your ideas in your notebook.

Step 2: Obtain the plastic bags, index cards or notebook, pencil, ruler, waterproof markers, and thermometer from your teacher.

Step 3: Go to one leaf litter sample area selected by your teacher.

Step 4: Use the ruler to mark off a 15-cm by 15-cm square of ground to sample.

Step 5: Student-Led Planning: Discuss with your partner how you will record the following information in your notebook or on the Leaf Litter Sample Information Form (Figure 21.1).

- Date and time of day
- Location
- Type of ecosystem
- Temperature
- Sky conditions
- Atmospheric conditions
- Ground conditions

Also, make a quick drawing or take a photograph of the area that you will be sampling.

Table 21.1: Leaf Litter Sample Information Form

Your Name (s):		Sample #
Date:	Time:	Location:
Circle Type of Ecosystem: (Forest) (Grassy field) (Swamp) (River bank) (Beach) (Desert) (Other)*		
Temperature	Sky: <i>Check one:</i> (Full sun) (Partly cloudy) (Overcast) (Night sky)	
Circle Atmospheric Conditions: (No rain) (Drizzly) (Slight rain) (Moderate rain) (Heavy rain) (Snow) (No wind) (Slight breeze) (Moderate wind) (Heavy wind) (Dry) (Slightly humid) (Humid)		
Ground conditions: (Full shade) (Partial shade) (No shade)		
*Explain other:		

Step 6: Collect leaf litter from the 15-cm by 15-cm area. Remember to include the uppermost layer of the soil. Place the sample in the plastic bag. Use the waterproof marker to write your name or names on the bag and provide a sample number. For example, if collecting multiple samples, label the first bag Site 1 and the second bag Site 2.

Step 7: Critical analysis: Explain why it is important to record the date and time of day for your sample. Describe how temperature, atmospheric conditions, and ground conditions could affect the organisms found in your sample. Record your answers in your lab book.

Constructing the Berlese funnel:

Step 8: Use the scissors to cut off the bottom of a 2-liter plastic soda bottle.

Step 9: Cut and bend the mesh screen so that it fits snugly inside the upside down two-liter plastic soda bottle. Make sure the mesh screen is completely in contact with the plastic walls of the bottle. This will prevent leaves from going past the mesh screen.

Step 10: Pour approximately 50 mL of the ethanol or isopropanol solution into the 1-quart jar or 500-mL beaker. This is your collecting container.

Step 11: Place the upside down two-liter plastic soda bottle into the mouth of a one-quart jar or 500-mL beaker. It should look like the Berlese funnel image in Figure 21.1. Use the tape and the ruler to secure the two-liter plastic soda bottle and hold it in place.

Step 12: Carefully carry your Berlese funnel to a location designated by your teacher. It may need readjustment to make sure that it is secure. Place the leaf litter from one of the samples into the 2-liter plastic soda bottle.

Step 13: Place the moveable neck lamp near your Berlese funnel. Adjust the light so that it directly shines on the leaf litter sample in the funnel. The light should be about 20 cm above the top of the Berlese funnel. It will take about two days of incubation for a majority of leaf litter organisms to enter the collecting container.

Step 14: Clean up:

- Clean and return any of the supplies that you do not need.
- Pour any excess ethanol or propanol down a sink unless your teacher instructs otherwise. Run water into the sink until the smell of the ethanol or propanol is not evident.

Step 15: Critical Analysis: Use the Internet to research the types of organisms that live in leaf litter. Discuss with your partner what you would expect to find in the leaf litter sample you collected. Why might your sample be lacking in some of the leaf litter organisms you researched on the Internet? Discuss with your partner why your sample may differ from samples collected by other students. How could the size of the openings in the mesh screen affect the results of an analysis using a Berlese funnel? Write your answers in your lab notebook.

Assessments

1. A student read that oak leaves release harmful substances upon decaying. The student wants to determine whether these substances from the oak leaves reduce the biodiversity of leaf litter organisms in a forest. How would you design an experiment to test this hypothesis? [SP 3, Questions and Methods]
2. Based on the experimental protocol you followed using the Berlese funnel, what environmental conditions are likely preferred by leaf litter organisms? How do you know? [SP 4, Representing and Describing Data]
3. What adaptation of leaf litter organisms is exploited by the Berlese funnel? [SP 1, Concept Explanation]
4. Your teacher asks you to investigate whether heat or light is the major factor in separating leaf litter organisms in a Berlese funnel. Explain how you would design an experiment to test this. [SP 3, Questions and Methods]
5. Pretend you are working as a scientist in a botanical garden. The director asks you to test the effects of fertilizer on the biodiversity of leaf litter organisms in the botanical garden. Explain how you would design an experiment to test this. Write your answers in your notebook. [SP 3, Questions and Methods]

[Answer Key]

1. The most appropriate design would include a long-term field experiment with two sample plots in an area that has a variety of trees, including oak. One plot would be the control, an area that is undisturbed. In the other plot, the student would regularly remove the oak leaves. The student would then perform the analysis using a Berlese funnel. The student could also design an experiment in a terrarium containing leaf litter organisms. In this setup, oak leaves and other types of leaves can be tested separately.

2. The Berlese funnel dries and heats the leaves, which repels the leaf litter organisms. This suggests that the leaf litter organisms are adapted to cooler and more moist conditions.
3. The leaf litter organisms have evolved behavioral mechanisms to avoid unfavorable conditions. They move away from areas that are hot and dry, preferring to stay cool and moist. This behavior allows the Berlese funnel to trap leaf litter organisms.
4. The student would have to set up two different Berlese funnels. One would use a cool light source, and another would have a non-illuminating heat source above the leaf litter.
5. The student would first set up several plots having the same environmental conditions in a particular region of the botanical garden. The student would then apply different amounts of fertilizer to the plots. One plot must be kept as a control with no fertilizer. Then the student would use a Berlese funnel setup to determine whether biodiversity differences exist.

Activity 2: Pre-Assessment

1. **Answer the following question in your notebook:** Explain how the temperature and rainfall of an ecosystem could affect the biodiversity of leaf litter.
2. **Answer the following question in your notebook:** Describe how the type of vegetation in an area can affect the biodiversity of leaf litter organisms.
3. **Be prepared to discuss the answers to questions 1 and 2 with the class.**

Activity 2: Estimate of the Index of Biodiversity and Independent Project

As in any environment, the biodiversity of the leaf litter habitat is affected by interactions between each organism and between the organisms and the physical features of the environment. In this activity, you will compare leaf litter from different areas to determine whether location affects the biodiversity of organisms living in the leaf litter. You will collect the organisms separated by the Berlese funnel and calculate the overall biodiversity of your sample. Biodiversity will be measured using a mathematical tool called the **Simpson Diversity Index**. It is one of many ways of representing biodiversity. Calculating the Simpson Diversity Index requires you to keep track of the number of different groups of organisms collected and the abundance of each. The Simpson Diversity Index provides a number scaled from zero to one, with zero being the lowest biodiversity and one being the highest. In this activity, you will characterize and count the leaf litter organisms in order to calculate the Simpson Diversity Index.

Safety Precautions

- Wear safety goggles throughout the laboratory activity.
- It is highly recommended that you wear rubber gloves and a lab apron when handling any chemicals.
- Immediately inform your teachers of spills.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.

- Clean up any spilled water or other fluids to prevent slippage.
- Immediately wash your hands if they directly come in contact with any chemicals. It is important to wash your hands after the laboratory activity to avoid any possible contamination of other surfaces with chemical residues that might be on your hands.

For this activity, you will need the following:

- Berlese funnel with leaf litter sample from Activity 1
- Several Petri dishes per group
- Dissecting microscope or hand lenses
- Forceps or tweezers
- Dissecting pins
- Pipette or eyedropper
- Small jar with a screw top
- Blank piece of paper
- Ethanol (70–95 percent) or isopropanol (70 percent)

Supplemental Information

- Soil or leaf litter organism keys, charts, and information:
<http://www.amnh.org/content/download/35188/518925/file/LifeInTheLeafLitter.pdf>
<http://www.blm.gov/nstc/soil/communities/index.html>
<http://www.hope.edu/academic/biology/leafitterarthropods/>
<http://treesforlife.org.uk/forest/forest-ecology/decomposition-and-decay/>
http://www.una.edu/faculty/microaquarium/leaf_litter_arthropods.htm
- Internet access to Simpson's Diversity Index website (optional):
<http://www.countrysideinfo.co.uk/simpsons.htm>
- Internet access to Biodiversity Calculator:
<http://bpmsg.com/academic/div-calc.php>

For this activity, you will work *alone, in pairs or in groups*.

Activity 2 Instructor Preparation and Teaching Notes

The following are recommendations for the Structured Inquiry for Activity 2:

- It will likely be difficult for students to identify many of the leaf litter organisms down to the species level, and they will need help with the characteristics in question. Review your chosen key(s) carefully and investigate some organisms under the microscope before lab so you can help students with this step.
- To clarify relative abundance to students, perhaps using the number of girls versus boys in your class as a quick example.

- Emphasize that a Simpson's Diversity Index value close to zero means very low biodiversity, while a value close to one is a very high biodiversity.

The following are recommendations for the Structured Inquiry for the Guided Inquiry for Activity 2:

- Students could remain in groups while doing the Guided Inquiry, but you may wish to have each student write their own paper or have each student graded individually on their contributions during a presentation. This is to ensure that each student contributes equally to a group project.
- You will want to check each student's hypothesis before he or she proceeds with research to ensure that it is testable, given the available equipment and time. The student will also likely need advice on site selection.
- Students often will not include all vital components of a research paper or presentation due to their inexperience. Providing a grading rubric for papers or presentations can guide students to include the proper analyses and critical thinking in their papers/presentation.
- In Step 6, clarify to students the difference between error analysis (conditions in the experiment they just performed that could have skewed the results) compared to creating new questions. Students will often come up with new research questions that simply involve re-running their experiment with the errors corrected. Tell students that they should come up with a completely new hypothesis based on the results of their first study.

Structured Inquiry

Now that you have collected your leaf litter organisms, it is time to identify the organisms in your sample. This involves categorizing the organisms into groups at the species or genus level. It is important to record your information in a data table to help organize the data for biodiversity calculations. Additionally, you should keep your sampled organisms in a container in case you need to confirm your data later. It also helps to have your sample in case you made an error identifying an organism. You can calculate the Simpson Diversity Index by either using the mathematical formula or entering the data in an online diversity index calculator.

Step 1: Draw a data table in your lab notebook like that shown in Table 21.2. Make enough rows to account for up to 20 different organisms that you may collect from the leaf litter.

Table 21.2

Species	Absolute Abundance (n)	Relative Abundance
Species 1		
Species 2		
Species 3		
Species 4		
Total number of different species =	Total number of organisms collected (N) =	Should total roughly 1.00

Step 2: Move your incubated Berlese funnel to your workstation.

Step 3: Carefully disassemble the unit so that you can remove samples from the collection container.

Step 4: Add about 20 mL of ethanol (70–95 percent) or isopropanol (70 percent) to the screw top container.

Step 5: Use forceps or a pipette to place small samples of organisms from the collecting container into a Petri dish.

Step 6: Place the Petri dish under the dissecting microscope.

Step 7: Use the suggested online identification charts or keys to categorize the organisms in the leaf litter sample. You should do your best to identify the organisms down to the species or genus levels.

Step 8: Record the information in your data table. Carry out the calculations on your data as shown in Table 21.3.

Table 21.3

Species	Absolute Abundance (n)	Relative Abundance
Species 1	36	$36/87 = 0.413$
Species 2	27	$27/87 = 0.310$
Species 3	10	$10/87 = 0.115$
Species 4	14	$14/87 = 0.162$
Total number of different species = 4	Total number of organisms collected (N) = 87	Should total roughly 1.00

Step 9: Pour the contents of the Petri dish into the screw top jar and repeat. Continue until you have collected all the organisms from your collecting container.

Step 10: Clean up:

- Dispose of the used leaf litter in a garbage basket.
- Clean the disassembled Berlese funnel and return any reusable components.
- Clean and return any other supplies.
- Pour any leftover alcohol solutions in a sink and flush the sink with water until the alcohol smell is not evident.
- Store your screw top container in a designated area as instructed by your instructor. When directed by your instructor, the organisms should be poured into a sink and washed down the drain.

Step 11: Using the information in your data table, calculate the Simpson's Diversity Index value for your sample. This can be done in one of the following ways:

- Mathematically: Read the information on the Simpson's Diversity Index website at <http://www.countrysideinfo.co.uk/simpsons.htm>. Then use the following formula to determine the Simpson's Diversity Index value (n = total number of organisms of a particular species, N= the total number of organisms of all species).

Simpson's Diversity Index = $1 - [\text{Sum of } n(n-1) / N(N-1)]$. For example, using Table 21.2, the calculation would be:

1. Simpson's Diversity Index = $1 - [(36 \times 35) + (27 \times 26) + (10 \times 9) + (14 \times 13) / (87 \times 86)]$
2. Simpson's Diversity Index = $1 - (2,234 / 7,482)$
3. Simpson's Diversity Index = $1 - 0.30$
4. Simpson's Diversity Index = 0.70

- Online Simpson's Diversity Index Calculator:
 1. Go to the online calculator at <http://bpmsg.com/academic/div-calc.php>.
 2. Use the New Input area to enter the number of categories of organisms in the designated box. For the sample in Table 21.2, you would enter 4 and press the Submit button for the New Input area.
 3. In the data input chart, enter the name of the category (species or genus) and the number of organisms in the category (n). For our example, we would enter the information as shown in Table 21.4.

Table 21.4

No.	Category	Value
0	Species 1	36
1	Species 2	27
2	Species 3	10
3	Species 4	14

4. When done entering your data, press Submit in the Submit Values area.
5. Subtract the Simpson Dominance (R1) value from 1 to calculate the Simpson's Diversity Index. For our example, the Simpson Diversity Index is 0.3017. So, the Simpson's Diversity Index = $1 - 0.30$ which is 0.70.

A Simpson's Diversity Index value close to zero means very low biodiversity, while a value close to one is a very high biodiversity.

Step 12: Record the Simpson's Diversity Index value for your sample (or samples, if you analyzed multiple samples).

Step 13: Critical Analysis: Compare the biodiversity of your sample to the biodiversity observed in samples collected by other students in class. Use the information from each sample's Leaf Litter Sample Information Form to explain any similarities or differences between your sample and the other samples. Discuss with your partner how differences in the environment may explain the variations in the biodiversity data from different samples collected by other students.

Guided Inquiry

Step 1: With your teacher's direction, you will now perform an independent research project comparing the biodiversity of leaf litter organisms among different leaf litter sites using the Berlese funnel and Simpson's Diversity Index. Based on the results of your Structured Inquiry, was any particular organism predominant in the leaf litter? What could be a reason for one particular organism dominating the leaf litter community? Using the Internet, search for resources describing the diets of leaf litter organisms. Compared to the population of organisms that feed on decaying leaves, would you expect the predator population to be larger or smaller? Also, research how the physical conditions of the leaf litter, such as temperature and moisture, may affect leaf litter biodiversity. Take notes in your notebook and print off your resources as needed.

Step 2: Hypothesize/Predict: Based on your research, think of a question you wish to explore about the leaf litter community, such as how does the diversity of leaf litter species change in sites that have different moisture levels. Write your question in your notebook. Then create a clear and specific hypothesis that you will test for your project. Present both your question and your hypothesis to your teacher for approval.

Step 3: Student-Led Planning: Discuss with other students the best way to test your hypothesis. Try to think of ways to control for external variables, especially when determining where and how you will collect your leaf litter. Your teacher may have you write and submit an experimental plan for review.

Step 4: Collect leaf litter according to your experimental plan. Then, run your leaf litter through one or more Berlese funnel setups as described by your experimental plan. Set up your funnel as described in Activity 1. Remember to set up each funnel in the same way for each leaf litter sample and run each for the same amount of time. Then, determine the diversity of leaf litter organisms as described above. Calculate the Simpson's Diversity Index for your samples.

Step 5: Combine and analyze your data as necessary, based on your hypothesis. You may be presenting your data to the class or writing it up as a lab report as instructed by your teacher. Create graphs, if possible, or use other methods to neatly and efficiently present your results.

Step 6: Critical Analysis: Was your hypothesis supported or unsupported by your results? What do your results indicate about the diversity of leaf litter organisms under different environmental conditions? How could you improve your experiment and what question could you investigate next? Include answers to these questions in your presentation or lab report, as directed by your teacher.

Assessments

1. Describe an experiment to determine whether evolution is occurring in the leaf litter for an environment in which the type of vegetation dominating the area is changing. [SP 3, Questions and Methods]
2. Describe an experiment to determine how the nutrient levels of leaves affect the biodiversity of leaf litter. [SP 3, Questions and Methods]
3. Describe an experiment to determine the effect of moisture levels on the types of insects and spiders in leaf litter. [SP 3, Questions and Methods]
4. Explain why the leaf litter you analyzed produced the Simpson's Diversity Index value that it did. [SP 1, Concept Explanation; SP 5, Statistical Tests and Data Analysis]

[Answer Key]

1. An appropriate design would include a long-term biodiversity study to determine whether one or more organisms are changing in some type of characteristic that correlates to environmental change. The phenotypic change would then require further study.
2. An appropriate design would include the collection of many leaf litter samples in the same region, and measurement of the leaves for their nutrient contents. A biodiversity study could then be performed to determine whether variations in biodiversity correlate to leaf litter nutrient levels, as measured by the amount of carbohydrates, lipids, or proteins in the leaves.
3. An appropriate design would include a series of studies on the populations of insects and spiders in leaf packets having different moisture levels. The relative dominance of the insects and spiders could then be measured to determine whether the populations respond to the different moisture levels.
4. Answers will vary. Environmental factors can affect one or more types of organisms in the leaf litter, based on the organisms' adaptations to a particular environmental condition. For example, a lack of moisture might decrease the earthworm population but not the spider population. Earthworms are much more sensitive to drying than spiders. Also, the decrease in one species may affect the populations of organisms that interact with it.

Extension Activities:

1. Design a study to show how pesticide applications could affect the arthropod biodiversity of soils. Explain why it is important to demonstrate this using a controlled experiment and explain the accurate procedures for sampling soil arthropods.
2. Design a study to show how the biodiversity of plants in a vacant lot varies over a five-year period. Use that information to explain how local animal biodiversity may be affected by changes in the vacant lot's plant biodiversity.