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Biology

for AP® Courses

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College Biology for AP® Courses Lab Manual

Student 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 testable.

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^{o} ramp will be lower than the speed of the vehicle traveling down the 60^{o} 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

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In this lab, you will learn

- how to safely work in the lab;
- how to use the scientific method;
- how to measure weight and volume.

Activity 1: Pre-Assessment

1. Name one piece of lab safety equipment. What is it used for?
2. What is one hazard of the lab and why it is hazardous?
3. Discuss the answers to questions 1 and 2 with the class.

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

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. Search online for *Lab Techniques & Safety: Crash Course Chemistry #21.*

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.”

Assessments

1. Describe what you would do in each of the following situations.
    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.
3. Name one rule that you learned from the video.

Activity 2: Pre-Assessment

1. When doing an experiment, why is it beneficial to alter only one experimental condition at a time?
2. 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 the class.

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*.

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, 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? 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?
2. You observe that plants sitting in one location in your house grow faster than plants sitting in another location. Create a testable hypothesis to test why this occurs. Then, describe an experiment that would test your hypothesis that includes controls.

Activity 3: Pre-Assessment

1. What are the conversions for the following measurements?
    a. 50 ml = ___ L
    b. 20 g = ___ mg
    c. 700 µl = ___ ml
    d. 1 gal = ___ pt
    e. 2000 ml = ___ L
2. **V**isit 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 the class.

Activity 3: Measurement

In the 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

Figure 1.3

Figure 1.4

Figure 1.5

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*.

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 to the amount 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, take the measurements you need to calculate the density of both objects. Write your final densities in your notebook.

Step 3: 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?
2. What is the most likely instrument used to measure the following volumes?
    a. 200 ml
    b. 50 µl
    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.

Lab 2:
Introduction to Microscopy

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Activity 1: Pre-Assessment

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.

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*.

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.

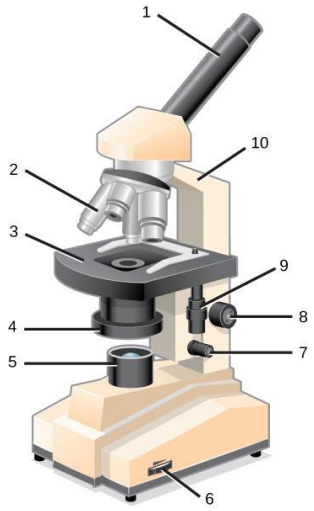

Figure 2.1: Can you identify all of the parts of the microscope?

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.

Assessments

1. What are three precautions you must take to prevent damage to the microscope?
2. A scientist uses a 40× objective to observe his specimen. He has a 10× ocular lens. What is the total magnification of the object?
3. A magnifying glass is also used to magnify objects. How is a compound microscope different from a magnifying glass?
4. Draw how Figure 2.2 would appear under a compound microscope.

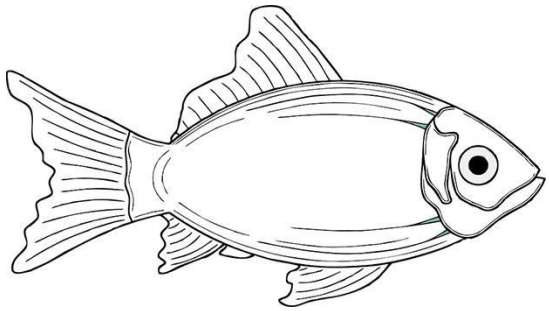

Figure 2.2: This illustration shows a simple line drawing of a fish facing right.

Activity 2: Pre-Assessment

1. 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. 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*.

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.
2. As you increased in power on the microscope, the image became darker. Why do you suppose this is so?

Activity 3: Pre-Assessment

1. 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. 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*.

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?
2. *Elodea* plants appear as solid green objects to the naked eye, but appear mostly clear under a microscope. Explain this phenomenon.
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?
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 of in the open.

    Explain how the invention of the microscope would lead to the replacement of both of these theories with the cell theory.

Lab 3:
Macromolecules in Common Food

Bio-
logy

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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

1. A chemical indicator called Benedict's solution turns from blue to brick red in the presence of certain monosaccharides. How would the 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 the 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 carbohydrates activities while the other team member does the tests on the lipids and the proteins.

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.

Step 1: Collect eight test tubes, a test tube rack, the grease pencil or marker, and a 10 ml graduated cylinder.

Step 2: Write out a procedure for calibrating your eight test tubes.

Step 3: Get your procedure approved by your teacher before you continue with the calibration.

Step 4: Use the grease pencil or marker provided by your teacher to draw the calibration lines.

Step 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.

Test tube number	1	2	3	4
Test solution	Distilled water	Glucose test solution	Distilled water	Starch test solution

Table 3.1

Step 2: Hypothesis/Predict: 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^{o}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 3.1 and Figure 3.2. 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?

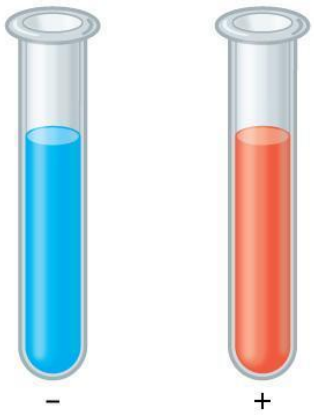

Figure 3.1: Benedict's test results.

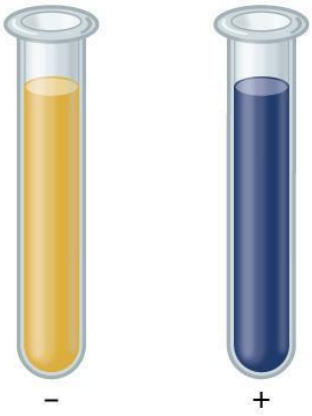

Figure 3.2: Iodine test results.

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.

Test tube number	**1**	**2**	**3**
Test solution	Distilled water	Dilute protein solution	Concentrated protein solution

Table 3.2

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 3.3. 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 3.3: 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 3 clean test tubes in the test tube rack and use the grease pencil or marker to number each test tube as shown here.

Test tube number	**1**	**2**	**3**
Test solution	Distilled water	Water mixed with vegetable oil	Vegetable oil

Table 3.3

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 3.4.

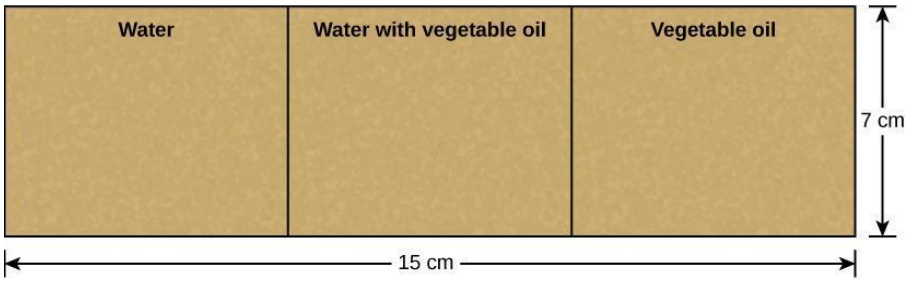

Figure 3.4: Labeled section of brown paper bag for lipid test.

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 3.5. 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.

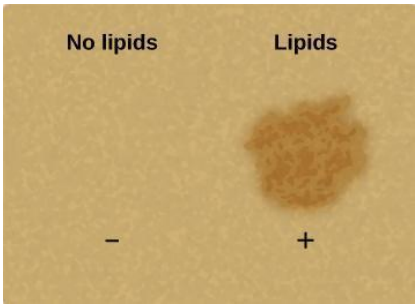

Figure 3.5: Paper spot test for lipids.

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?
    b. Which would be the correct apparatus for calibrating a test tube in two 1 ml increments?
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.
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.
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.
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.
6. Use the image of the Benedict's test Figure 3.6 to help you design an experiment to compare the glucose levels of different foods.

Figure 3.6: Benedict's test results for glucose concentration.

Lab 4:
Prokaryotic and Eukaryotic Cells

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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 your observations
- how and why microscope stains are used when viewing cells under the microscope

Activity 1: Pre-Assessment

1. What is the difference between the cells of a bacterium and the cells of your own body?
2. 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 4.1, 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.

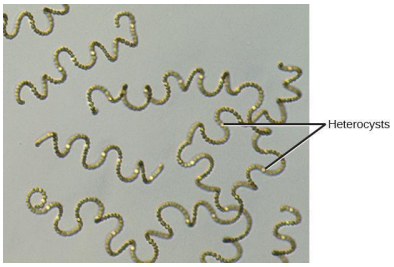


Figure 4.1: Cyanobacteria *Nostoc* with larger heterocysts.

Safety Precautions

- Be careful when 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.
- Wash your hands with soap and water after handling live organisms.

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

For this activity, you will need the following:

- Light compound microscope (10×, 40×, 100×)
- 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)

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 4.2: 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 in 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 4.3. 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.

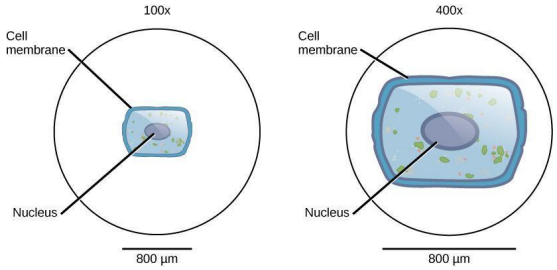


Figure 4.3: Example of a student drawing including labels and magnification.

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 the color what 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.
2. How would internal membrane-bound structures, such as chloroplasts and mitochondria, allow chemical reactions to occur more efficiently in cells?

Activity 2: Pre-Assessment

1. What new structures would you observe in *Elodea* cells which are not present in a cyanobacterium cell?
2. 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 4.4, also have a **nucleus** with DNA, a **large central vacuole** full of water and other important substances for maintaining life such as carbohydrates, non-nutrients, wastes, and help maintain cell pressure.

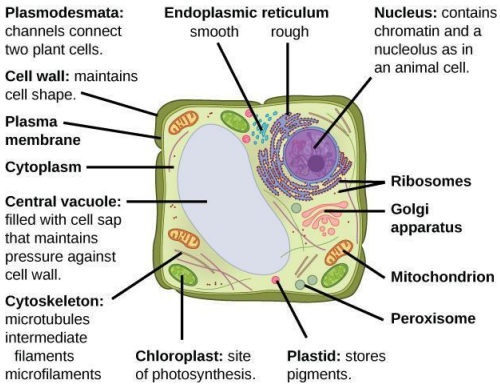


Figure 4.4: Structures found in a typical plant cell.

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.*

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 are you expecting 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 4.4 can you identify in the *Elodea* cells? What about the onion cells? Why do you think this is the case?
2. What are the similarities and differences between cyanobacterium and a plant cell?

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 4.5. 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.

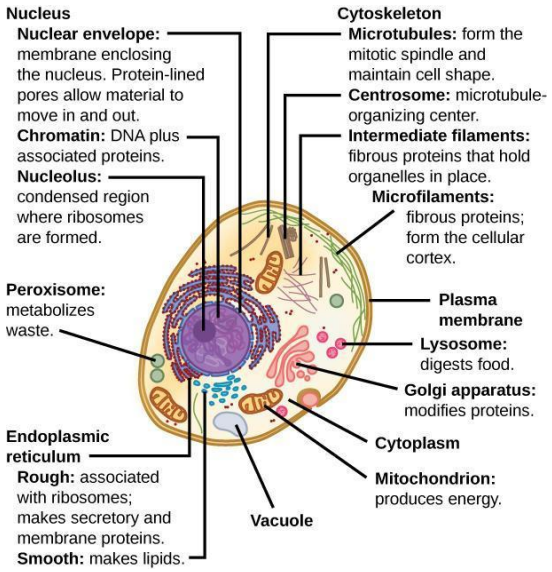


Figure 4.5: Diagram of the parts of an animal cell.

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.
- 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.*

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 4.6 and Figure 4.7 and outlined below.

a. Take a clean cotton swab and gently scrape the inside of your mouth.

b. Smear the cotton swab on the center of the microscope slide for 2 to 3 seconds.

c. Add a drop of methylene blue solution (a dye) and place a coverslip on top.

d. Remove any excess solution by allowing a paper towel to touch one side of the coverslip.

e. View the slide at all magnifications.

f. 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.

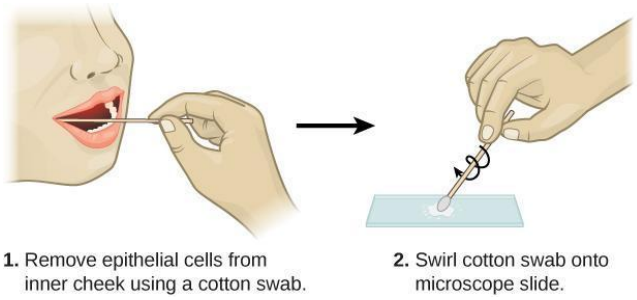


Figure 4.6: How to prepare a cheek swab.

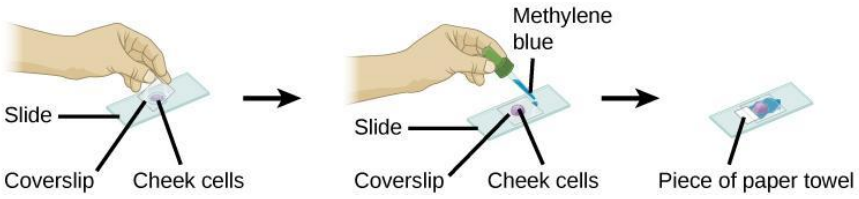


Figure 4.7: How to stain cheek cells with methylene blue dye.

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.

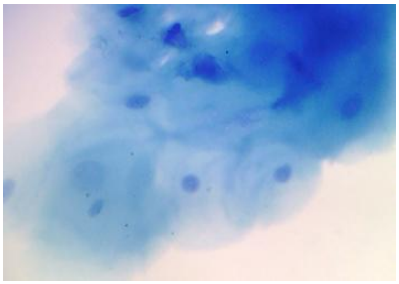


Figure 4.8: Cheek cells stained with methylene blue dye.

Assessments

1. Based on the staining technique you performed in this activity, how could you distinguish stained prokaryotic cells from stained eukaryotic cells?
2. What do all cells have in common, whether prokaryotic or eukaryotic? What major differences would you expect to find?
3. Identify whether the following images (Figure 4.9a, Figure 4.9b, and Figure 4.9c) show an animal cell, a plant cell, or a prokaryote cell. Explain how you know the difference.

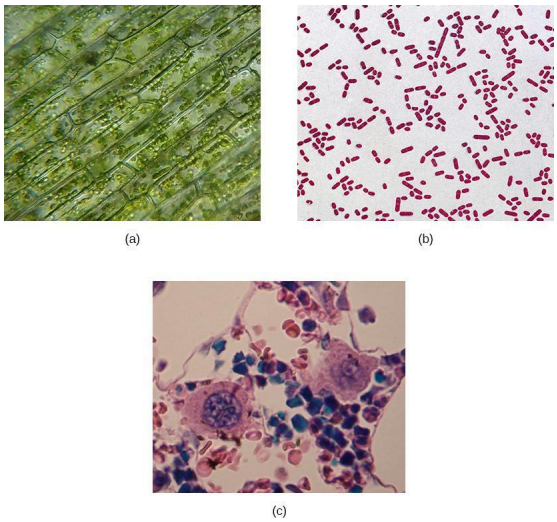


Figure 4.9: This figure shows three photos of different cell types. The photo in part (a) shows green cells with smaller organelles within. The photo in part (b) shows numerous tiny oval-shaped cells. The photo in part (c) shows a complex arrangement of different types of cells, some with a nucleus.

Lab 5:
Subcellular Structures

Bio-
logy

for AP® Courses

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. Why would pigments make visualizing subcellular structures easier under the light microscope?
2. 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 5.1, 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 5.2. 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 5.3 and Figure 5.4, 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.

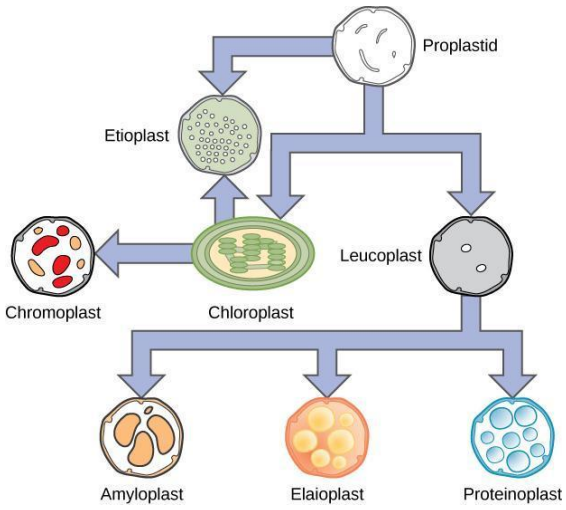


Figure 5.1: Protoplasts are the stem for several other plastids.

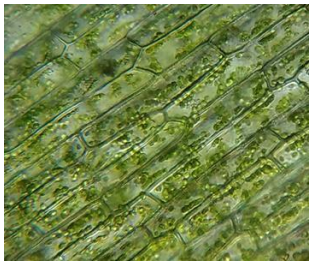


Figure 5.2: A micrograph of chloroplasts within plant cells

Figure 5.3: A micrograph of chromoplasts within plant cells

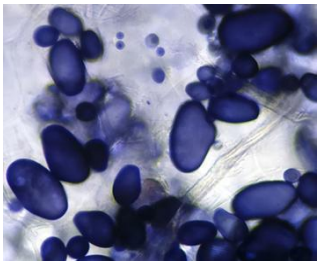


Figure 5.4: A micrograph of amyloplasts within plant cells

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
- Carrot and potato
- Iodine stain (or Lugol's stain) in dropper bottle
- Paper towels

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

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 (100×) and high power (400×) 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 (100×) 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 5.5. Observe the slice under high power and draw and label what you observe in your notebook, recording the magnification.

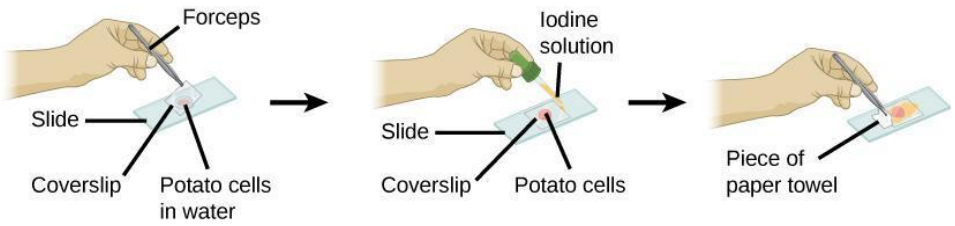


Figure 5.5: Process for making a wet mount cell of potato tissue

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 5.2, Figure 5.3, and Figure 5.4? How are you convinced that the chromoplast pigments were lipids?
2. Differentiate between the pigment functions in chromoplasts and in chloroplasts.
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?

Activity 2: Pre-Assessment

1. How do plant cells and animal cells capture energy that they use to survive?
2. 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 5.6) full of water and other important substances (such as carbohydrates, non-nutrients, wastes) for maintaining life and to help maintain cell pressure.

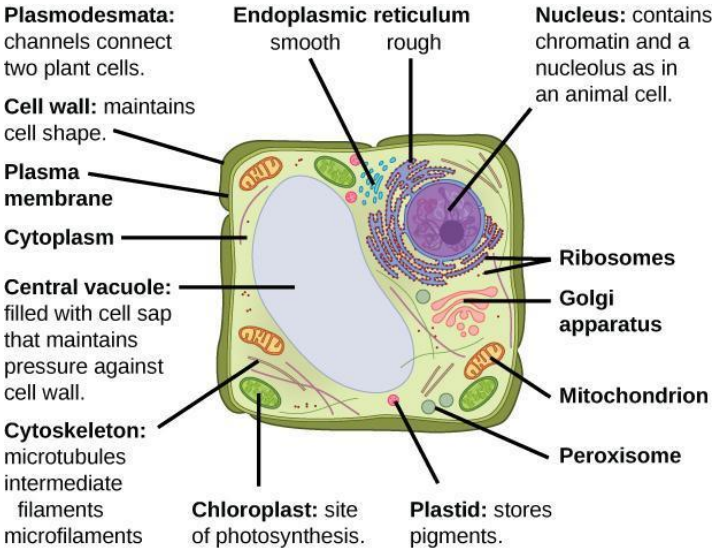


Figure 5.6: Structures of a generalized plant cell.

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*.

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 5.7. 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.

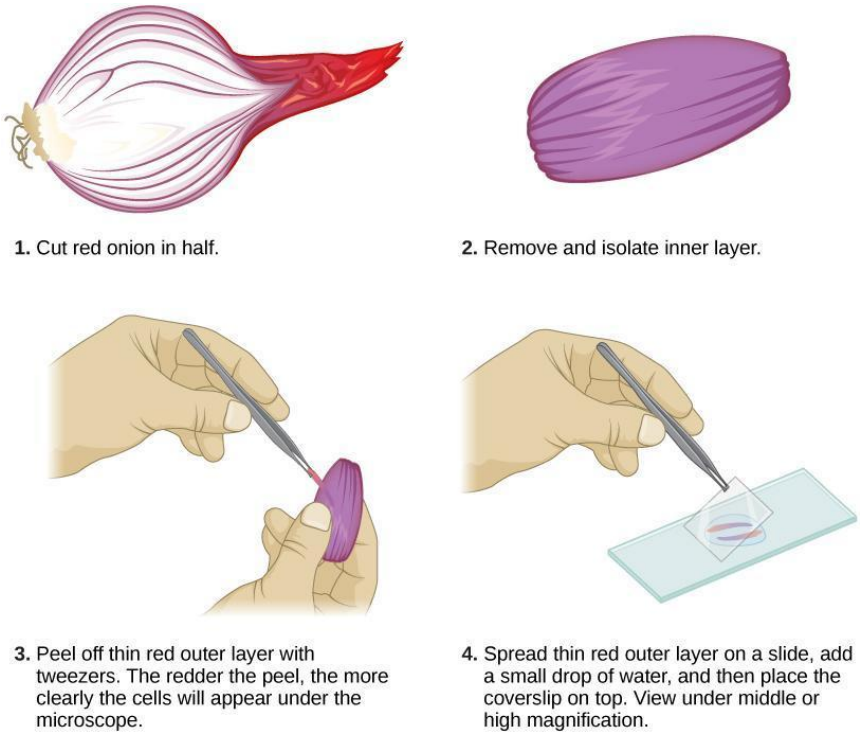


Figure 5.7: Process for preparing red onion wet mounts

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 date 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 5.7 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?
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.

Activity 3: Pre-Assessment

1. What structures would you expect to find in plant cells but not animal cells?
2. 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 5.8, 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.

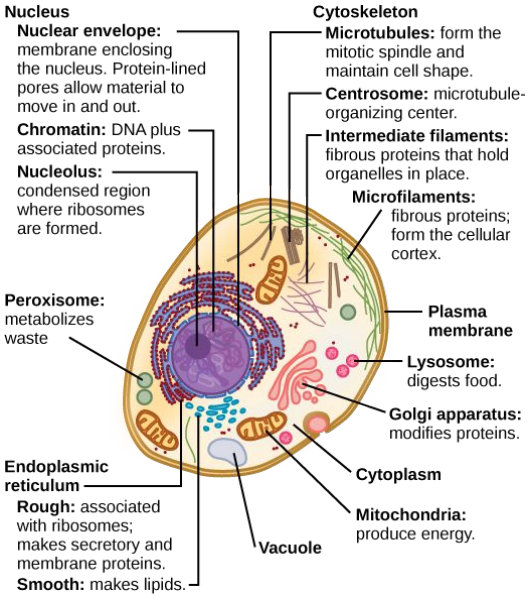


Figure 5.8: Structures of a generalized animal cell

Nissl bodies, shown in Figure 5.9, 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.

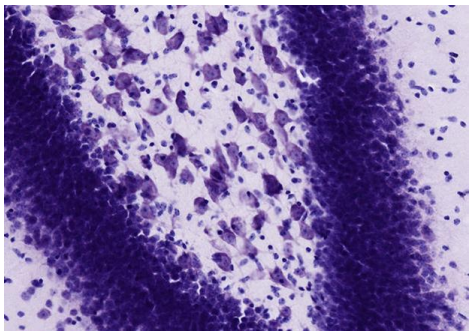


Figure 5.9: In this cross section from a rodent brain cells containing Nissl bodies are stained purple.

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*.

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 5.9. 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.
2. Describe some of the functions of thin and thick filaments in the muscle cells, based on your observations of their structure.

Lab 6:
Diffusion and Osmosis

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. 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. 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

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 6.1. 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.

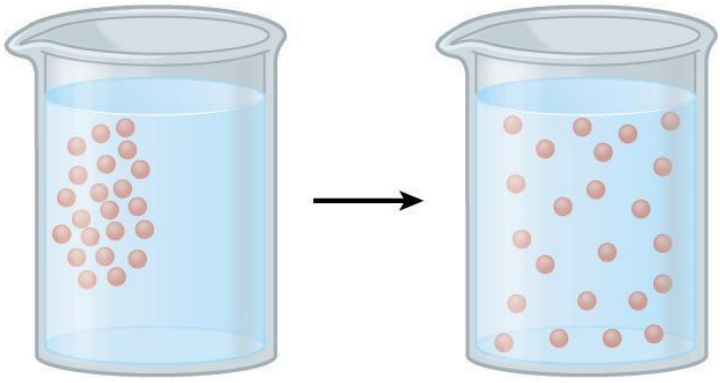


Figure 6.1: In diffusion, molecules move from areas of high concentration to areas of low concentration.

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).

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?
2. Diffusion is affected by what factors?
3. Dye tends to move faster in warmer temperatures. Why is this?

Activity 2: Pre-Assessment

1. 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 6.2). 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 6.3). 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.

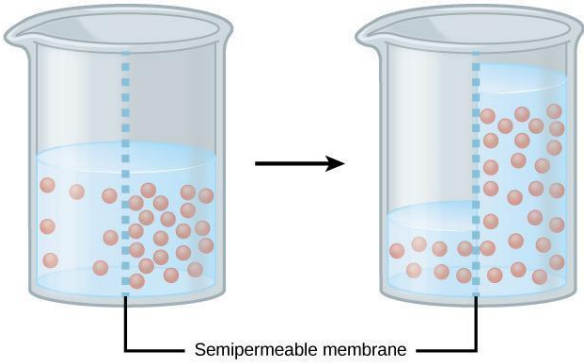
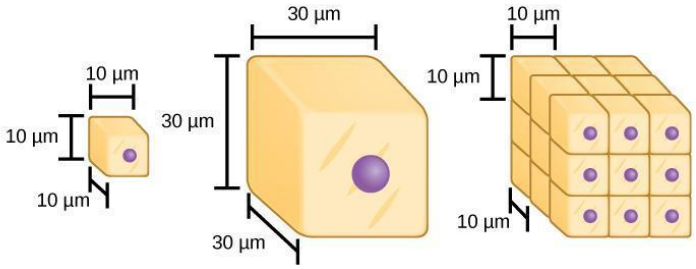


Figure 6.2: Osmosis is the diffusion of water across a semipermeable membrane. Water moves from an area of high water and low solute concentration to an area of low water and high solute concentration. Note that the solute does not move.



Surface area = height × width × number of sides × number of cubes	600 µm^2	5400 µm^2	16,200 µm^2
Volume = height × width × length × number of cubes	1000 µm^3	27,000 µm^3	27,000 µm^3
Surface area-to-volume ratio = area ÷ volume	0.6	0.2	0.6

Figure 6.3: 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*.

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 6.4). Develop and implement the use of an appropriate controlled experiment to go alongside your experiment. Allow both to sit for 20–30 minutes.

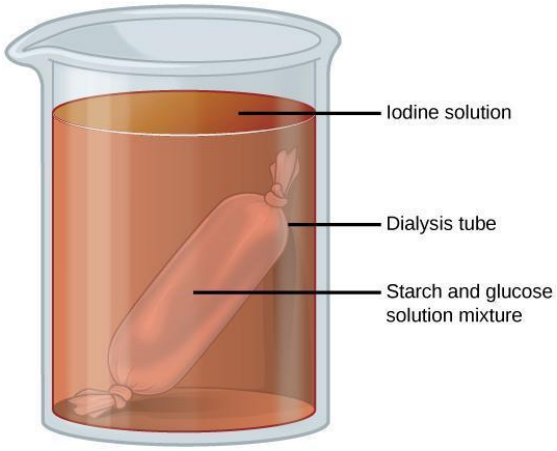


Figure 6.4: Your dialysis tube should be placed into the beaker and allowed to sit for the time specified.

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?
2. Why do smaller cells have more efficient diffusion compared to larger ones?

Activity 3: Pre-Assessment

1. 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*.

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?
2. How can you predict whether osmosis will occur into or out of a cell?
3. Describe 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?

Lab 7:
Factors Affecting the Enzymatic Activity of Lactase

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

Activity 1: Pre-Assessment

1. How would the concentration of an enzyme, its substrate(s), and its product(s) change over time as an enzymatic reaction takes place?
2. 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 7.1). 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.

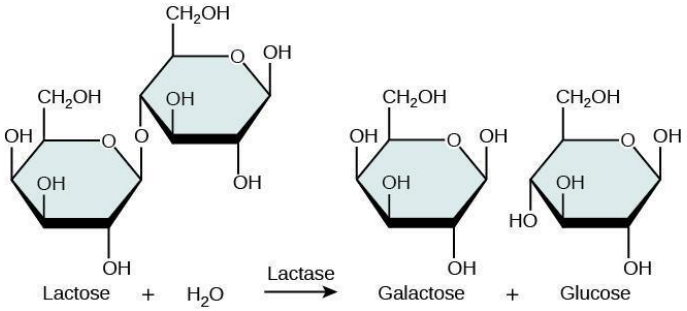


Figure 7.1: The enzyme lactase degrades the disaccharide lactose into the monosaccharides galactose (1) and glucose (2).

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 7.2). 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 7.2: 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*.

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 decreased 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.
2. In this experiment, describe the role of each of the following:
    a. The water-only tube
    b. The tube of 20 mg/mL glucose solution

Activity 2: Pre-Assessment

1. Which environmental conditions could alter the rate at which an enzymatic reaction takes place? Why would this occur?
2. 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*.

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?
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?

Activity 3: Pre-Assessment

1. 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 7.3). What does this suggest about how specific enzymes are to their substrates?

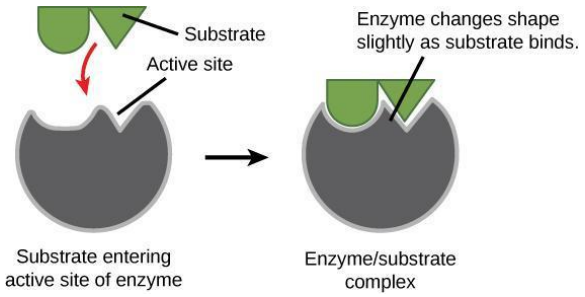


Figure 7.3: The three-dimensional shape of the active site is also important in determining which substrates will bind to the active site.

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*.

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 how you know in your notebook.

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.
2. What might you conclude if you observed glucose in a lactose-free food at the start of the experiment?
3. How could enzyme specificity be used to determine the concentration of a substrate in a food product?

Lab 8:
Cellular Respiration

Bio-
logy

for AP® Courses

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. What function do mitochondria fulfill in the cell? What kind of staining would allow visualization of mitochondrial activity?
2. 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

- Use single edge razor blade with caution. Do not leave blades exposed on the bench. When you are finished using the razor blade, dispose of it as instructed by your teacher.
- 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*.

Structured Inquiry

Step 1: Preparation and observation of wet mount:

1. Slice a layer from an onion with the single edge razor blade and 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 8.1. Place a piece of filter paper or tissue on one side of the cover slip. Add one or two drops of Janus Green on the opposite site of the coverslip close to the edge. The stain solution will flow in by capillary action.

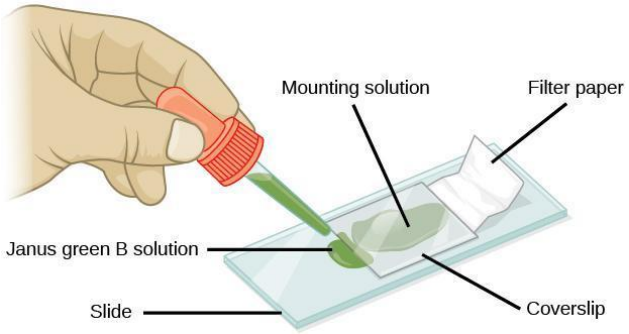


Figure 8.1: Staining a wet mount by capillary action.

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?
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?
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?

Activity 2: Pre-Assessment

1. 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. 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 rates 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 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 CO_2 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 CO_2 gas which forms will be removed by adding $Ca(OH)_2$ that reacts with carbon dioxide to form the insoluble salt $CaCO_3$, 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 8.2, 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 8.2: 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 [$Ca(OH)_2$] 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 non absorbent 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*.

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 8.3 as a guide and following the steps below.

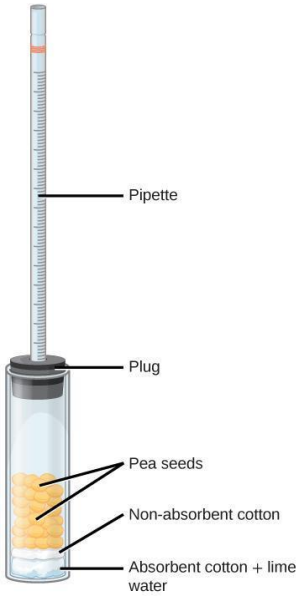


Figure 8.3: Assembled respirometer.

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 $Ca(OH)_2$, being careful not to oversaturate the pad or drip the lime water on the side of the tube.

b. Insert a thin layer of non absorbent 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 8.3.) 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 8.4. The goal is to keep the pipettes out of the water while the test tubes remain submerged.

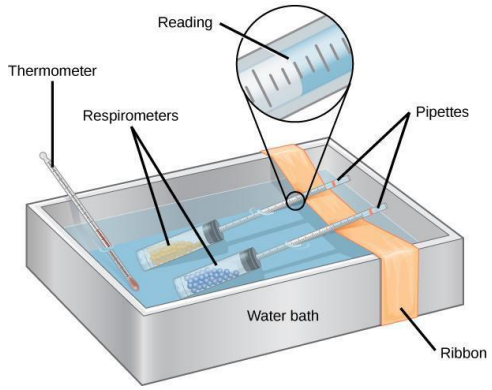


Figure 8.4: Respirometers in water bath.

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?
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?
3. A classmate insists that there are no mitochondria in leaves because chloroplasts produce ATP through photosynthesis. How would you experimentally disprove this claim?

Lab 9:
Fermentation in Yeast

Bio-
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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. What is the difference between real sugar and a sugar substitute? Could either substance be used during cellular respiration?
2. 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.

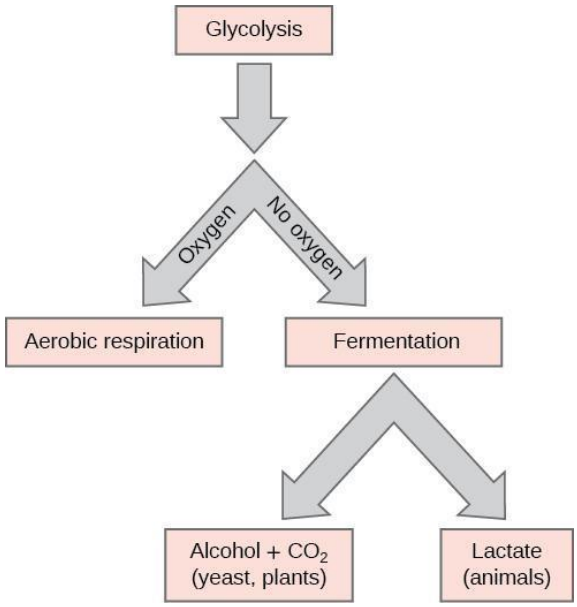

Figure 9.1: Glycolysis is the first step of both aerobic respiration and fermentation. Aerobic respiration only produces carbon dioxide as a toxic byproduct, but fermentation also produces alcohol or lactate.

Safety Precautions

- Use care when using glassware.
- Be careful when inverting the graduated cylinder into 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*.

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 at 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.

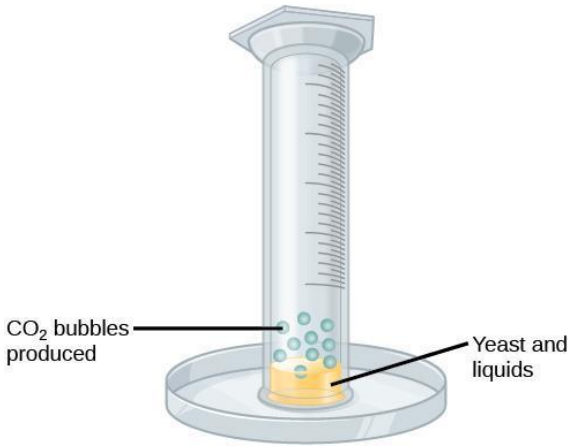

Figure 9.2: As fermentation takes place, carbon dioxide bubbles will rise into the cylinder. Volume measurements can be taken at specific time increments.

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 makeup 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?
2. What do you think would happen to the activated yeast if no carbon-based molecules were provided?
3. What environmental reasons would cause yeast to go dormant?

Activity 2: Pre-Assessment

1. How does temperature affect the rate of chemical reactions? Explain your answer.
2. 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*.

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?
2. How could this experiment better measure the effect of temperature changes in the natural habitat of the yeast?
3. What other biotic and abiotic factors would likely affect the rate of cellular respiration in yeast? Explain your answers.
4. What patterns can you identify in the data you collected between the yeast and the abiotic factor of temperature?

Lab 10:
Plant Pigments

Bio-
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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. 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. 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 10.1, 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?

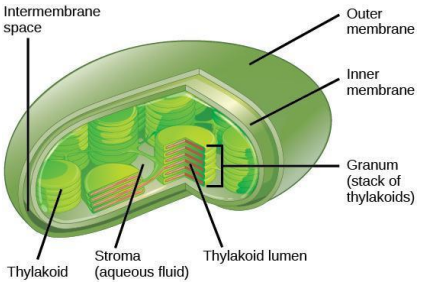


Figure 10.1: A section through a chloroplast shows the arrangement of the thylakoid membranes in stacks and single membranes. The thylakoids are pockets enclosed by membranes.

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*.

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 10.2 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 10.2. 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 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.

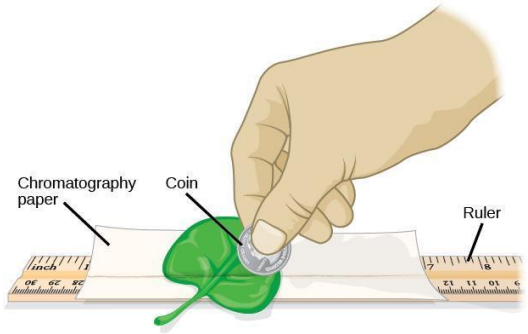


Figure 10.2: Extract pigments by rolling a coin along a ruler. Make sure that the leaf does not cover the entire paper and align the ruler with the starting line to avoid edge effects.

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 10.2 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?
2. You analyze a pigment extract from a mutant plant and discover that the plant 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?
3. The Rhodophyta are algae that can grow deeper in the ocean than other plants. Based on the information shown in Figure 10.3, 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?

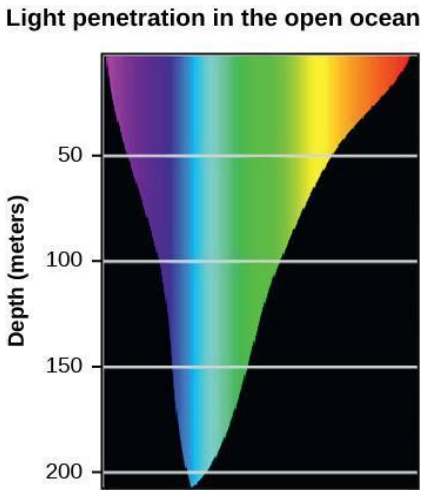


Figure 10.3: This graph shows the light penetration in the open ocean as a spectrum of color from violet to red. The depth of the ocean is measured in meters, and the peak light penetration occurs in blue light, which progresses past 200 m.

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 10.1. 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 10.4).

We separate the compounds based on how quickly they move across the paper. Compounds which are soluble in the solvent mixture be 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*.

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 10.4.

Figure 10.4: 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.

1. Label the beaker with a piece of tape with your initials and your partner's initials.
2. 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 notebook.
3. Cover the container tightly with a piece of aluminum foil.
4. Track the rising of the solvent front. Can you see a separation of colors on the paper?
5. 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.
6. 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.
7. 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 10.5. 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 (Rf) is the ratio of the distance traveled by a colored band to the distance traveled by the solvent front. Calculate $\mathbf{R_f}$ values for each pigment using the following equation:

$$R\,f = \text{Distance traveled by colored band} / \text{Distance traveled by solvent front}$$

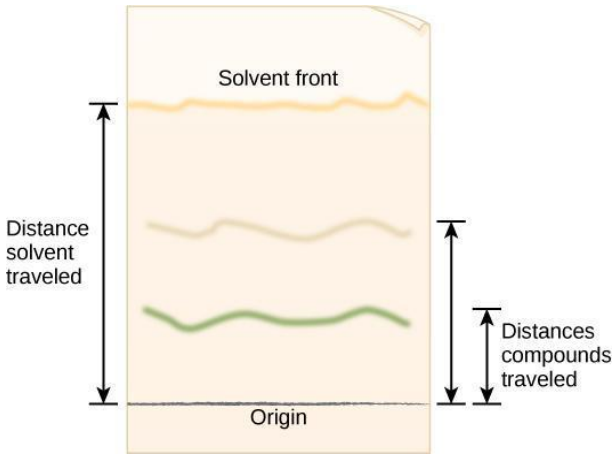


Figure 10.5: Chromatogram shows the distance traveled by the solvent front and the compounds separated by chromatography.

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?
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?
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?

Lab 11:
The Light Reaction of Photosynthesis

In this lab, you will learn

- how to measure the dependence of CO_2 fixation on the light-dependent reaction
- how to explain the chemical principles of pH indicators and the bicarbonate-CO_2 equilibrium
- how to analyze the dependence of O_2 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 CO_2 is released in water? What is the connection between an increase in CO_2 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 CO_2

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

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H+ + HCO_3-.$$

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 CO_2 molecules are consumed by photosynthesis, the reaction shifts to the left. Hydrogen ions and bicarbonate ions combine to replace CO_2 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 11.1). 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 you partner and write your answer in your notebook.

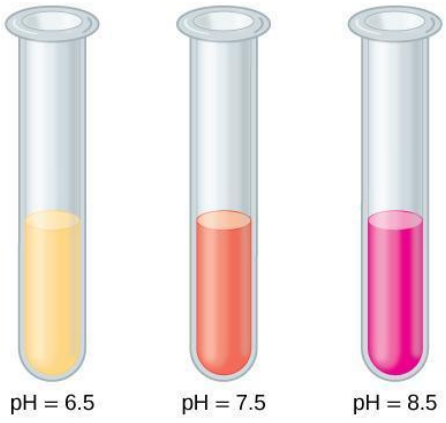


Figure 11.1: The pH indicator phenol red appears yellow at an acidic pH, pinkish orange at a neutral pH, and bright red or pink at a basic pH.

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***.**

Structured Inquiry

Step 1: Hypothesize/Predict: In this experiment, you will measure CO_2 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 CO_2 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 CO_2 to adjust the pH. The reason for using distilled water is that freshly distilled water has a pH of 7.0. As CO_2 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 11.2. 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 phenol indicator solution every 30 minutes. To compare CO_2 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 *Elodea* exposed to light	Tube without *Elodea* exposed to light	Tube with *Elodea* in the dark	Tube without *Elodea* in the dark
0				
30				
60				
90				
120				

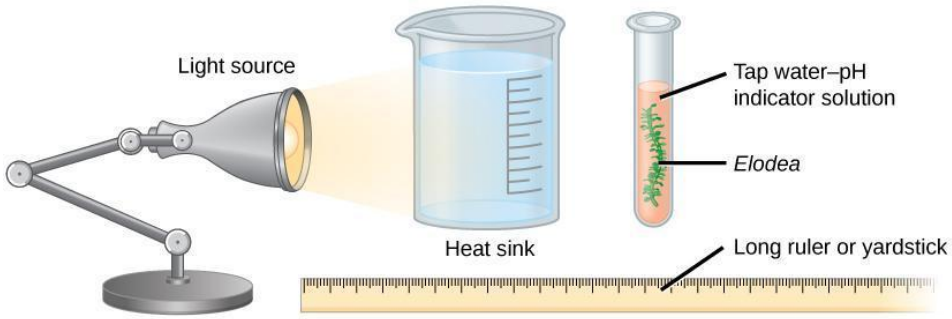


Figure 11.2: Experimental set up to measure release of CO_2 during photosynthesis.

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 modify the composition of the *Elodea* environment?
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?
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?

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 $1/ET_{50}$ 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*.

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 11.3.

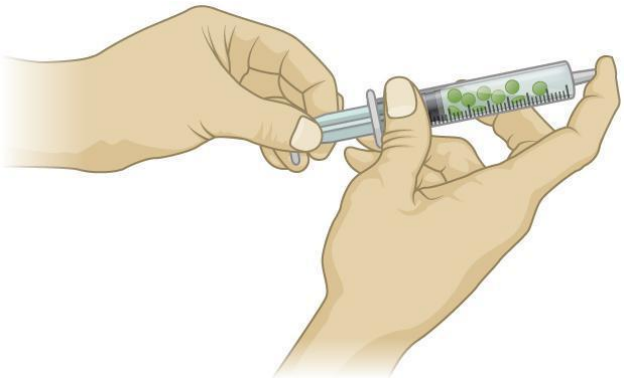


Figure 11.3: Pulling the barrel of the syringe and capping the tip with a finger creates a vacuum inside the syringe.

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 concentration 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_02, 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?
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.
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 the interaction between the *Elodea* and the water snail.

Extension Activities

1. Measure the ET_{50} 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 ET_{50} 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 O_2?

Lab 12:
Photosynthesis: The Dark Reactions

Bio-
logy

for AP® Courses

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. Why might the presence of starch serve as a good indicator for the products of the dark reactions in plants?
2. 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 12.1, where a starchy seed has been stained with a drop of iodine.

Figure 12.1: 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*.

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.
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?
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?

Activity 2: Pre-Assessment

1. Explain how the dark reactions got their name? Can they happen independently of exposure to the sun? Explain.
2. 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 CO_2 to carry out the dark reactions with their need to retain water. While opening stomata on leaf surfaces to get CO_2 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 O_2 produced as a by-product of the light reactions can accumulate inside plant cells and serve as an alternative substrate to RuBisCO when CO_2 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 CO_2 (Figure 12.2), while also using O_2 that otherwise would be used as a terminal electron acceptor during respiration to make ATP. Photorespiration is problematic for **C_3 plants**, those lacking any mechanism to avoid this, which are named as such because the first fixed carbon product contains 3 carbons.

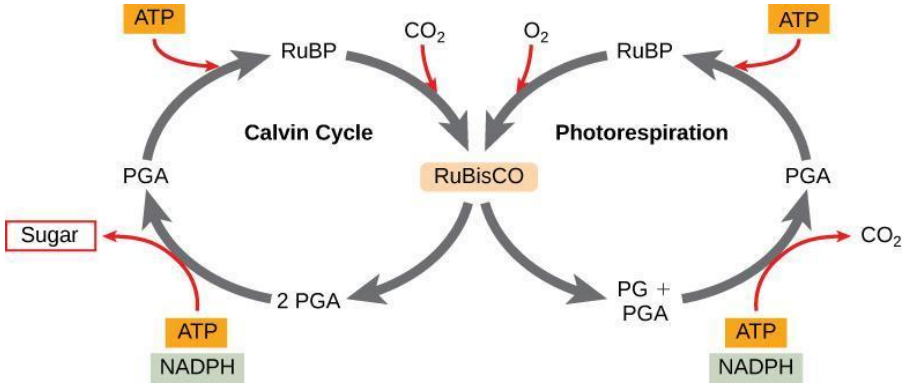


Figure 12.2: Photorespiration occurs when cellular O_2 levels are high and CO_2 levels are low. When this occurs, RuBisCO may bind to O_2 instead of its typical substrate CO_2. This results in the use of NADPH and ATP without the production of carbohydrates.

Some plants have evolved mechanisms to overcome photorespiration (Figure 12.3). 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.

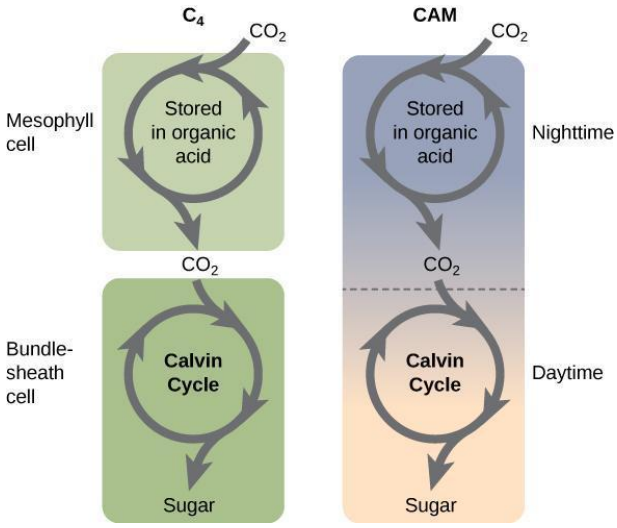


Figure 12.3: To avoid photorespiration, plants have evolved strategies to either spatially (C_4 plants) or temporally (CAM plants) separate the incorporation of CO_2 into a 4-carbon molecule, which subsequently serves as a CO_2 source, from the Calvin cycle.

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
    - C_3 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*.

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 12.4, 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.

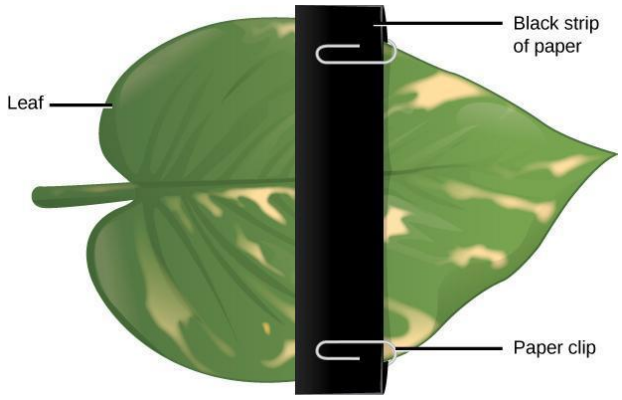


Figure 12.4: Cover several leaves of a potted plant with black strips, securing with paper clips.

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.
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?
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?

Lab 13:
Mitosis and Meiosis

In this lab, you will learn the following:

- 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

Activity 1: Pre-Assessment

1. List three reasons why organisms need to produce new cells.
2. 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.

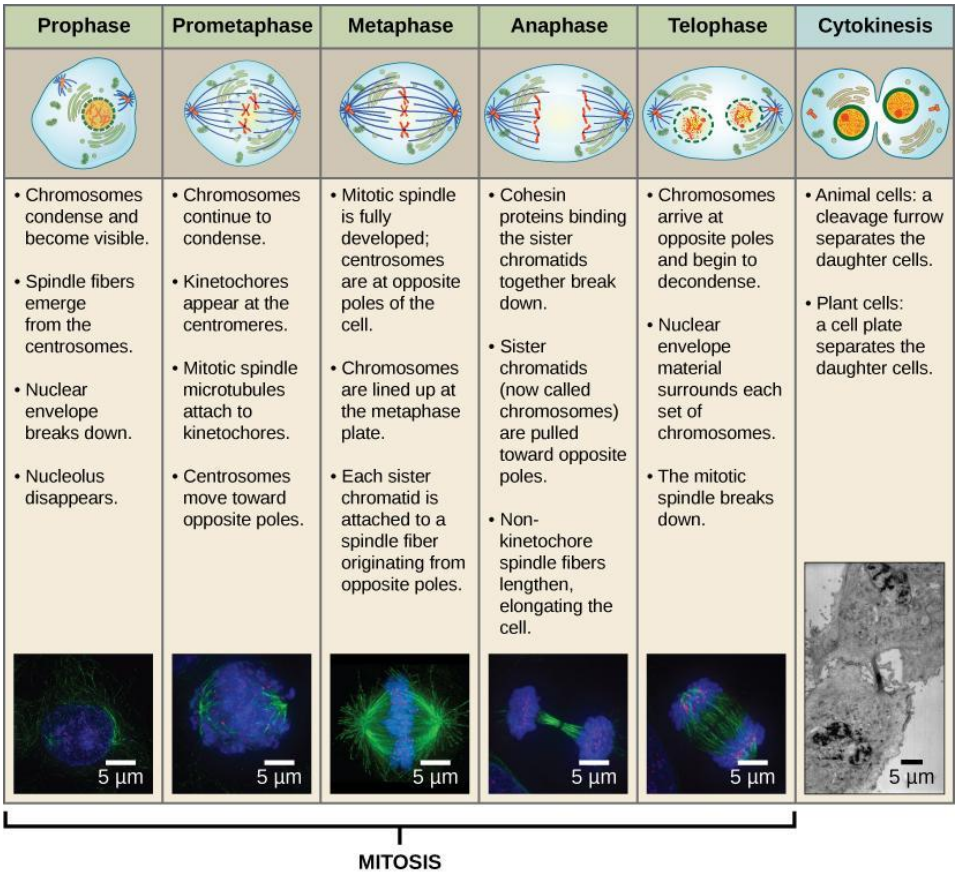


Figure 13.2: Mitosis is divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase.

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*.

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.

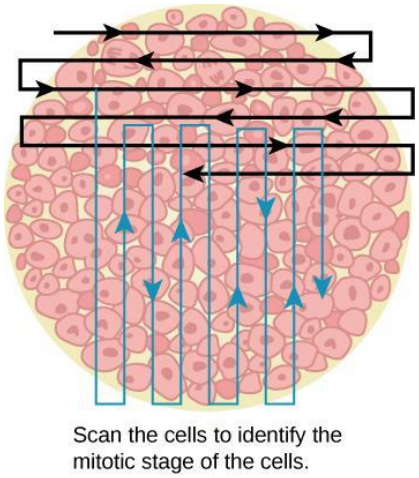


Figure 13.3: Scan and count cells by using either a horizontal (black line) or vertical (blue line) back and forth scanning technique with your eyes.

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. [APLO 3.7][APLO 3.8]
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. [APLO 3.7][APLO 3.8]

Activity 2: Pre-Assessment

1. In a plant, where would you likely find cells that actively undergoing mitosis? Where would you likely find cells that aren't actively undergoing mitosis? Explain your answers.
2. 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.

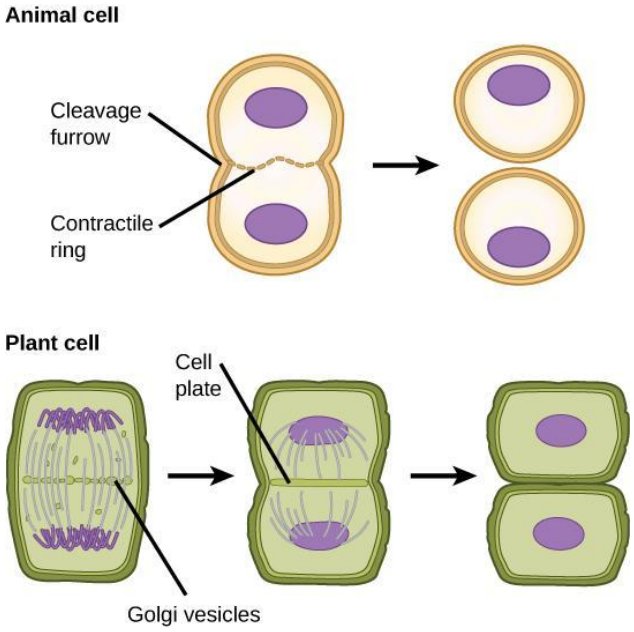


Figure 13.4: Cytokinesis differences between plant and animal cells

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.*

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.
2. Based on your observations, explain how onion roots grow at the cellular level.

Activity 3: Pre-Assessment

1. Looking at Figures 13.2 and 13.9, compare the outcomes of mitosis versus meiosis.
2. 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.**

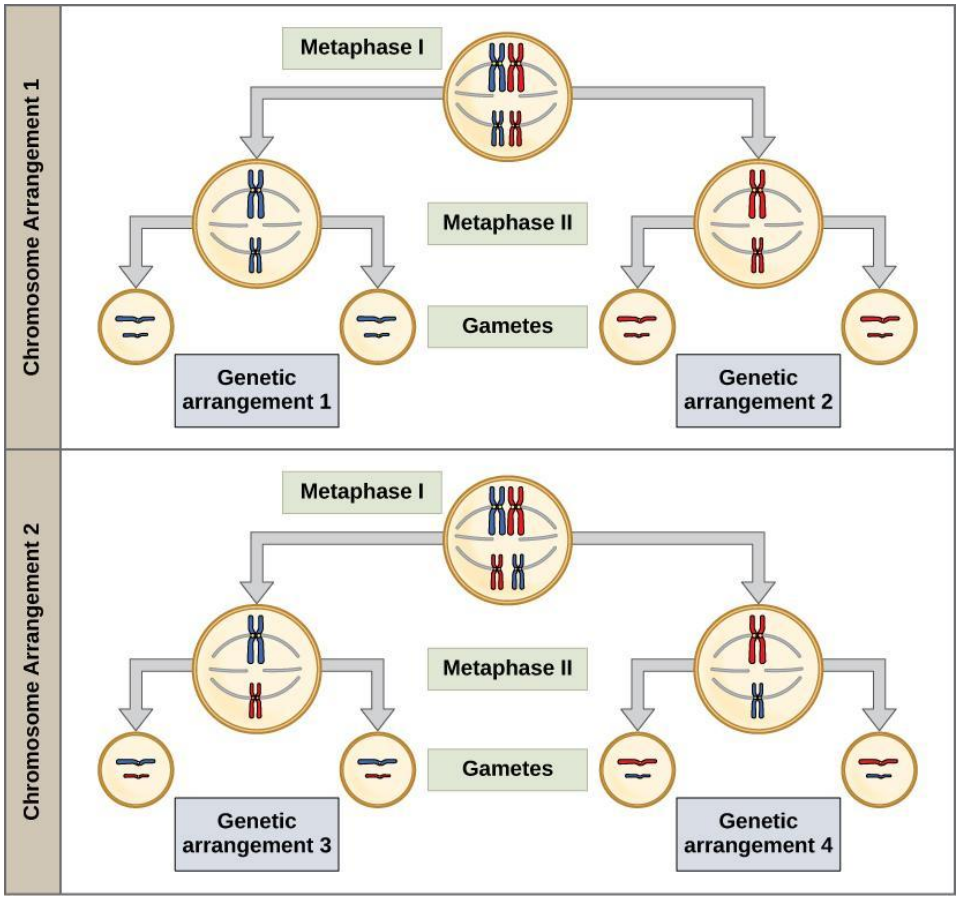


Figure 13.9: Overview of meiosis

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.

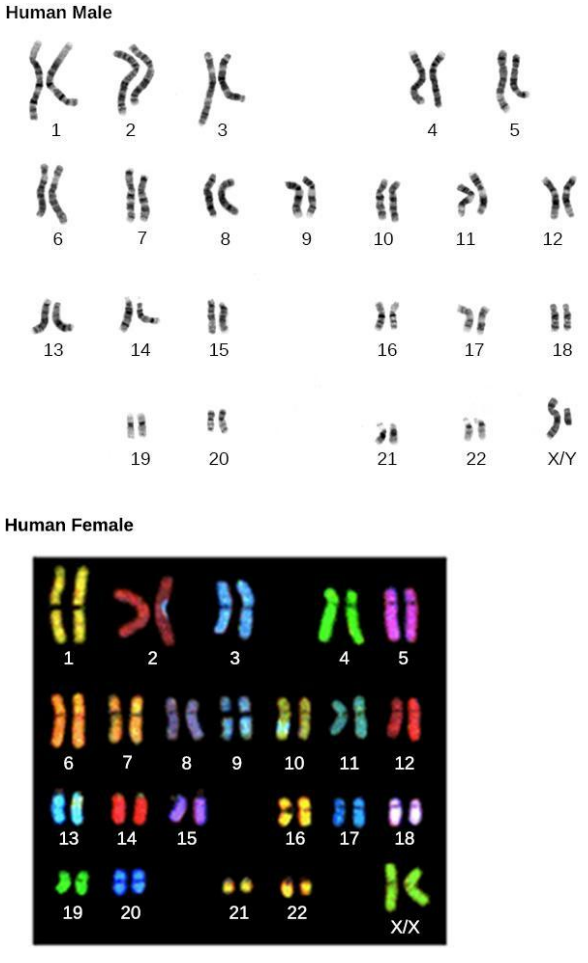


Figure 13.10 Sorted human male and female chromosomes

Safety Precautions

None

For this activity, you will need the following:

- Images of karyotypes

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

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.

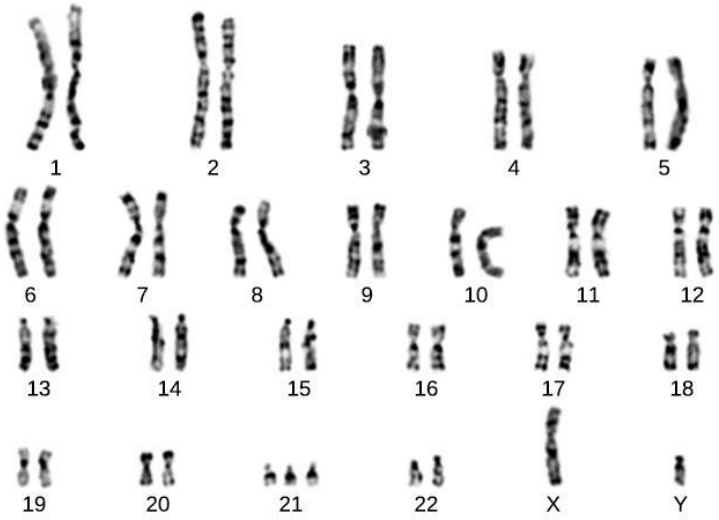


Figure 13.12: Karyotype 1

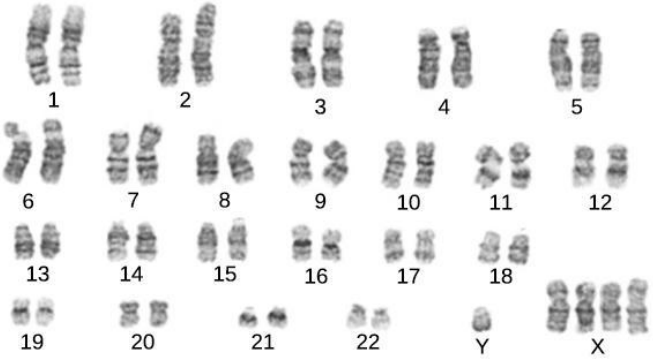


Figure 13.13: Karyotype 2

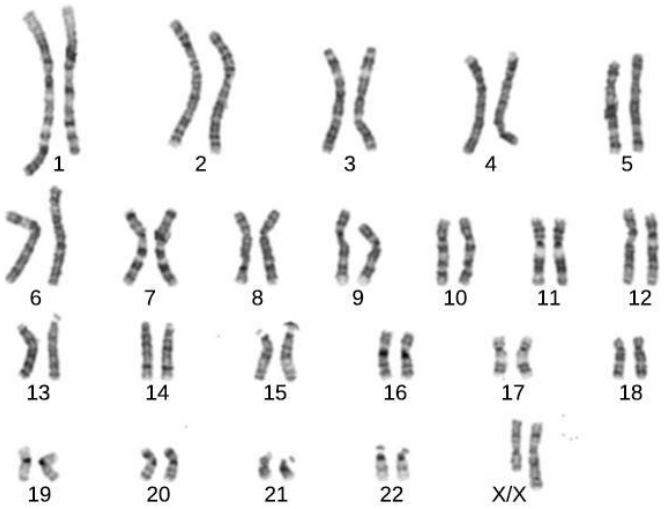
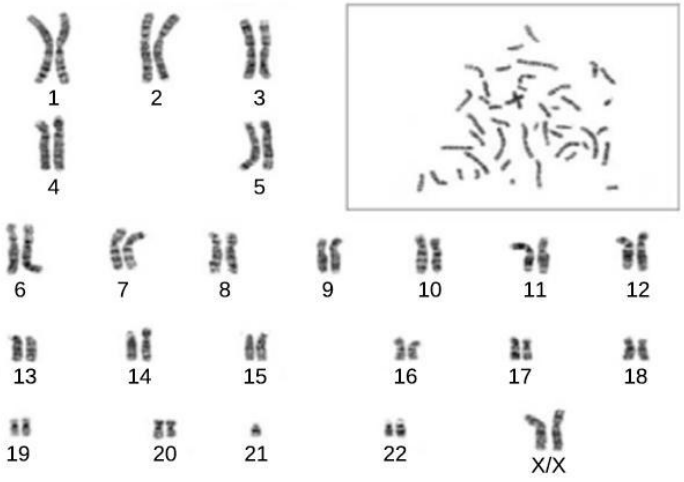


Figure 13.14: Karyotype 3

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.



2. Crossing over of chromosomes during prophase I is common. How does this process increase the genetic diversity of the daughter cells? [APLO 3.7, 3.8, 3.11]

Lab 14:
Mendelian Genetics

Bio-
logy

for AP® Courses

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. 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. 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 × 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 14.1). 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.

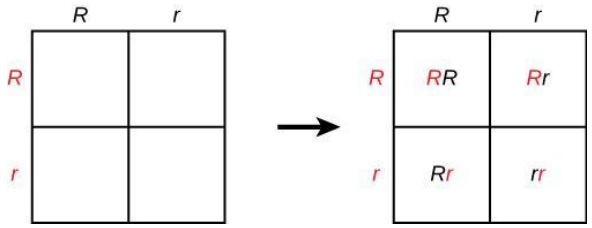


Figure 14.1: A Punnett square is used as a visual representation of crossed traits and the results of the crosses. Capital R represents the dominant trait, and lowercase r represents the recessive trait. The first square is a cross between the two dominant traits R and R. RR is the result.

Safety Precautions

- Do not let the flies fly off.
- 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.*

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 vermillion/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 14.1). 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.)

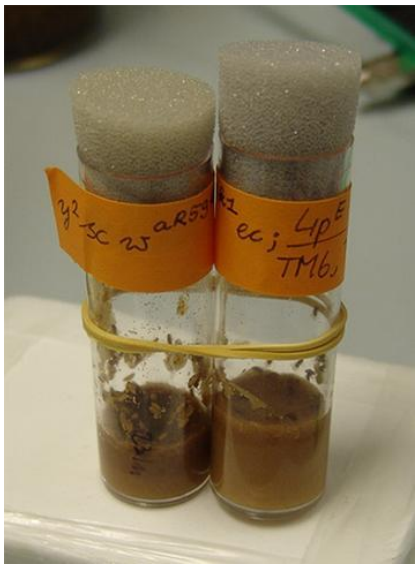


Figure 14.2: A typical vial of *Drosophila melanogaster*. The food medium is at the bottom of the vial, with fly larvae (maggots) on its surface. The fruit fly pupae can be seen adhered to the inner wall of the vial.

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?
2. Answer the following about the assumptions of Mendelian inheritance:
    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?

Activity 2: Pre-Assessment

1. What does the chi-square test tell you?
2. Explain the difference between expected and observed data.
3. 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.*

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?
2. Describe another situation, outside the realm of science, where a chi-square test would be useful.
3. What would change the degree of freedom in a chi-square test?

Lab 15:
DNA Restriction Analysis

for AP® Courses

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. How could we measure the size of an unknown fragment of DNA?
2. 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 box and load samples with the electrodes disconnected.
    - Turn off power supply before connecting or disconnecting 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 µL micropipette with µL 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*.

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 15.1). 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.

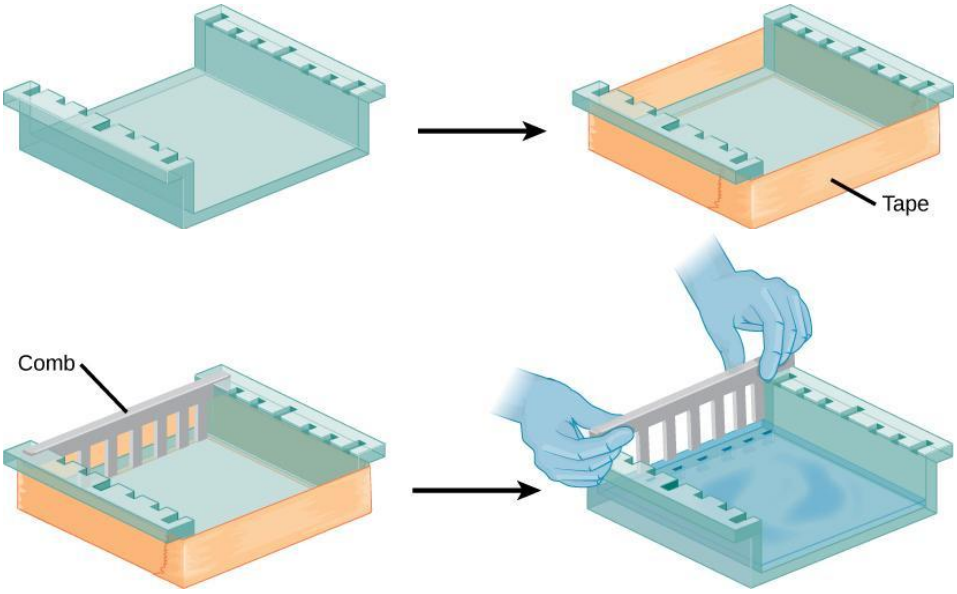


Figure 15.1: If your gel trays need to be taped, apply the tape straight to form a wall that will hold the gel. Be sure to insert the comb straight into the gel. All the comb teeth should penetrate an equal distance into the gel. Once the gel if fully set, remove the comb slowly by pulling straight up 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 if fully set, remove the comb slowly by pulling straight up on both ends of the comb (see Figure 15.1).

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 15.2 to coax the DNA and loading dye together into the bottom of the microfuge tube.

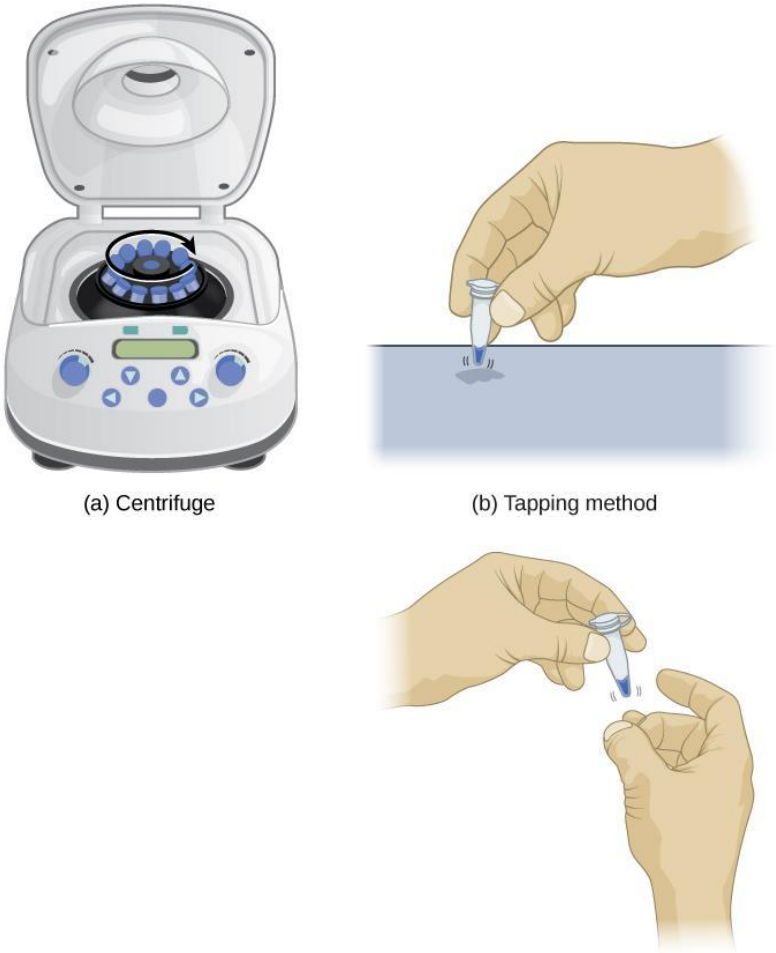


Figure 15.2: Centrifuge and tapping method to move small amount to the bottom of microfuge tubes. If no centrifuge is available, flick the bottom of the microfuge tube with your finger at least 20 times to thoroughly mix its contents.

Step 4: Student-led planning. Place your gel in the gel box as shown by your teacher (see Figure 15.3). 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 15.3. How can you ensure that the DNA will not run off the gel? Write you answer in your notebook.

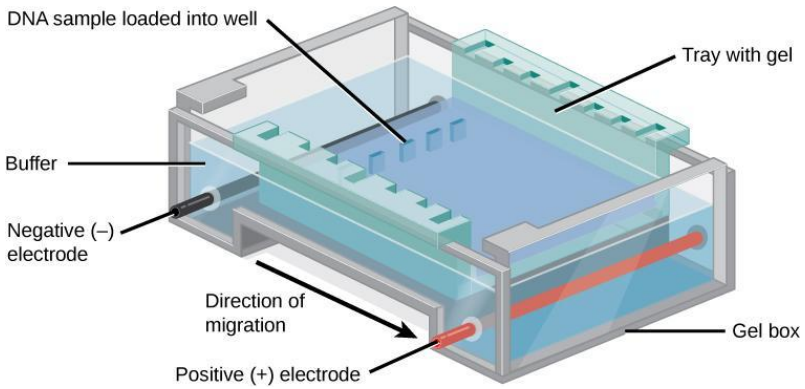


Figure 15.3: Place your sample tray so that the wells are located closest to the negative electrode of the gel box.

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_07). 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.

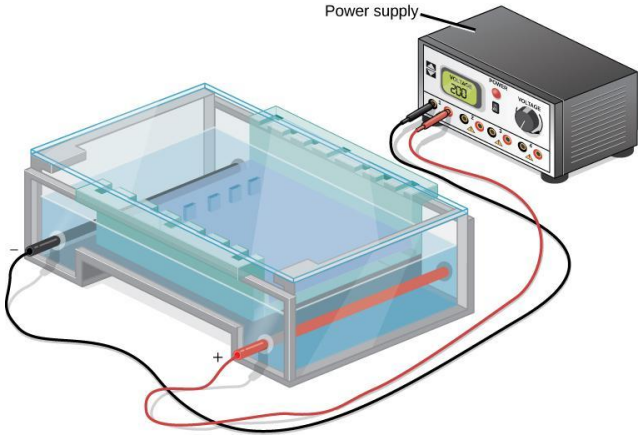


Figure 15.4: Be sure that the red and black wires and plugged into the corresponding colors on both the gel box top and the power supply (red to red and black to black).

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 Figures 15.5 and 15.6. Create a data table for your data in your notebook.

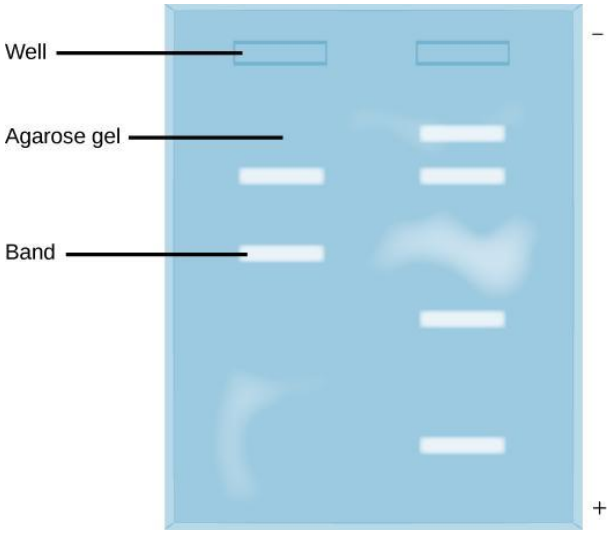


Figure 15.5: Diagram of a gel that has been run.

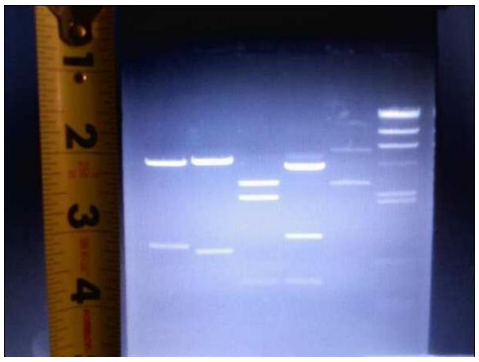


Figure 15.6: Example of a DNA Gel with Ruler. The standard lane is on the far left. This lane is used to determine the size of the DNA fragment, in base pairs.

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 15.7 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 15.8 shows the genes located on Lambda that provide the information on making new virus.

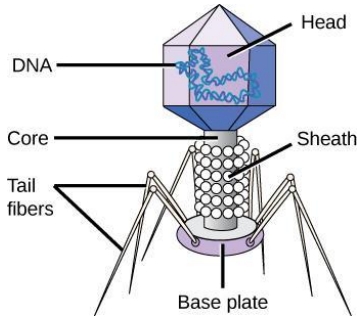


Figure 15.7: The Lambda phage is a virus that invades, and reproduces in, the cells of bacteria.

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_09 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 extra-chromosomal piece of DNA) in Figure 15.11. If you digested this plasmid with restriction enzymes BamHI, HindII, and ScaI, how many fragments would appear in a gel? How long (# of base pairs) would each fragment be? Draw a gel showing the digestion. [APLO 3.5]

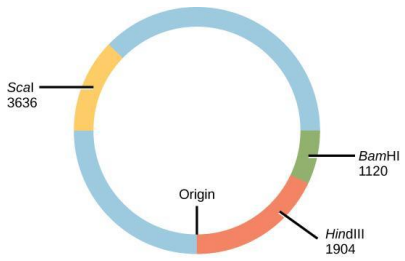


Figure 15.8: Partial restriction map for the Lambda phage.

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. [APLO 3.5]

Lab 16:
Bacterial Transformation

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. What techniques are used to transfer a gene from one bacterium into another?
2. 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 16.1). 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.

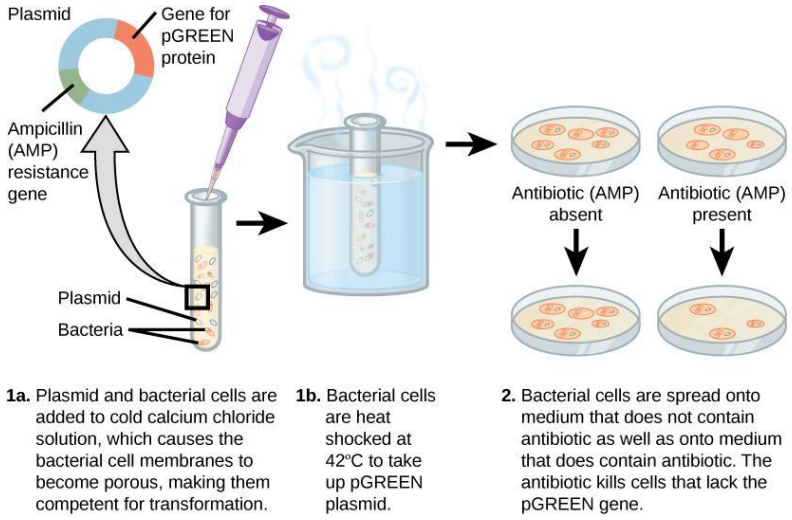


Figure 16.1: The transformation process, in Stage 1.

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 16.2. In this stage of transformation, you will make competent cells and then store them in the refrigerator until you complete the transformation process.

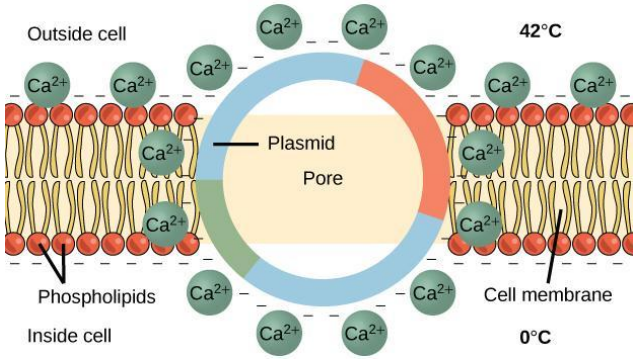


Figure 16.2: Heat shock transformation of *E. coli*.

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 µl micropipette (one per class)
- Micropipette tips for class (for 20 µl micropipette)
- 42 ^{o}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 µg/µl; 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*.

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 $CaCl_2$ 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 ^{o}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 ^{o}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 ^{o}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 16.3 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.

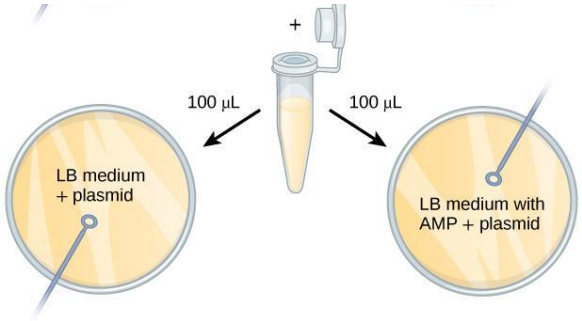


Figure 16.3: Illustration of process for steps 7–10.

Step 4: After the 10-minute incubation period, use a pipette to transfer 100 µL 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 µL 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 ^{o}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 16.4.

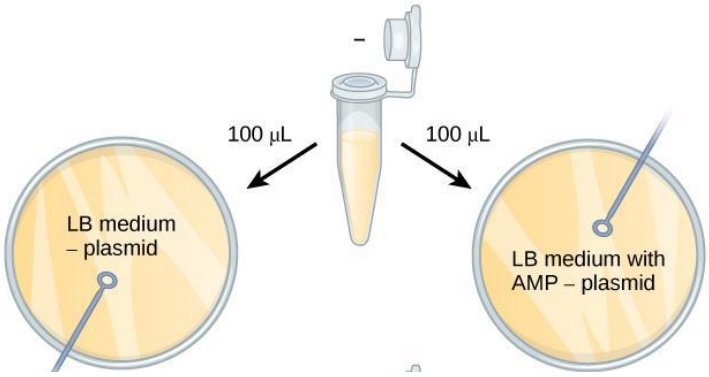


Figure 16.4: Experimental treatments for Guided Inquiry.

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?
2. Explain why the heat shock step is essential for transformation to occur?
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?
4. Describe how your results would most likely change if the ampicillin-resistance gene underwent a severe mutation within the bacteria?
5. Draw the gene sequence that shows why expression of the ampicillin-resistance gene also causes the expression of the green fluorescent protein.
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?

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

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: Pre-Assessment

1. Why might the allele frequencies from a sample population not match those from the general population?
2. 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:

$$p2+2pq+q2=1.0$$

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**).

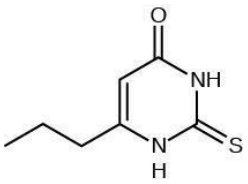


Figure 17.1: Chemical structure of PROP (6-n-propylthiouracil)

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

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, $p+1=q$ and $p2+2pq+q2=1.0$, 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?
2. What are the limitations of Hardy-Weinberg equilibrium?
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.
    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?

Lab 18:
Natural Selection Simulation

Bio-
logy

for AP® Courses

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: Pre-Assessment

1. 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. Why would the frequency of physical characteristics in a population change over several generations?
3. 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. 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

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 decided 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.

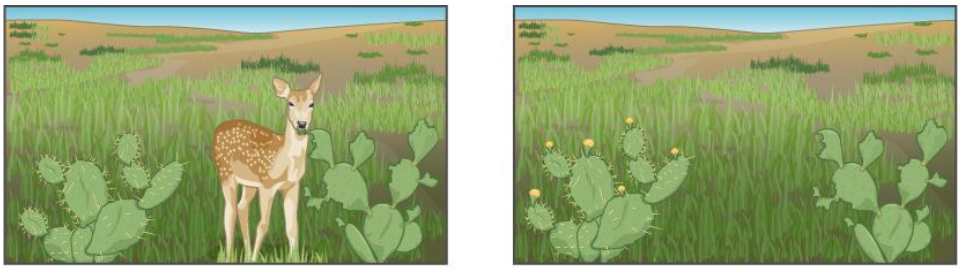
Step 2: Hypothesize/Predict: Predict how natural selection would occur with the new phenotype and hypothesize how it will affect the phenotypic frequencies of the population after 10 generations. Use the same simulation procedure from Guided Inquiry 1. How would the frequency of brown and green change 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?
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?
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?
4. Observe what happened in Figure 18.2 and explain how it represents an example of natural selection.



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.

Lab 19:
Plant Transpiration

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. 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. 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.

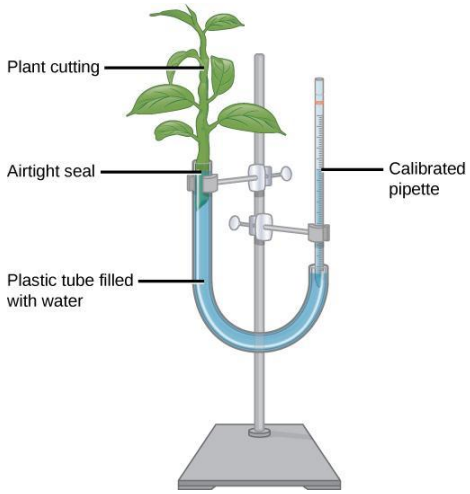


Figure 19.1: When transpiration takes place, water evaporates from open stomata. As the water potential difference increases between the bottom of the stem and the leaves, the stem draws in water from the tube, and the water level drops in the pipette

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.*

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 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 4: Collect the transpiration data using the potometer as in the Structured Inquiry. Record the measurements in your data table.

Step 5: 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 6: 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?
2. Explain whether your prediction in the Guided Inquiry was accurate. Include a reason based on how the variable affected transpiration.
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.

Activity 2: Pre-Assessment

1. What environmental conditions could be applied to a plant that would cause it to open more of its stomata?
2. 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, stomata will open to release the pressure. If water pressure is low, 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.

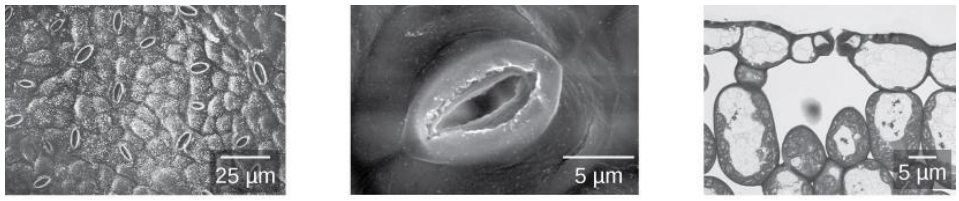


Figure 19.2: Transpiration takes place through open stomata, (a) generally scattered across the underside of leaves. (b) A single stoma. (c) A cross section of a stoma shows the two small guard cells above the large open air space within the leaf tissue. The guard cells contain darkened areas.

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*.

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?
2. Could a researcher estimate the how many plants in an enclosed area that have their stomata open using the concentration of carbon dioxide in the air? Explain your answer.
3. How could a research scientist use the methods in this lab to determine how to care for a given species of plant?

Lab 20:
Biodiversity in Leaf Litter

Bio-
logy

for AP® Courses

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*.

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: *Check one:* (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 15cm by 15cm 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]

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)

For this activity, you will work *alone, in pairs or in groups*.

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 – [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 × 35) + (27 × 26) + (10 × 9) + (14 × 13) / (87 × 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]

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.