Advanced Biology through Inquiry

Student Guide

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1. ENZYME ACTIVITY

Background

Cells have to carry out thousands of chemical reactions very quickly to sustain life. Enzymes are vital to this operation. Enzymes are protein catalysts—they increase the rate of the reaction by lowering the activation energy of the reaction. An enzyme's shape is critical to its ability to catalyze reactions.

Enzymes are *hyper-specific*, that is, usually an enzyme interacts with only one substrate. Like catalysts in other chemical reactions, enzymes are not consumed during the reaction but help turn the substrate into the final product. Notice in the following reaction that the enzyme is present before and after the reaction.

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

Hydrogen peroxide (H_2O_2) is a byproduct of aerobic respiration in cells and is used in cell signaling and apoptosis. Hydrogen peroxide is highly reactive and can produce free radicals that damage nucleic acids, so cells must carefully regulate its concentration. To remove excess hydrogen peroxide, cells produce an enzyme (called *catalase* in animal cells and *peroxidase* in plant cells) which breaks down H_2O_2 into oxygen (O_2) and water (H_2O), as shown above. This reaction proceeds spontaneously without the enzymes at a very slow rate. This uncatalyzed reaction will serve as the baseline, or control, in the initial investigation.

Driving Question

How does the catalyzed decomposition rate of hydrogen peroxide compare with the spontaneous (uncatalyzed) decomposition rate?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

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

Safety

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

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

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles.

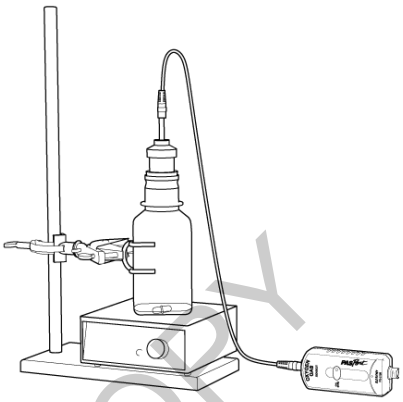
2. Connect the oxygen gas sensor or a pressure sensor to your data collection system. Build a page to show a graph of the appropriate sensor measurement versus time in minutes.

3. Adjust the sample rate to 1 sample every 15 seconds. If possible, set an auto-stop condition for three minutes.

4. Set up the equipment as shown.

5. Calibrate the oxygen gas sensor.

6. Put the magnetic stirring bar in the sampling bottle.



Setup with O_2 gas sensor

7. Using a graduated cylinder, transfer 20.0 mL of 1.5% H_2O_2 into the clean 250-mL sample bottle. If the bottle is on a stir plate, set the stir speed to a medium setting.

8. Use a pipet to add 2.0 mL of catalase and quickly insert the sensor into the opening of the bottle. Begin data collection.

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

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

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

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

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

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

Sensor	Spontaneous Rate of Decomposition	Catalyzed Rate of Decomposition	Increase in the Catalyzed Rate
Pressure sensor	4.26×10^{-5} kPa/min	RECORD ANSWERS & DATA IN	
Oxygen sensor	0.33 ppm/min	YOUR NOTEBOOK.	

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

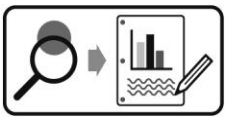
   b. Explain why the reaction is so much faster when an enzyme is present.

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

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

Design and Conduct an Experiment

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



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and data,

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

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

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

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

Synthesis Questions

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

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

   a) What does this indicate about the enzyme?

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

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

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

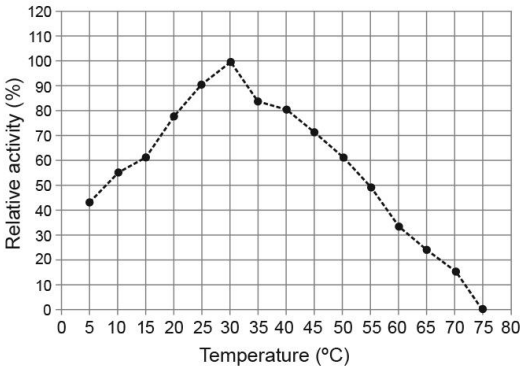


Figure 1. Amlyase activity in *Bacillus subtilis*

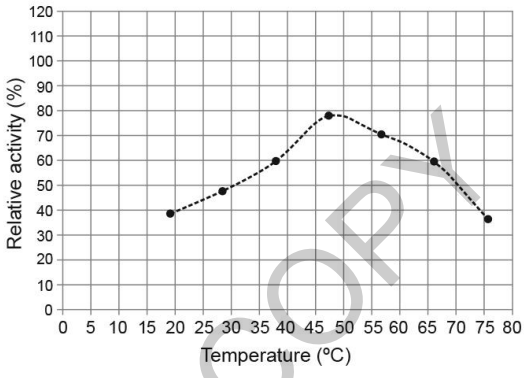


Figure 2. Amylase Activity in *Heliodiaptomus viduus*

   a. Discuss how and why temperature affects enzyme activity.

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

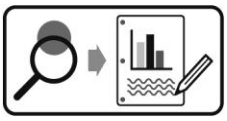
[1] Femi-Ola, T. O.; Olowe, B. M. Characterization of Alpha Amylase from *Bacillus subtilis* BS5 Isolated from *Ameritermes evuncifer* Silvestri. *Research Journal of Microbiology* 6 (2011): 140–146.

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

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

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



Develop and conduct your experiment using the following guide.

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

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

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

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

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

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

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

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

_____

_____

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

_____

_____

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

_____

_____

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

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

2. DIFFUSION

Background

Consider the simple act of breathing. With each inhalation and exhalation, your body operates under the principles of diffusion. Thanks to this process, oxygen molecules in an area of high concentration (the atmosphere) move to an area of lower concentration—your lungs, and then into the blood flowing through capillaries in the lungs. Oxygen then diffuses from the capillaries into the body's cells. Likewise, waste products from cell activity (CO_2) diffuse out of cells, into capillaries, travel to the lungs and diffuse out to the atmosphere. Both oxygen and carbon dioxide move along a concentration gradient (from high concentration to low concentration). Fortunately for you, this diffusion of gases is efficient and keeps you alive.

Most intercellular traffic occurs via diffusion. Therefore, it is important to understand how this process occurs. It is also important to understand what factors affect diffusion rates, such as the size of the molecule, the "steepness" of the concentration gradient, the distance the molecules must travel, the permeability of the membrane, and the temperature of the environment.

In this activity, you will use dialysis tubing to simulate the cell membrane and apple cider vinegar to represent intracellular fluid, a fluid containing a mixture of substances. With a pH sensor, you can determine the rate of diffusion for one of these intracellular substances: hydrogen ions (H^+).

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

Driving Question

How quickly do substances diffuse across a membrane?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

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

Safety

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

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

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles.

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

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

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

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

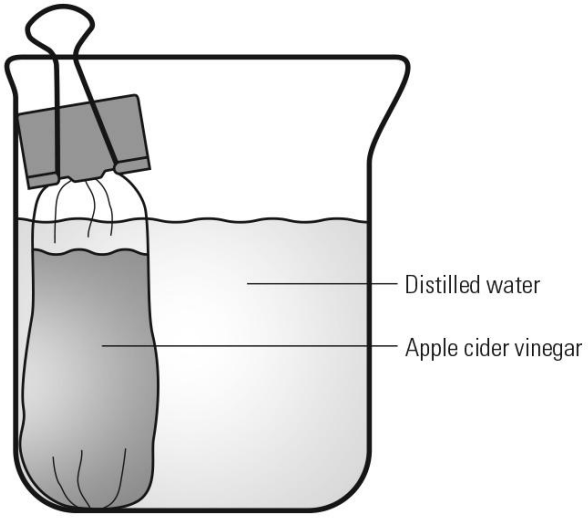
6. Use a clean pipet or syringe to add approximately 15–20 mL of vinegar from the graduated cylinder to the dialysis bag. Close the bag by tying a knot or by twisting the tubing and closing it with a binder clip. Rinse the outside of the bag with distilled water.

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

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

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

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



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

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

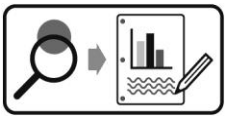
? 11. If the experiment is repeated with 20 mL of pickle juice in a dialysis bag, how do you expect the results to compare to the first experiment with apple cider vinegar? Explain the basis for your prediction.

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

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

Design and Conduct an Experiment

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



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

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

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

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

Synthesis Questions

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

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

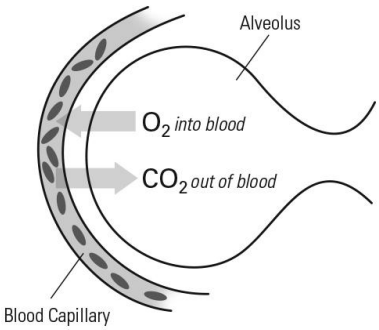
    b. Provide specific examples of molecules or other particles that enter or exit cells and for each example describe the mechanism of transportation.

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

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

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

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



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

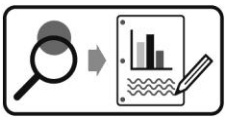
Table 1: Atmospheric oxygen concentration at various elevations

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

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

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



Develop and conduct your experiment using the following guide.

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

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

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

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

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

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

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

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

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

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

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

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

3. OSMOSIS

Background

Osmosis is the movement of water through a semipermeable membrane. During osmosis, water molecules may move from outside a cell to the inside or vice versa. The direction of the net movement of water depends on the concentration of water and solutes inside the cell compared to outside the cell.

A number of terms in biology describe the state of the extracellular fluid compared to the intracellular fluid. One set of terms is: *hypertonic*, *hypotonic*, and *isotonic*. In an isotonic situation, there is a state of dynamic equilibrium. Water molecules cross the membrane in both directions, but there is no net movement of water. In hypertonic and hypotonic situations, there is a net movement of water driven by a difference in water concentration on the two sides of a membrane. Water concentration is lower in a fluid with a high amount of dissolved solutes, and higher in a fluid with a low amount of dissolved solutes.

In this investigation, you will be given two pairs of solutions. In each pair, one solution represents extracellular fluid. The other solution will be put into a bag made from dialysis tubing, and will model the intracellular fluid of a cell. From your observations, you should be able to determine whether the extracellular fluid is hypertonic or hypotonic to the model cell.

Driving Question

Which extracellular fluid represents fluid that is hypertonic to the cell? When blood becomes hypertonic, how does the body respond to maintain homeostasis?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

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

Safety

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

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

Investigation

Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles.

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

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

4. Calibrate the colorimeter.

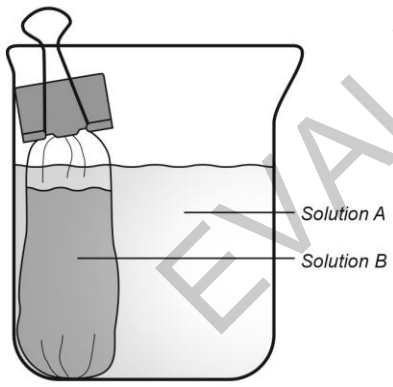
5. Obtain two graduated cylinders and pour 20 mL of Solution B into one and 20 mL of Solution D into the other.

6. Use a plastic pipet to fill a clean, dry cuvette with approximately 5 mL of Solution B. Place the cuvette into the colorimeter and record the transmittance of green light. Remove the cuvette.

7. Use a different pipet to fill a clean, dry cuvette with Solution D. Measure and record the transmittance of green light through the solution.

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

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



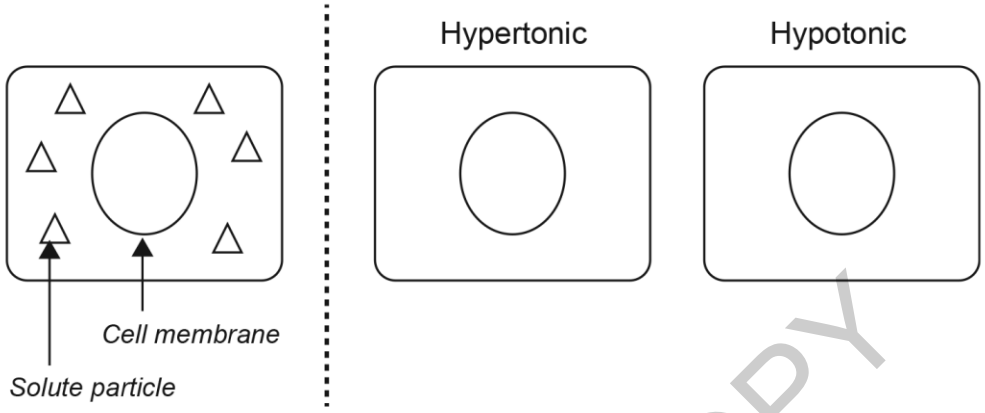
Dialysis bag, submerged in beaker

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

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

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

    NOTE: Draw the diagrams in your lab notebook.



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

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

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

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

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

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

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

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

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

Data Analysis

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

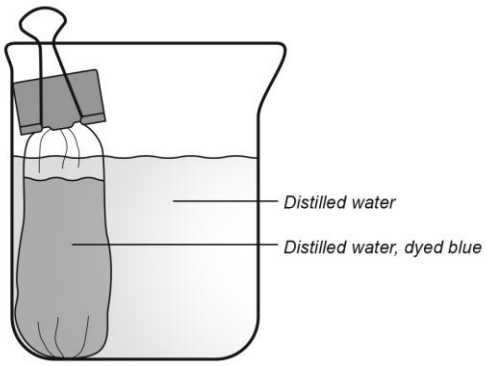
   b. If transmittance decreased, did the solution become lighter in color or darker in color?

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

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

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

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



Dialysis bag, submerged in beaker

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

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

Synthesis Questions

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

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

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

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

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

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

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

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

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

4. PLASMOLYSIS

Background

Water potential is an important concept for plant scientists and farmers. Water potential influences plants in many ways, from the cellular to the whole organism level. For plants to take in water from soil, the water potential of the soil must remain higher than the water potential inside the plant cells. This is due to the fact that during osmosis, water molecules diffuse down a water potential gradient, from a region of higher water potential to a region of lower water potential.

Water potential is influenced by two variables: the presence of solutes in a solution and pressure. Typically, the presence of solutes, and the concentration of these solutes, is the factor most responsible for determining if osmosis occurs. Solutes lower the water potential of a solution because *hydration shells* around solute particles make some water molecules less free to move, lowering the potential for water movement from that solution to another region. Distilled water has a water potential of zero; all solutions containing solutes have a negative water potential.

Plant cells and animal cells are affected similarly when surrounded by fluid hypertonic to the cell. A hypertonic solution has a relatively high amount of solute and, therefore, a low water potential. The low water potential causes water to leave cells. In plants, the result is called *plasmolysis*. Due to the loss of water, the volume of the cytosol decreases and the cell membrane pulls away from the cell wall. Plasmolysis can be observed by looking at plant tissue with a microscope. If a plant or animal cell loses too much water, the cell dies.

Turgor pressure is a characteristic of plant cells but not animal cells. Plant cells are turgid in hypotonic environments. (Animal cells burst, or *lyse*, in this condition.) The high water potential of a hypotonic solution causes water to enter plant cells, increasing the volume of the cytosol. Turgor results from the cell membrane and cytosol pushing against the cell wall. If plant cells experience plasmolysis, they lose turgor pressure—which is why a plant wilts in dry soil.

Driving Question

How can the water potential of plant cells be determined?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- Conductivity sensor
- Microscope, 400× magnification
- Microscope slides and cover slips, 4
- Plastic pipet or eye dropper
- Three salt solutions of unknown concentration, several drops
- Red onion section
- Water, several drops
- Paper towel

Safety

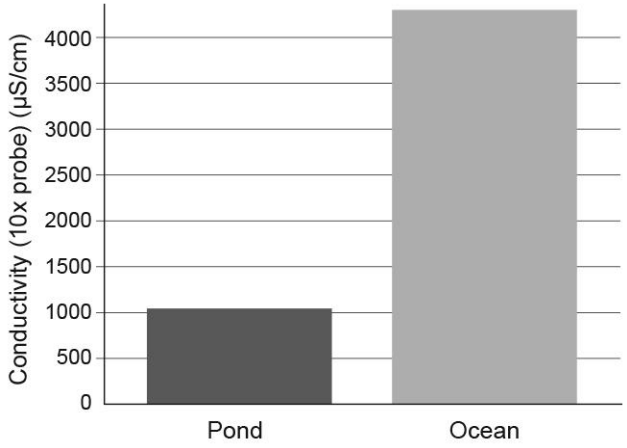
Follow these important safety precautions in addition to your regular classroom procedures:

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

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

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



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

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

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

3. Prepare three additional microscope slides with onion tissue.

   a. For each of these slides, use one of the three "unknown" solutions (A, B, or C) as the liquid for the wet mount.

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

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

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

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

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

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

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

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

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

The equation used to calculate water potential is

$\Psi = \Psi_s + \Psi_p$

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

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

i is the ionization constant

C is the molar concentration

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

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

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

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

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

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

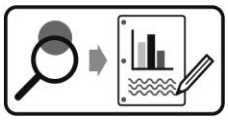
    b. How does the water potential of Unknown C compare to that of Unknown A? Support your claim with evidence and clear reasoning.

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

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

Design and Conduct an Experiment

A number of methods have been developed to experimentally determine the water potential of plant tissues. One method, the gravimetric technique, involves measuring the change in mass that occurs in tissue samples exposed to solutions of known concentration. For your experiment, devise a method to use the gravimetric technique to compare the water potentials of two or more types of plant tissue.



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

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

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

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

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

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

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

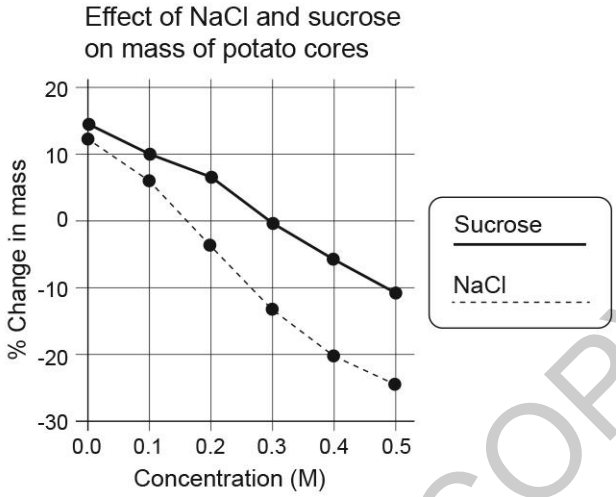
Synthesis Questions

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

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

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

4. Potato cores were placed in solutions with varying concentrations of sodium chloride (NaCl) and sucrose ($C_{12}H_{22}O_{11}$) for 24 hours. The percent change in mass of the cores in each solution was calculated and plotted on a graph.[3] Consider the equation for water potential and the properties of sodium chloride and sucrose as solutes, and explain the different results for the cores in the two solutions.

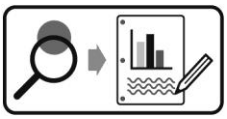


[3] Kosinski, R.J.; Osmosis and the Water Potential of Potato Tissue. Clemson University. Last update: October 2013. Retrieved February 6, 2014 from http://biology.clemson.edu/bpc/bp/lab/110/osmosis.htm.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

A number of methods have been developed to experimentally determine the water potential of plant tissues. One method, the gravimetric technique, involves measuring the change in mass that occurs in tissue samples exposed to solutions of known concentration. For your experiment, devise a method to use the gravimetric technique to compare the water potentials of two or more types of plant tissue.



Develop and conduct your experiment using the following guide.

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

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

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

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

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

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

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

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

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

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

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

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

5. CELL SIZE

Background

Virtually every biology student has heard the following statements from his or her teacher, "The cell is the basic unit of all life," and "A cell's structure is related to its function." Both statements are absolutely true and fundamental to understanding biology.

One of the most interesting things about cells is their size. Why are cells so small? How can cells accomplish so many metabolic functions within such a small space? The answers to these questions boil down to two fundamental concepts: (1) Biological membranes regulate traffic into and out of a cell and compartmentalize cellular activities and (2) the ability of a cell to procure nutrients, eliminate wastes, and perform metabolic processes at a sufficient rate is governed by a cell's surface area (its membrane) relative to its volume (the cytosol enclosed by the membrane). In fact, the SA:V ratio is so fundamental to survival that sizes, shapes, and structures that maximize this ratio are exhibited within all biological systems, from the sub-cellular level to the whole organism. It is a factor that drives adaptations as diverse as the size and shape of a single cell and the size and body shape of animals. It is a factor that explains how the body systems in the large bodies of mammals are adapted to meet the challenge of supplying trillions of cells with their need for nutrients and oxygen.

In this activity you will use potato cubes as models for cells, and investigate how a difference in surface-area-to-volume ratio affects a cell's interaction with its environment.

Driving Question

Do small and large cells lose heat to the environment at the same rate?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

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

Safety

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

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

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles.

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

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

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

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

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

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

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

RECORD ANSWERS & DATA IN YOUR NOTEBOOK.

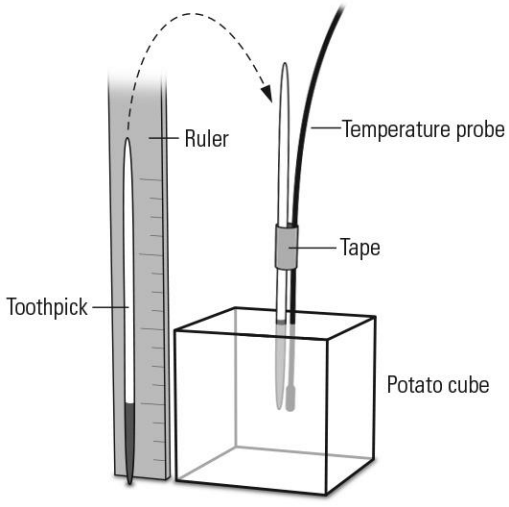
6. Calculate the surface area, volume, and surface-area-to-volume (SA:V) ratio for each cube. Record these values in Table 1. Which has a greater SA:V ratio, a large cube or a small cube?

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

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

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

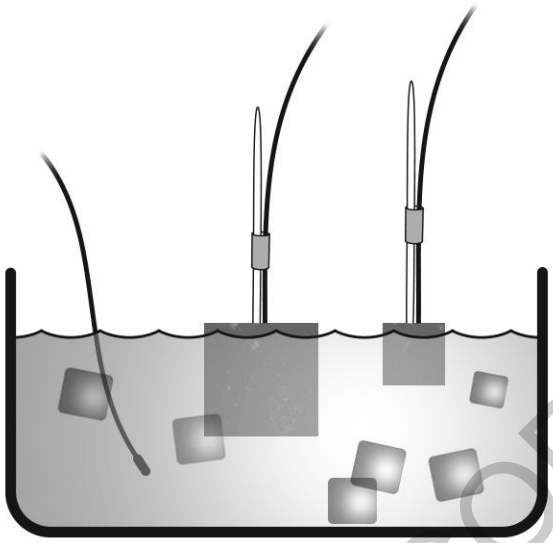
   c. Remove the toothpick and insert a temperature probe into the hole. Reinsert the toothpick into the hole and use tape to secure the wire of the probe to the toothpick.



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

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

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



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

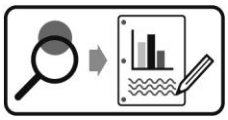
11. What is the relationship between the cooling rate and the SA:V ratio? Use evidence from the investigation to support your claim.

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

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

Design and Conduct an Experiment

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



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

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

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

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

Synthesis Questions

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

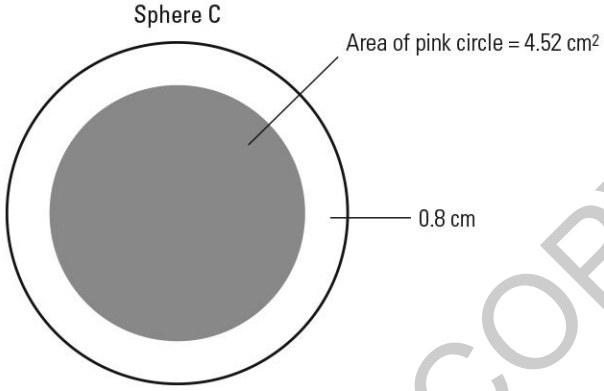
   Table 2: Surface-area-to-volume ratios of different sized spheres

Sphere	Radius (cm)	Surface Area (cm^2)	Volume (cm^3)	SA:V Ratio
A	0.5 cm			
B	1 cm			
C	2 cm			
D	4 cm			
E	8 cm			

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

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

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



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

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

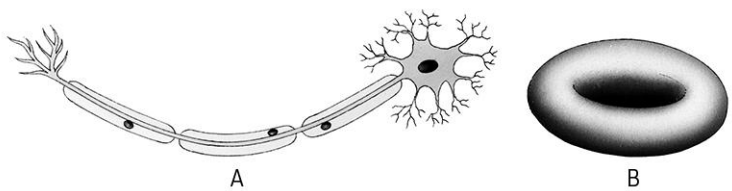
a. The largest penguin on earth is the Emperor penguin with an average height of 1.1 m and a body mass of 27–41 kg. Emperor penguins live in the very cold climate of Antarctica. Galapagos penguins live in a much warmer climate and average 0.5 m in height, and 1.7–2.6 kg in body mass. Based on their body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

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

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

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

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

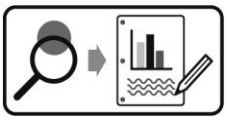


c. The respiratory, circulatory, digestive, and excretory systems of mammals all contain specialized structures that are highly branched to maximize their membrane surface area relative to their volume. Describe two examples of highly branched structures in these systems and explain how the SA:V ratio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

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

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



Develop and conduct your experiment using the following guide.

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

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

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

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

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

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

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

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

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

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

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

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

6. HOMEOSTASIS

Background

Your body produces heat as a byproduct of metabolism. Your body also loses heat to the environment. These events may seem to balance one another; however a person's metabolism changes with activity, and the temperature of the environment does not remain constant, resulting in greater or less heat loss. In spite of these ever-changing internal and external conditions, the body's core temperature remains very near 98.6 °F. An organism's ability to maintain its body temperature within a narrow range is known as thermoregulation. Thermoregulation is one example of homeostasis: the property of a cell or an organism to maintain equilibrium in its internal environment.

Mammals have a variety of mechanisms to maintain homeostasis with regard to body temperature, water balance, blood volume, blood pH, and other internal conditions. The brain and nervous system play an important role in the body's response to stimuli and or other events that change internal conditions in some way. In the case of thermoregulation, the hypothalamus is vital to homeostasis. It receives information about the external environment, interprets that information, and responds to changes by sending signals to multiple organ systems. For example, the hypothalamus may send signals that adjust blood flow to compensate for changes in the body's core temperature.

In this lab you will analyze the body's response to a cold stimulus, using multiple temperature probes to measure the surface temperature of the skin at two locations, and relate the results to thermoregulation.

Driving Question

How does the hypothalamus respond to help maintain homeostasis when temperature changes occur in the extremities?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

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

Safety

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

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

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

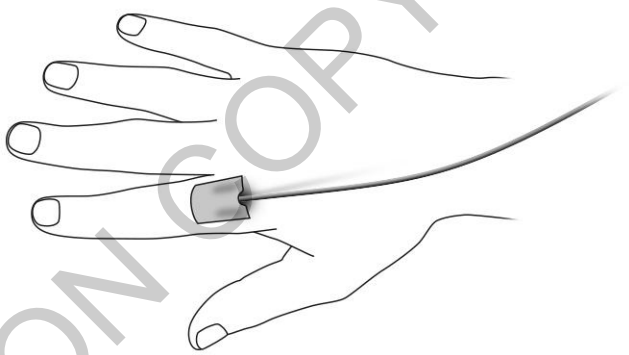
1. Put on your safety goggles.

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

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

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

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

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

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

5. Build graph displays for each temperature sensor.

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

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

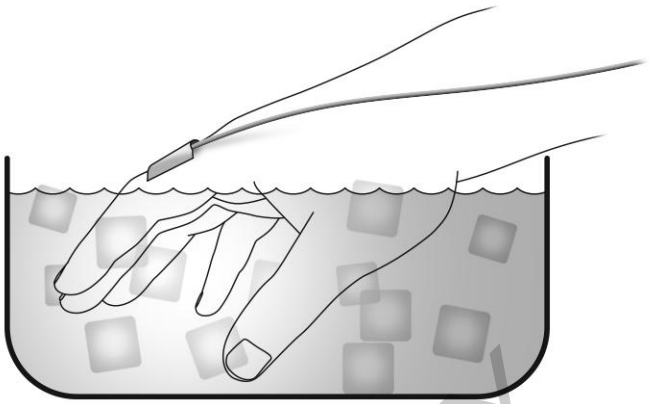
? 8. Is the skin temperature of the hand the same as the body's core temperature? If not, explain why not.

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

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

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

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



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

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

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

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

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

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

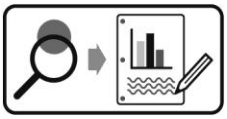
12. Did the temperature of the right (experimental) hand change when it was submerged in ice water? If yes, describe the change that occurred.

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

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

Design and Conduct an Experiment

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



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

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

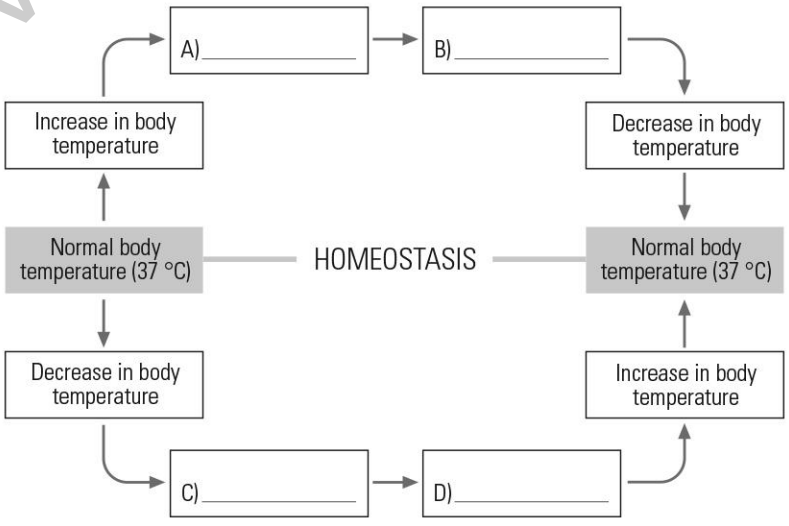
2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

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

Synthesis Questions

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

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



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

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

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

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

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

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

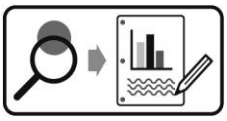
   b. Mammals are endotherms and have evolved a wide variety of adaptations to deal with the different challenges to thermoregulation in the world's biomes. Identify three biomes with distinctly different climates. For each biome, name a mammal that lives there and list at least two adaptations each mammal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

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

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

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



Develop and conduct your experiment using the following guide.

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

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

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

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

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

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

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

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

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

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

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

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

7. CELLULAR RESPIRATION

Background

Seeds may lay dormant for months or years following dispersal, waiting for the right conditions to promote germination. But what factors affect the embryo's chances of breaking the soil's surface and developing into a seedling? What changes are occurring within the cells of the germinating seed, and how are those changes affected by the environment?

One indication of cellular activity is cellular respiration, a process that breaks down sugars in the presence of oxygen to produce ATP.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{Energy (36 ATP)}$$

Cellular respiration is just one example of how biological systems transform free energy into a usable form. Energy transformations in living systems, or "bioenergetics," is one of the themes you will observe throughout your investigations. Despite the complexity of bioenergetic processes, the following fundamental concepts apply:

- Bioenergetic processes are governed by enzyme activity.
- Because bioenergetic processes are governed by enzyme activity, the rate of these processes will change as environmental parameters change.
- In cellular respiration, enzymes facilitate the process of catabolizing high-energy organic molecules to low-energy carbon dioxide and water.
- By measuring the products of a bioenergetic process, such as cellular respiration, the rate of that bioenergetic process can be determined.

Driving Question

How does germination affect the rate of cellular respiration in seeds?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- Carbon dioxide gas sensor
- Sensor extension cable
- Sample bottle, 250 mL
- Balance, readability: 0.01 g
- Paper towel
- Germinating pinto beans (50)

Safety

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

- Wear safety goggles at all times.
- Handle living organisms with care.

Initial Investigation

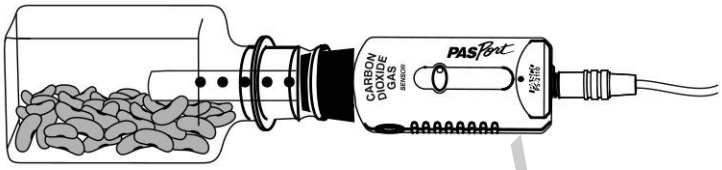
Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles.

2. Connect and calibrate the carbon dioxide gas sensor.

3. Create a display of the sensor measurement in ppm versus time in minutes, and adjust the sample rate to one sample every 15 seconds.

4. Select 50 germinating seeds. Dry the seeds with a paper towel and record their mass.



Setup with CO_2 gas sensor

5. Place the seeds in the sample bottle with the sensor and lay it horizontally on your table as shown.

6. Wait for 1 minute and then start data collection; record data for 5 minutes.

7. The cells within germinating seeds carry out cellular respiration to acquire adenosine triphosphate (ATP).

   a. Identify the organelle in which cellular respiration occurs in eukaryotic cells and describe the structure of this organelle.

   b. Summarize how ATP is produced within this organelle and describe the importance of ATP for the germinating seeds.

   c. Explain why carbon dioxide is produced during the process of cell respiration.

8. After 5 minutes, compare your data for germinating seeds to the data for dormant (dry, non-germinating) seeds in Table 1.

   NOTE: To make the comparison, you will first need to normalize *the data by finding the respiration rate per gram.*

Table 1: Dormant seed respiration data

Condition	Seed Quantity	Seed Mass (g)	Seed Respiration Rate (ppm CO_2/min)	Normalized Respiration Rate [(ppm CO_2/min)/g]
Dormant	50	19.01	22.8	
Germinating (24 hr)	RECORD ANSWERS & DATA IN YOUR NOTEBOOK.			

* Dormant seed respiration data was collected using *Phaseolus vulgaris* in a 250-ml sample bottle over 6 hours. The rate was determined from a linear regression of the data. Given the difficulty of measuring the low rate of respiration in dormant seeds, a research grade respirometer was used.

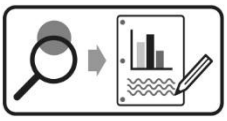
9. How do you explain the difference in the rate of respiration between germinating seeds and dormant seeds?

10. What other ways could the data be normalized to enable us to make some comparisons across trial groups? What are the limitations and assumptions of each approach?

11. If a similar experimental protocol was repeated after the seed had sprouted and matured into a seedling with several leaves, how would you expect the results to change?

Design and Conduct an Experiment

Cellular respiration is critical to utilizing stored energy for cells and organisms. It is a process that can be affected by a number of factors. Identify factors that might change the rate of respiration in seeds or develop a related question using another model organism.



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

   b. Explain why the results occurred.

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

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

Synthesis Questions

1. A yeast culture is placed into a flask attached to an apparatus that detects bubbles released by the solution. Twenty grams of glucose are added to the culture and the temperature is incrementally increased and monitored by a sensor. The results are shown below.

Table 2: Counting bubbles to measure the effect of temperature on yeast respiration

Temperature °C	5	15	25	35	45	55	65	75
Five Minute Bubble Count	0	18	38	61	33	24	3	0

   a. Draw a graph showing the effect of temperature change on the rate of respiration in yeast cells.

   b. Using your knowledge of enzymes and the data provided, explain the results of the experiment.

2. The breakdown of sugars to carbon dioxide and water during respiration releases energy. Much of this energy is captured by the cells to generate ATP through oxidative phosphorylation, but some energy is lost as heat.

   a. Describe a procedure to use temperature to measure metabolism in a human.

   b. Explain how the laws of thermodynamics apply to cellular respiration in this example.

3. An experiment was carried out to compare the effect of temperature on respiration rate in crickets and in mice. The experiment showed that at cold temperatures the respiration rate in crickets decreased. However, in mice the respiration rate increased at colder temperatures. Do you think the results of the experiment are valid? Explain your position.

4. Free energy *G* is an important aspect of understanding how organisms obtain, use, and transform energy to maintain their complex levels of organization and grow and develop. Table 1 shows the change in free energy that accompanies two processes that take place in cells.

   Table 1: Free energy changes

Reaction	ΔG
ADP + P_i → ATP	7.3 kcal/mol
Glucose → 2 Pyruvic acid	–32.1 kcal/mol

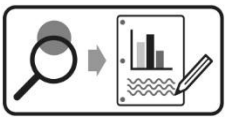
   a. Which reaction is more energetically favorable? How do you know?

   b. In cells, a number of reactions are "coupled." What purpose does coupling reactions serve?

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

Cellular respiration is critical to utilizing stored energy for cells and organisms. It is a process that can be affected by a number of factors. Identify factors that might change the rate of respiration in seeds or develop a related question using another model organism.



Develop and conduct your experiment using the following guide.

1. For the reaction shown below, which reactants or products can you measure with the available equipment? Explain which sensor, procedure, or equipment can be used and what variable would be measured.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{Energy (36 ATP)}$$

2. Based on your knowledge of cellular respiration and biological systems, what environmental factors (abiotic or biotic) could affect this process?

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

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

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

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

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

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

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

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

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11. Describe at least 3 potential sources of error that could prevent you from gathering accurate and reliable data.

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

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

8. FERMENTATION

Background

Yeast are single-celled eukaryotic organisms classified as *facultative aerobes*; they carry out both aerobic respiration and fermentation, depending on whether oxygen is readily available. When yeast ferment sugars, such as glucose, they produce ethanol and carbon dioxide. The fermentation pathway is paired with glycolysis and supplies the cells with a small amount of adenosine triphosphate (ATP) for each glucose molecule converted to ethanol.

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

You likely know that yeast are commonly used in baking. A basic bread recipe calls for sugar and flour (starch) as ingredients. When yeast use one or more ingredients in the bread dough for energy, carbon dioxide gas is produced and causes the bread to rise. The ethanol evaporates due to the heat used for baking.

This investigation, comparing the use of sugar and starch by yeast for fermentation, uses an ethanol sensor rather than a carbon dioxide gas sensor, since carbon dioxide is a product of both fermentation and aerobic respiration.

Driving Question

Can yeast use both sugar and starch for fermentation?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- Ethanol sensor
- Graduated cylinders (2), 50-mL
- Sampling bottle or glass flask (125-mL or 250-mL)
- Plastic pipet
- Small beaker
- Magnetic stir plate and stir bar
- Rod stand and 3-finger clamp *(optional)*
- 1% Ethanol (for calibration)
- Yeast suspension, 40–60 mL
- 2% Sucrose solution, 30 mL
- 2% Starch solution, approximately 150 mL
- Iodine indicator (IKI), 5–10 drops
- Water from germinating seeds, 5 mL
- 2% Starch solution mixed with amylase, 30 mL *(optional)*

Safety

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

- Wear safety goggles at all times.
- Do not get the end of the ethanol sensor wet; the sensor is a gas sensor and should never be submerged in a liquid.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

Part 1

1. Put on your safety goggles.

2. Connect the ethanol sensor to your data collection system. (If possible, connect the data collection system to a power source during data collection, rather than relying on the system's battery power.) Allow the sensor to "warm up" for at least 5 minutes. After the warm-up period, calibrate the sensor with a 1% ethanol solution.

3. Set up the data collection system to measure percent ethanol over time.

4. Add 30 mL of 2% sucrose solution to a clean, empty container (sampling bottle or flask). Place a magnetic stir bar into the bottle and place it on a magnetic stir plate.

5. Add 20 mL of yeast suspension to the sampling bottle and set the stir speed to a low–medium setting.

   NOTE: Be sure the bottle and sensor do not tip over. If possible, use a rod stand and 3-finger clamp to secure the bottle. The sensor is a gas sensor and should NOT be immersed in a liquid.

6. Seal the sampling bottle with the ethanol sensor. The seal between the stopper of the sensor and the bottle's opening should be air tight. Leave the system undisturbed for 1–2 minutes before starting data collection.

7. Begin data collection. Collect data for at least 15 minutes. Answer the following questions as you wait for data collection to end.

8. Describe how ethanol is formed during fermentation.

9. What type of molecule is sucrose? What would yeast have to do with sucrose in order to utilize it as an energy source for fermentation?

10. After at least 15 minutes has transpired, stop data collection. Thoroughly rinse the sampling bottle. Then add 30 mL of 2% starch solution and 20 mL of yeast suspension to the bottle.

11. Measure the rate of ethanol production over the course of 15 minutes (or longer).

12. Sketch or print a record of your data. Which is a better energy source for yeast, sucrose or starch? Describe evidence that supports your answer.

Part 2

13. Fill a small beaker halfway with starch solution. Add 5–10 drops of iodine indicator (IKI) to the beaker and mix. Record the color of the mixture.

14. Add 5 mL of water from germinating seeds to the starch/IKI solution. Slowly swirl the beaker until you observe a change. Record your observations.

15. What effect did the water from germinating seeds have on the starch solution? How do you know this?

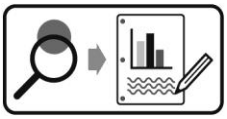
16. What enzyme is present in germinating seeds that helps explain the color change that occurred when seed-water was added?

17. If you added seed-water to a starch solution and then provided this solution to yeast for fermentation, how do you think the results would compare to the original yeast-starch results? Explain your prediction.

18. Depending on the time available, your teacher may have you carry out this experiment or may simply provide data for you to compare to your prediction. Based on the data collected or provided, how do the results compare to your prediction?

Design and Conduct an Experiment

In addition to the types of compounds present in the environment, there are myriad factors that may affect the efficiency of fermentation in yeast. Many industries depend on optimum fermentation rates to produce products. Consider a factor that you think affects ethanol production by yeast and design an experiment to test that factor.



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

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

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

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

Synthesis Questions

1. Yeast are used to ferment sugars as part of the production process of many human-made products including biofuels—fuels made from organic "feedstocks" such as corn or sugarcane.

   a. These feedstocks contain a large amount of sugar, but also contain starch and cellulose. What is the purpose of using enzymes such as amylase or cellulase in the biofuel production process?

   b. What is the role of genetic engineering in biotechnological methods that efficiently produce products such as ethanol?

2. Compare and contrast lactic acid fermentation and alcohol fermentation. What determines the type of fermentation carried out by an organism?

3. Free energy *G* is an important aspect of understanding how organisms obtain, use, and transform energy to maintain their complex levels of organization and grow and develop. Table 1 shows the change in free energy that accompanies two processes that take place in cells.

Table 1: Free energy changes

Reaction	ΔG
Glucose + Oxygen → Carbon dioxide + water	–2870 kJ/mol
Glucose → Ethanol + Carbon dioxide	–285 kJ/mol

   a. Explain the relationship between the data provided in Table 1 and the difference in ATP production for aerobic respiration compared to fermentation.

   b. How does the information above help explain why fermentation is limited to microorganisms or to brief periods of time in certain cells of multicellular organisms?

4. Metabolic pathways are closely regulated to maintain homeostasis within organisms.

   a. Describe two ways in which the activity of metabolic enzymes can be altered, thereby providing a mechanism for a cell to control a biochemical pathway?

   b. Phosphofructokinase 1 (PFK1) is an enzyme which catalyzes a step of glycolysis that is highly regulated by the cell. If the enzyme is active, glycolysis takes place. If the enzyme is inhibited, glycolysis is inhibited. Use the data below to draw a graph of PFK1 activity versus ATP concentration. Explain the shape of the curve from 0.4 µM to 2 µM. Also explain why the activity of one enzyme in a pathway would affect the entire pathway, not just the step it catalyzes.

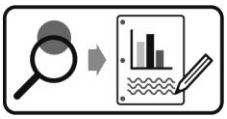
Table 2: PFK1 activity versus intracellular ATP concentration

PFK1 Activity (% of V_{max})	ATP Concentration (µM)
40	0.2
50	0.4
30	0.8
20	1.2
15	1.6
5	2.0

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

In addition to the types of compounds present in the environment, there are myriad factors that may affect the efficiency of fermentation in yeast. Many industries depend on optimum fermentation rates to produce products. Consider a factor that you think affects ethanol production by yeast and design an experiment to test that factor.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of fermentation, what environmental factors (abiotic or biotic) could affect this process?

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

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

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

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

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

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

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

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

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

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

9. PHOTOSYNTHESIS

Background

Plants and other producers utilize the sun's energy to build the complex organic compounds that serve as a source of energy for the organism. Chloroplasts are the site of this energy capture and biosynthesis. Chlorophyll and other pigment molecules play a vital role in the light reactions of photosynthesis, capturing energy that drives an electron transport chain and ATP synthesis. Ultimately, through light-independent reactions, the chemical energy is transferred and stored within molecules such as glucose. This entire process is summarized by the chemical equation:

$$6CO_2 + 6H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6O_2$$

The uptake of carbon dioxide is an indication that photosynthesis is occurring within the chloroplasts of a leaf. The reactions that fix carbon into organic compounds depend on the products of the light reactions and are therefore dependent on the absorption of light by pigments.

While sunlight is composed of many different wavelengths of light, not all wavelengths are equally available to a plant. This investigation compares the amount of photosynthesis that occurs when different colors of light are provided to a plant.

Driving Question

Does the color of light affect the rate of photosynthesis in green leaves?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- Carbon dioxide gas sensor
- Sensor extension cable
- Sampling bottle, 250-mL
- Box, foil, or cloth for shading the setup
- Light source
- Compact fluorescent light bulb, 60 W equivalent (or higher), red
- Compact fluorescent light bulb, 60 W equivalent (or higher) green
- Fresh spinach leaves
- Forceps or pencil

Safety

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

- Wear safety goggles at all times
- Allow the light bulb to cool before removing it from the light source

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles.

2. Connect the carbon dioxide gas sensor to your data collection system and calibrate the sensor.

3. Display a graph of Carbon dioxide (ppm) on the *y*-axis versus Time in minutes on the *x*-axis. Adjust the sample rate to one sample every 5 seconds.

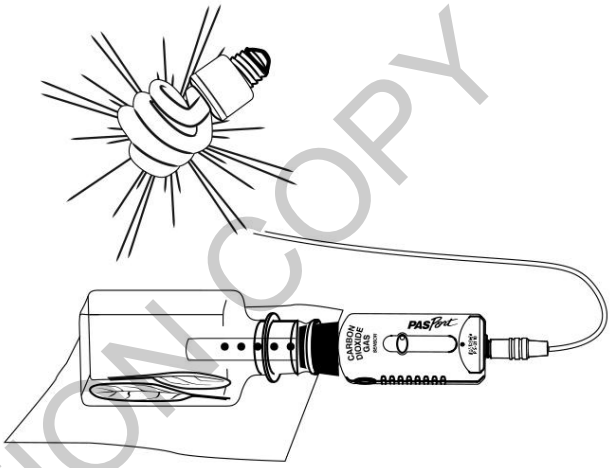
   NOTE: If your data collection system allows you to set an automatic stop condition, set the stop time for 7 minutes.

4. Holding the sampling bottle horizontally, place two fresh leaves into it. The leaves should lay flat and overlap as little as possible.

5. Seal the sampling bottle with the carbon dioxide sensor and stopper. Rest the bottle on its side on a flat surface.

6. Arrange the light source directly above the sampling bottle.

   NOTE: Make sure a colored light bulb (red or green) is in the light source.



7. Shade the bottle from ambient white light in the room.

8. Turn on the light source. Wait approximately 15 seconds and then start collecting data. Adjust the scale of the graph to show all data. Collect data for 7 minutes.

9. After 7 minutes, stop recording data and turn off the light source.

10. Refresh the air in the bottle by waving the bottle through the air a number of times. If necessary, readjust the leaves so they lay flat.

11. Remove the colored bulb from the light source and replace it with a different colored bulb.

12. Repeat the data collection using the other colored bulb.

13. Draw or print a record of the data.

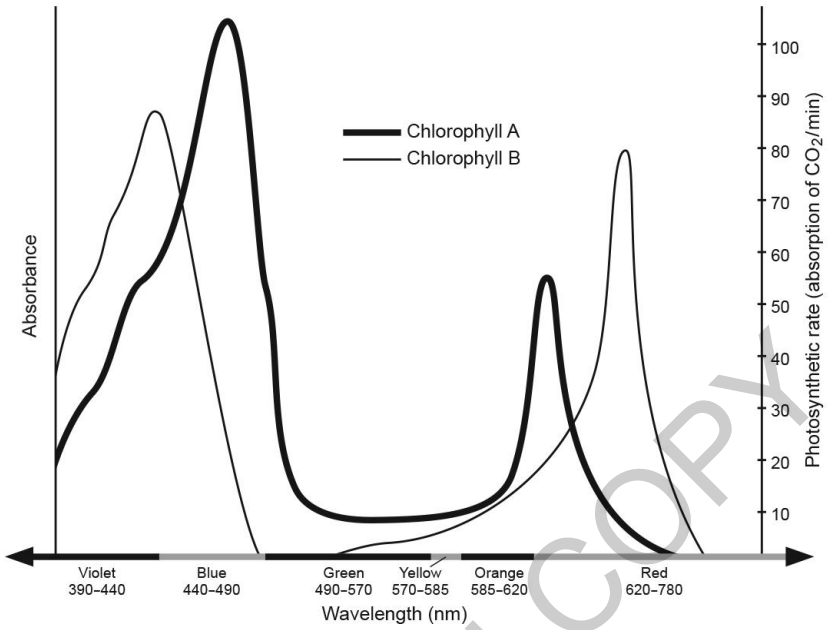
14. Describe the apparent trend of the data.

15. How can you quantify the results? What type(s) of mathematical analysis would be appropriate?

    a. Identify the method(s) chosen for quantification and analysis of results.

    b. Apply the method(s) chosen to the data collected in the initial investigation.

16. Create a graph that relates the absorbance spectrum to the action spectrum. The absorbance spectrum is provided in the graph below. For the action spectrum, draw a bar graph indicating the photosynthetic rate at the wavelengths tested in the investigation.

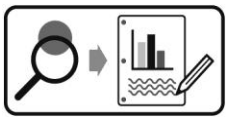


17. Based on a typical absorbance spectrum for leaves, explain the results of this investigation. Your response should provide a clear connection between light absorbance, the reactions of photosynthesis, and evidence from the investigation.

18. What could be done to confirm the relationship observed in the data collected by your group?

Design and Conduct an Experiment

Photosynthesis is critical for providing energy to organisms in an ecosystem. It is a process that can be affected by a number of factors. Think of possible factors and design an experiment to test one of them.



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data,

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

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

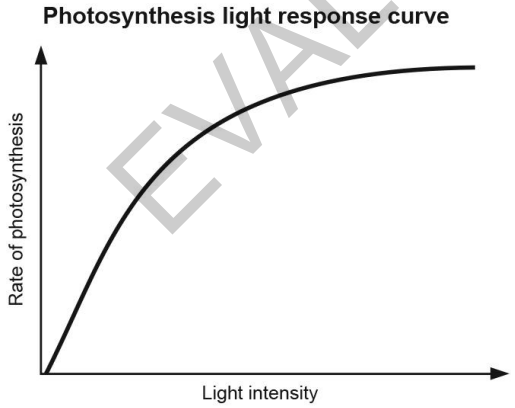
2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

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

Synthesis Questions

1. Incandescent light bulbs are inefficient light sources due to a large amount of energy released as heat into the environment. If you carried out a photosynthesis experiment using an incandescent bulb, how would you expect this to affect the results? Explain your reasoning.

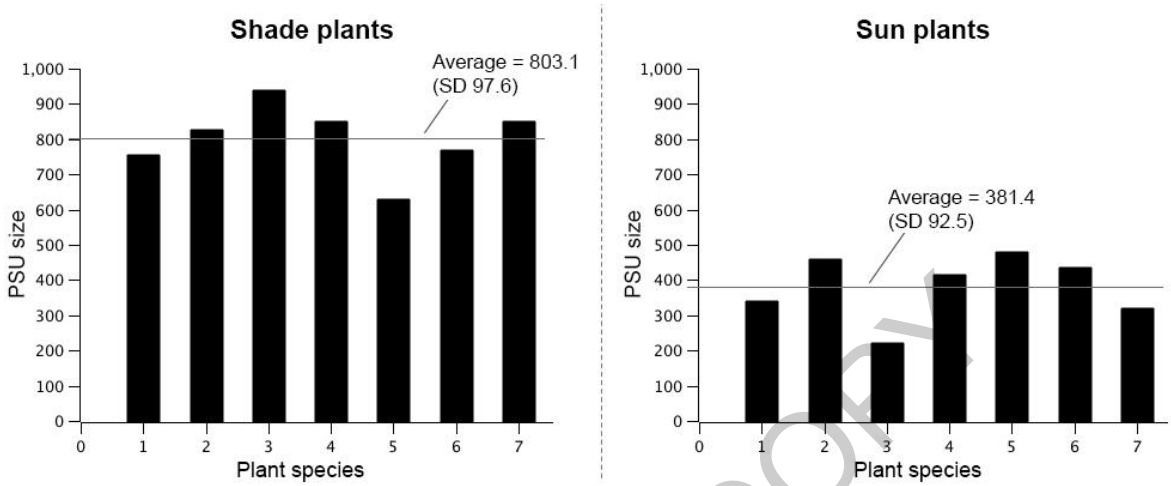
2. Refer to the generalized light response curve for photosynthesis to answer the following.



   a. For low to medium light intensity, explain the trend in the data.

   b. For higher levels of light intensity, explain the trend in the data.

3. A "photosynthetic unit" (PSU) is a complex of pigment molecules and one or more reaction centers. The reaction centers are responsible for passing excited electrons to an electron acceptor within a photosystem. Graphs of the data from a study that compared the PSU sizes for a variety of plants are shown below.[4] In the study, scientists measured the PSU size of seven different species of shade plants and seven different species of sun plants.



a. What conclusion can be made from the data acquired in this study?

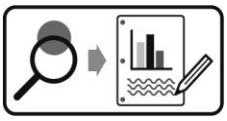
b. Considering the environments of the plants, provide an explanation for the difference in the mean PSU sizes.

[4] Malkin, S.; Fork, D.C. Photosynthetic Units of Sun and Shade Plants. *Plant Physiology* 67 (1981): 580–583.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

Photosynthesis is critical for providing energy to organisms in an ecosystem. It is a process that can be affected by a number of factors. Think of possible factors and design an experiment to test one of them.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of photosynthesis, what environmental factors (abiotic or biotic) could affect this process?

_____

_____

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

_____

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

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

_____

_____

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

_____

_____

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

_____

_____

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

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

_____

_____

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

_____

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_____

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

_____

_____

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

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

10. PLANT PIGMENTS

Background

Most plants have green leaves, a color that results from the presence of chlorophyll in the leaves. Leave may also contain accessory pigments that may not be visible to the naked eye but play an important role in acquiring energy for photosynthesis. Paper chromatography is a simple process that can be used to determine the pigments present in leaves.

Chromatography separates the components of a mixture using a solvent as a moving phase. The solvent moves up the paper and as it travels past the sample on the paper, it attracts the particles of the mixture. Differences in the chemical properties of these particles make some more attracted and others less attracted to the solvent and to the paper. Differences in attractions to the moving and stationary phases cause pigments in a mixture to travel at different rates up the chromatography paper, resulting in the separation of the pigments.

In the cells of plant leaves, chloroplasts carry out photosynthesis. Sunlight provides the energy needed for this process. Sunlight is composed of different colors (red, orange, yellow, green, blue, and violet)—each color corresponds to a narrow range of wavelengths. The structure of a pigment molecule allows it to absorb some wavelengths and reflect others. A colorimeter or spectrometer can be used to determine which wavelengths are absorbed.

A colorimeter shines four colors of light through a sample: blue, red, orange, and green. A sensor on the opposite side of the light source detects how much light of each color gets absorbed by or transmitted through the sample. A spectrometer is similar in function to a colorimeter. However, the spectrometer can determine the absorbance or transmittance for all of the colors (wavelengths) in the visible light spectrum.

Absorption of light excites electrons in pigment molecules. These excited electrons, and the electron transport systems within chloroplasts, are vital to the photosynthesis process. As in cellular respiration, certain molecules act as *electron acceptors* during the process. In this investigation, a colored compound, DPIP, acts as an electron acceptor to allow you to observe the effect of light on chloroplasts and photosynthesis.

Driving Questions

What pigments are present in spinach leaves and what colors of light are absorbed by these pigments?

What role do pigments play in the light-dependent reactions of photosynthesis?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

PART 1

- Data collection system
- Colorimeter
- Colorimeter cuvette
- PASCO Wireless Spectrometer and spectrometry software
- Spectrometer cuvette (1-cm glass cuvette)
- Plastic pipets (3), 1-mL
- Capillary tube or eye dropper without a bulb
- Chromatography chamber with solvent
- Chromatography paper
- Ethanol, 10 mL
- Colorimeter and spectrometer blanks (cuvettes with ethanol)[3]
- Pigment extract, 10 mL, or if necessary to prepare:
  Spinach leaves (3)
  Ethanol, 5–10 mL
  Beaker, small
  Mortar and pestle
  Cheesecloth or coffee filter paper
- Scissors
- Small stapler or paper clips
- Ruler
- Pencil
- Kimwipes®

PART 2

- Data collection system
- Colorimeter
- Colorimeter cuvettes (3)
- Plastic pipets (4), 1-mL
- Chloroplast suspension, 9 drops
- 0.1 M Phosphate buffer solution, 3 mL
- DPIP (2,6-dichlorophenolindophenol) solution, 2 mL
- Lamp with a compact fluorescent (CFL) light bulb
- Kimwipes
- Aluminum foil, to cover a cuvette
- Distilled water, 10 mL

Safety

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

- Wear safety goggles at all times.
- Never use ethanol near a flame; it is highly flammable.
- Work in a well-ventilated area, ideally a fume hood, when carrying out the extraction and chromatography procedures that use ethanol.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

Part 1 — Pigment Chromatography and Absorbance of Light

1. Put on your safety goggles.

2. If your teacher has prepared the pigment extract using ethanol, continue to the next step. Otherwise, follow the procedure below for preparing this extract yourself.

   a. Cut three large spinach leaves into small pieces. Discard the stem of the leaves. Place the leaves into a mortar and add approximately 5–10 mL of ethanol to soak the leaves.

   b. Use a pestle to grind the spinach to help the ethanol dissolve the pigments in the leaves. Continue grinding for at least 3–5 minutes.

   c. Place a piece of cheesecloth or a coffee filter over the top of a beaker. Filter the spinach mixture from the mortar, taking care to prevent the cheesecloth or filter paper from falling into the beaker.

      NOTE: You can squeeze the cheesecloth to help the liquid pass through.

3. Use a pencil to draw a line across a square of chromatography paper 2 cm above the bottom edge of the paper.

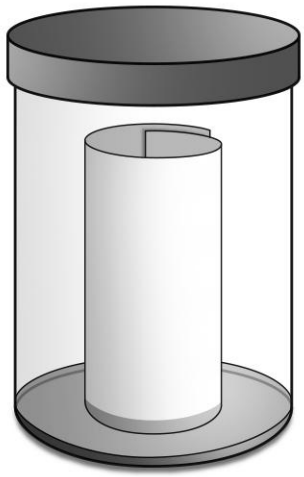
4. Make a green line of pigment extract on or slightly above the pencil line as follows:

   a. Place a capillary tube or the tip of an eye dropper into the pigment extract. Place your finger over the top of the tube or dropper to prevent the liquid from falling out when you remove it from the beaker.

   b. Place the capillary tube or pipet tip at the edge of the chromatography paper and release your finger slightly from the top of the tube, allowing a small amount of the green extract to absorb into the paper while moving the capillary tube or dropper across the paper. As needed, acquire more extract in the capillary tube or pipet tip. Be sure to keep the extract at or above the line.

   c. Let the extract dry on the paper and then repeat the process once.

5. Roll the paper into a cylinder and staple or paper clip the paper where the edges meet to prevent it from unrolling. Place the paper into a chromatography chamber that contains a small volume of solvent at the bottom. It is important that the pencil line be above the solvent.



6. Seal the chromatography chamber and observe the movement of the solvent up the paper. Leave the chamber undisturbed until the solvent moves most of the way up the paper. While you wait, continue with the questions and procedures that follow.

   NOTE: Do not allow the solvent to reach the top of the paper.

? 7. For some chromatography investigations, water is used as the solvent. In this investigation, the solvent is a solution that contains acetone (C_3H_6O). Why is an organic solvent used in this chromatography experiment rather than water?

? 8. Describe the location of pigments within plant leaf cells. Be specific.

9. Connect the colorimeter to your data collection system and calibrate the sensor with a blank that contains ethanol.

10. Fill a clean colorimeter cuvette about three-quarters full with ethanol. Add 10–15 drops of pigment extract to the ethanol, cover, and invert the cuvette to mix. The mixture should appear light green and transparent. Place the cuvette into the calibrated colorimeter.

11. Record the *absorbance* of light for each of the four colors the colorimeter detects.

? 12. For the pigment extract, which color of light has greatest absorbance? Which color of light has the lowest absorbance in the sample? Provide an explanation for these results.

13. When the paper chromatography is complete, remove the paper from the chromatography chamber and sketch the results in your lab notebook. Lay the paper flat to dry.

? 14. How many different types of pigments are present in spinach leaves? Provide evidence to support your claim.

? 15. The absorbance of green light is low for a sample extracted from green leaves. However, the absorbance is not zero. Which pigments absorb green light in the spinach leaves?

16. Use a PASCO Wireless Spectrometer and corresponding software to observe the full absorbance spectrum for the pigment extract:

    a. Connect the spectrometer to the PASCO spectrometer application using the Bluetooth® or USB connection. Conduct the light calibration using the cuvette blank—a glass cuvette that contains ethanol.

       NOTE: Do not use the plastic cuvettes with organic solvents.

    b. Use the same diluted extract as before (from the cuvette three-quarters full with ethanol plus 10–15 drops of pigment extract) and transfer the solution to a clean spectrometer cuvette. Draw a sketch or print a record of the spectrometer data.

Part 2 — Measuring Photosynthetic Activity with DPIP

NOTE: For this part of the investigation, use the spinach chloroplasts your teacher prepared by blending spinach with an ice-cold sucrose solution. Keep the sample on ice for the DPIP procedure.

17. DPIP (2, 6-dichlorophenolindophenol) is a blue-colored compound. When reduced, it turns colorless.

    DPIP (blue) + $2H^+$ + $2e^-$ → Reduced DPIP (colorless)

    In the following procedure, DPIP acts as an electron acceptor for excited electrons.

    a. Given what you know about the light-dependent reactions of photosynthesis, describe how electrons in chloroplasts become *excited*.

    b. In this investigation, excited electrons will be transferred to DPIP. In a typical photosynthesis reaction, what happens to these excited electrons?

18. Connect the colorimeter to your data collection system so you can measure the change in DPIP color under different conditions.

19. Label the caps of three colorimeter cuvettes "1," "2," and "3." Use two clean pipets to add phosphate buffer and distilled water to the cuvettes in the volumes specified in Table 1.

Table 1: Set up for the DPIP photosynthesis experiment

Cuvette Contents	Cuvette 1 (blank)	Cuvette 2 (placed in light)	Cuvette 3 (placed in the dark)
Phosphate buffer	1 mL	1 mL	1 mL
Distilled water	4 mL	3 mL	3 mL
Chloroplast suspension	3 drops	3 drops	3 drops
DPIP	none	1 mL	1 mL

20. Cuvette 1 is a blank, a cuvette that contains the same contents as the other cuvettes but lacks the colored DPIP. Add 3 drops of chloroplast suspension to Cuvette 1 and invert it a few times to mix the contents. Place the cuvette into the colorimeter and calibrate the sensor.

22. Add 3 drops of chloroplast suspension to Cuvette 2 and 3 drops to Cuvette 3.

23. Use a clean pipet to add DPIP to Cuvette 2 and invert the cuvette to mix the contents. Place Cuvette 2 into the colorimeter. Record the initial transmittance or absorbance for the suspension. Repeat the process for Cuvette 3.

    NOTE: Whether you record transmittance or absorbance, and for one color or more, are decisions left up to your group. Create a data table to organize the results of this part of the investigation.

24. Place Cuvette 2 near a bright light source. Wrap Cuvette 3 completely in foil and place it next to Cuvette 2.

25. After 5 minutes, 10 minutes, and 15 minutes, record the transmittance or absorbance of light for Cuvettes 2 and 3. Be sure to invert the cuvettes to mix the contents well before recording measurements. Also, unwrap Cuvette 3 each time, make the measurement, and then re-wrap it.

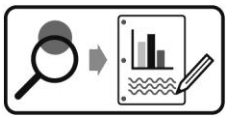
    NOTE: It is a good science practice to place the blank in the colorimeter each time to ensure the transmittance of the blank continues to read 100% (or the absorbance reads 0.00).

26. Create an appropriately labeled graph to illustrate the results of the DPIP investigation.

27. Explain any difference in results for Cuvettes 2 and 3.

Design and Conduct an Experiment

Use one of the tools or techniques from the Initial Investigation to explore a question of your own related to plant leaves and pigments, or to photosynthesis.



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

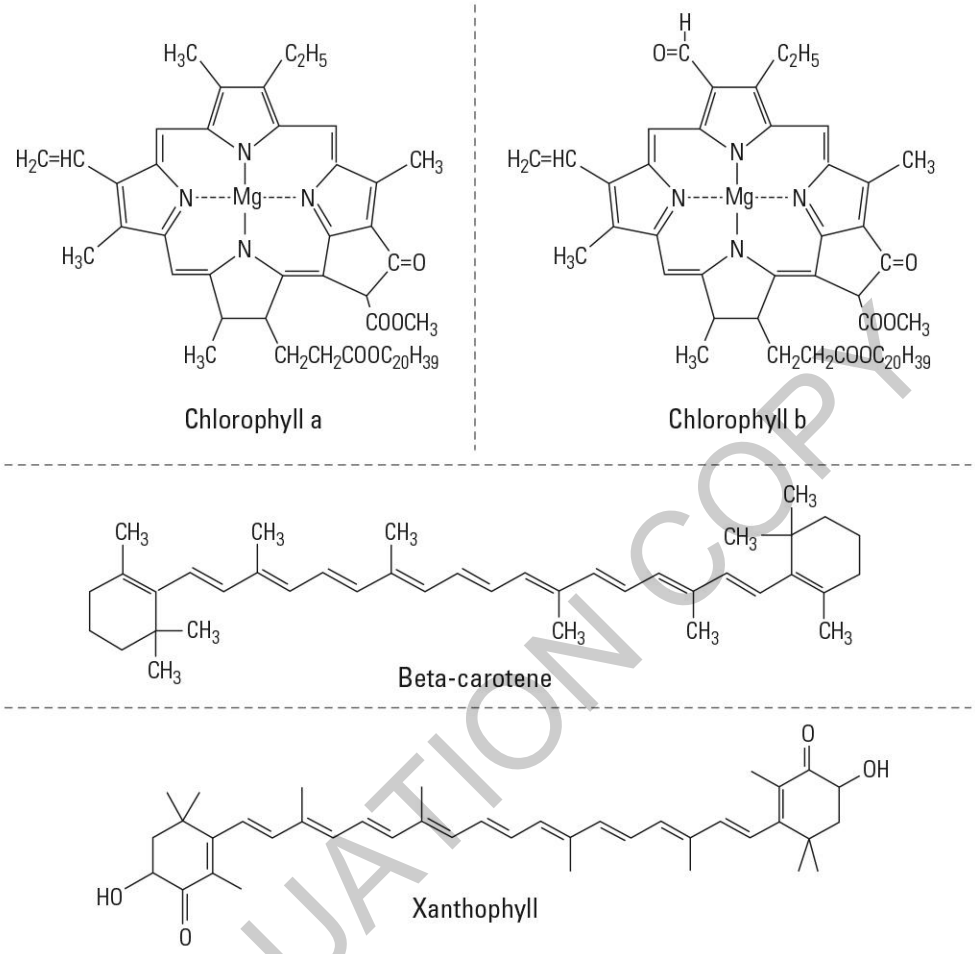
   b. Based on the evidence you collected, explain why the results occurred.

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

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

Synthesis Questions

1. Refer to the diagrams below illustrating the structures of four common plant pigments.

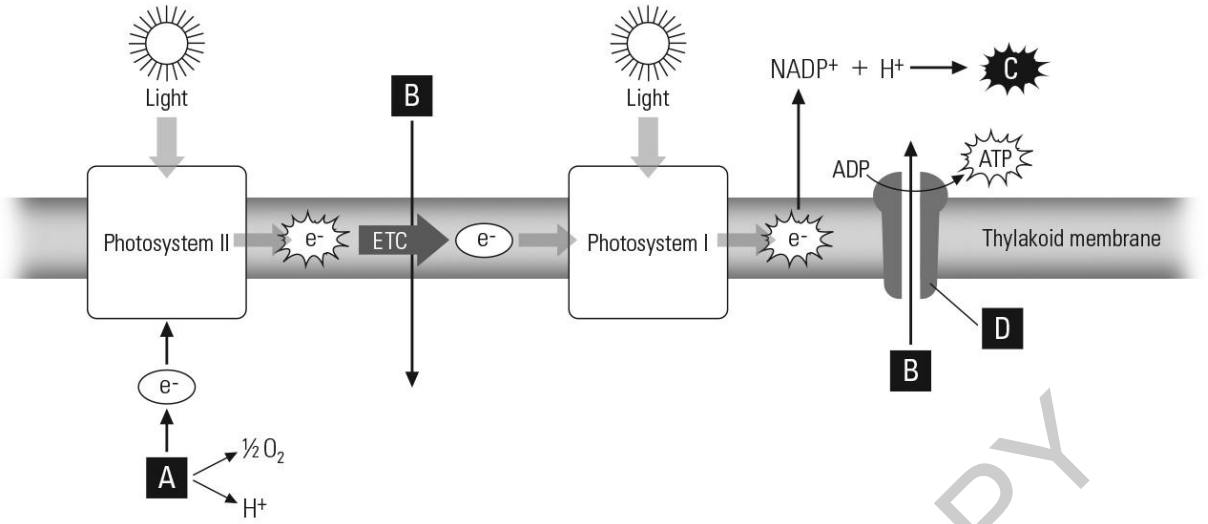


a. Which pigment is more polar, chlorophyll *a* or chlorophyll *b*? Justify your answer by describing aspects of the pigment structure that relate to its polarity.

b. Which pigment is least polar, beta carotene or xanthophyll? Explain your reasoning for your choice.

c. How do the structures of these pigments relate to the process of paper chromatography?

2. The diagram below shows events of the light reactions of photosynthesis.

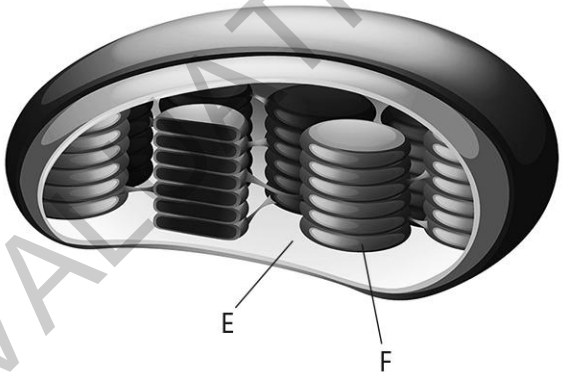


a. Identify the items labeled A, B, C, and D.

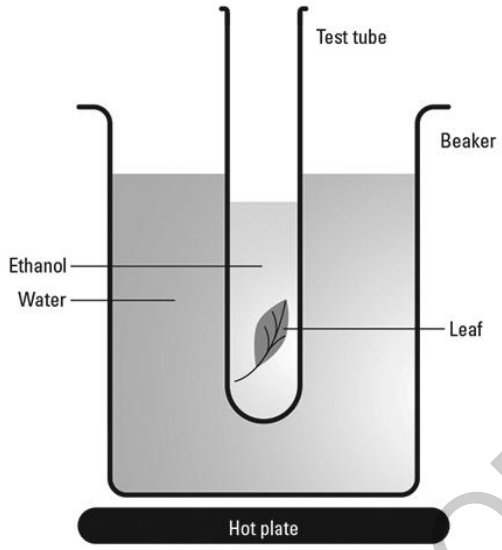
b. Of what importance is B to the function of D?

c. Of what importance are C and D to the reactions of the Calvin cycle—the series of reactions that follow the light reactions?

d. Identify labels E and F on the diagram of a chloroplast. Describe the processes that occur in each area.



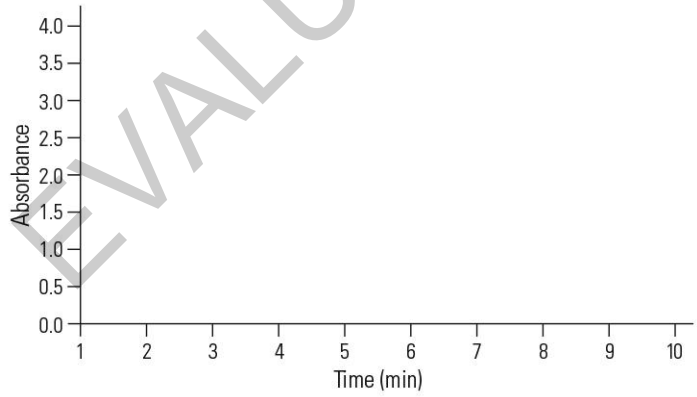
3. A student researches various methods of extracting chlorophyll from leaves. One method describes boiling leaves in ethanol, as illustrated:



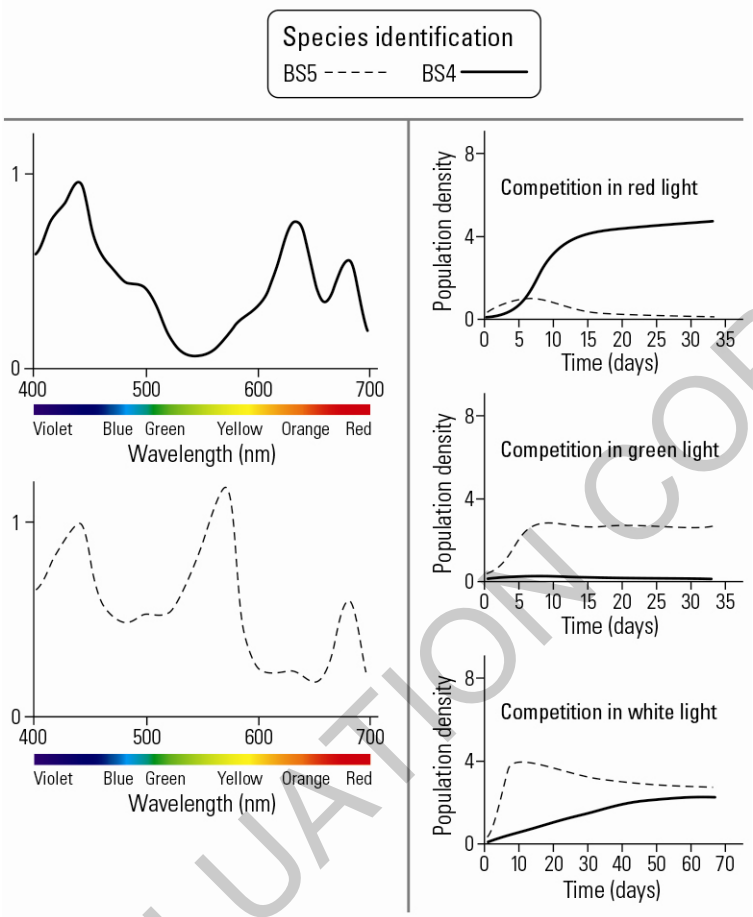
The boiling process causes the ethanol to turn green and "bleaches" the leaf.

The student reads about this method in an investigation that tests leaves for the presence of starch and wonders whether this method might be used for a DPIP investigation similar to the Initial Investigation. He sets up a cuvette with the following contents: phosphate buffer, distilled water, DPIP, and 3 drops of the green-colored ethanol obtained after boiling the leaf in ethanol in a test tube, as described in the investigation. The cuvette is placed under a bright light source and the student measures light absorbance in the solution at 1 minute intervals for 10 minutes.

Copy the graph below and sketch a line on the graph to illustrate what you predict will happen to the absorbance of the solution in the cuvette the student set up. Explain the reasoning for your prediction.



4. In the Baltic Sea, two similar species of photosynthetic picocyanobacteria were found to occupy water at a similar depth. One species, BS4, is blue-green in color and the other species, BS5, is red. Both species contain chlorophyll *a* but differ in the presence of certain accessory pigments: phycocyanin is found only in BS4 and phycoerythrin is found only in BS5. The absorbance spectra for the pigments in these species are shown below. Also shown are the results of experiments in which the species were grown together under different light conditions.[5]



a. Explain why the picocyanobacterium BS5 is red in color.

b. Describe evidence from the absorbance spectra that both of the species contain chlorophyll *a*.

c. Describe the results of growing BS4 and BS5 together in green light and provide a biological explanation for the results.

d. Describe the results of growing BS4 and BS5 together in white light and provide a biological explanation for the results.

e. If paper chromatography was performed using pigments extracted from BS5, what color or colors would you expect to see on the paper after chromatography is complete? Explain the reasoning for your choice(s).

[5] Stomp, M. et al. Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature* (Impact Factor: 38.6). 12/2004; 432(7013):104–7. DOI: 10.1038/nature03044

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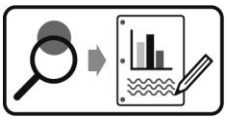
Design and Conduct an Experiment Worksheet

Use one of the tools or techniques from the Initial Investigation to explore a question of your own related to plant leaves and pigments, or to photosynthesis.



Develop and conduct your experiment using the following guide.

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

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

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

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

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

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

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

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

_____

_____

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

_____

_____

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

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

11. TRANSPIRATION

Background

Transpiration is the evaporative loss of water from the leaves of plants through tiny pores called *stomata*. Carbon dioxide and oxygen gas are also exchanged through these pores. Cells present in plant leaves use carbon dioxide and water to carry out the reactions of photosynthesis.

Water enters a plant at its roots and transpiration draws water up the xylem to the site of photosynthesis. The movement of water from a plant's roots to its leaves is driven by differences in water potential. Solute concentration and physical pressure are factors that contribute to water potential, which is different in the tissues in a plant, in the soil, and in the air that surrounds a plant. Water always moves from regions of high water potential to regions of lower water potential. Air surrounding a plant's leaves has the lowest water potential, given that the amount of water vapor in air is typically low. As water evaporates from the leaves, negative pressure is created within the xylem tube causing water to be pulled up from the roots.

A number of environmental conditions can affect water potential, and therefore transpiration. Plants monitor environmental conditions to balance their need for carbon dioxide and their need to limit the loss of water through evaporation. Changes in environmental conditions can upset this balance and plants have evolved a number of adaptations to defend against water loss while still acquiring the needed reactants for photosynthesis.

Driving Question

Does the relative humidity of the environment affect the rate of transpiration in a plant?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- Low pressure sensor (barometer)
- Weather sensor
- Sensor extension cables (2)
- Quick-release connector
- Clear plastic tubing, 40–50 cm, with a one-hole rubber stopper on one end
- Large tub or bucket (for water)
- Paraffin film or petroleum jelly *(if available)*
- Plant sample containing numerous leaves
- Base and support rod
- 3-finger clamps (2)
- Test tube clamp
- Clear plastic bag, 1 gallon
- Spray bottle with water
- Electronic balance, centigram
- Small syringe, 60-mL or larger, without needle
- Pipet
- Metric ruler
- Large scissors or small pruning shears

Safety

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

- Wear safety goggles at all times.
- Handle sharp objects carefully.
- Avoid contact with eyes or skin when handling plant materials. Wash hands thoroughly after touching plants.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles.

2. Use the two 3-finger clamps to attach the barometer (low-pressure sensor) and weather sensor to the base and rod stand. Connect the sensors to your data collection system using extension cables.

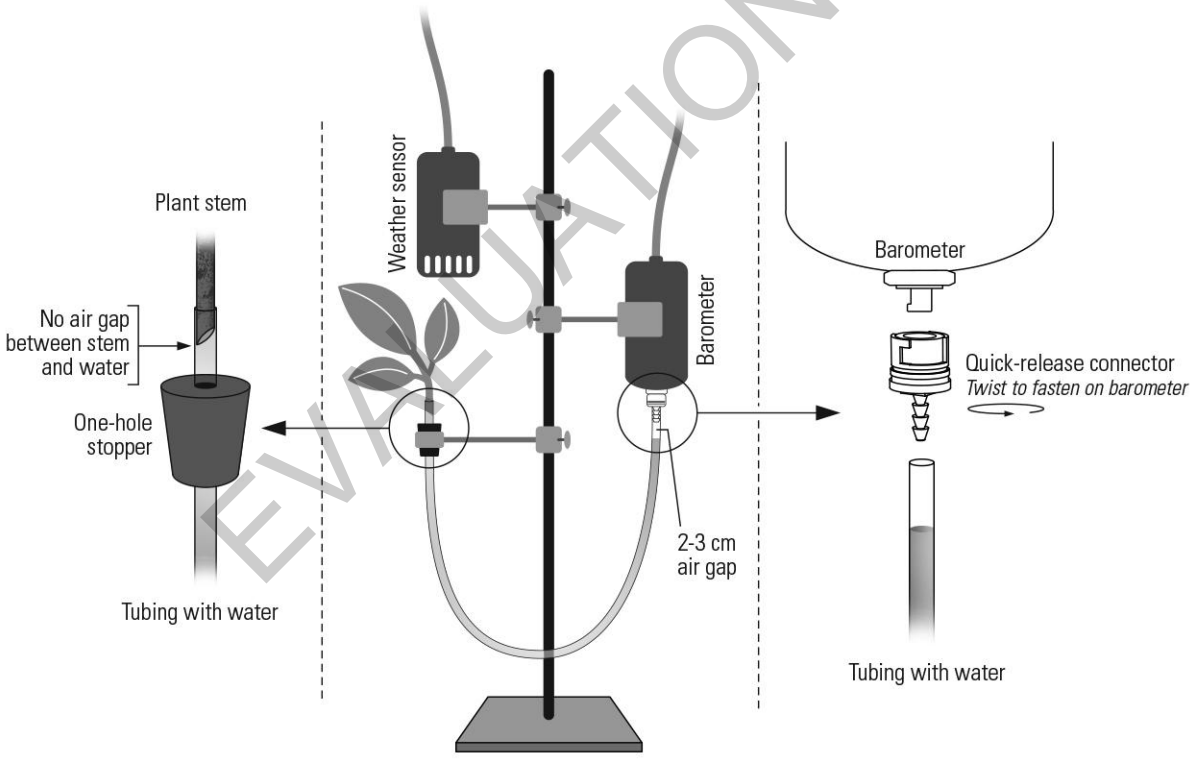
3. Create a graph display of Barometric pressure versus Time. If possible, set the sampling rate to one sample every 30 seconds. If your data collection system allows for an automatic stop condition, set the stop time for 10 minutes.

   NOTE: The units for barometric pressure default to inHg (inch of mercury) on the data collection system. These units can be changed within the system to the SI unit kPa (kilopascals).

4. Create one or more displays for Relative humidity (%) and Temperature (°C); these measurements are detected by the weather sensor and provide information about the microclimate surrounding the plant sample in the investigation.

SET UP THE POTOMETER

The diagram below illustrates the *potometer*, the apparatus that will detect transpiration in the plant sample.



5. To set up the potometer, perform the following steps using a large bucket or tub of water.

   a. Check that one end of the plastic tubing extends 3–5 cm past the stopper. Submerge the entire length of tubing in the tub of water.

      NOTE: It is important to keep the plastic tubing submerged in a tub or sink while preparing the plant and tubing of the potometer.

b. Fill the syringe with water and attach it to one end of the tubing. Push the plunger on the syringe to fill the tubing with water. Watch the open end of the tubing—the tubing is filled with water when air bubbles no longer exit the tubing. Keep the tubing submerged in the water.

c. Holding the stem of the plant sample under water, use sharp scissors to cut the plant stem at a 45° angle. Immediately insert the cut end of the stem into the short section of the tubing that extends past the rubber stopper. The stem should fit tightly in the opening of the tubing.

NOTE: If paraffin film or petroleum jelly is available, it can be used to help obtain an airtight seal. Use petroleum jelly carefully; it is difficult to remove from the tubing. Neither will create an airtight seal if the plant stem does not fit tightly in the tubing.

6. To prevent water from spilling out of the tubing, hold the tubing in a "U" shape and remove the tubing and plant sample from the water. Place the stopper in the test tube clamp on the base and support rod and tighten the clamp to secure the stopper. Be sure the plant remains upright.

7. Create a 2–3 cm air gap at the other end of the tubing—the end that will connect to the barometer. Either flick the tubing or use a pipet to remove a small amount of water, creating the air gap.

8. Insert the quick-release connector into the tubing far enough so it does not easily come out. Attach the connector to the barometer sensor and twist it to complete the connection between the sensor and the tubing.

   Check that your setup looks like the one illustrated on the previous page.

   - There must be an unbroken water column that extends from the water in the tubing to the water in the xylem of the plant sample in order for transpiration to occur. Any air bubbles in the tubing or at the surface of the cut stem will affect the data. Additionally, an air gap is important to have near the barometer sensor to be able to detect the small changes in pressure that result from the movement of water from the tubing into the plant xylem and through its leaves.

COLLECT DATA: NORMAL CONDITIONS

9. Leave the potometer undisturbed for at least one minute before beginning data collection. Collect data for 10 minutes. Answer the following questions while you wait.

? 10. If transpiration occurs in the plant sample, what will happen to the water in the tubing? What will happen to the pressure in the tubing? Explain your answer.

? 11. What will happen if the seal between the plant stem and tubing is not air tight?

? 12. Consider the microscopic structure of a leaf.

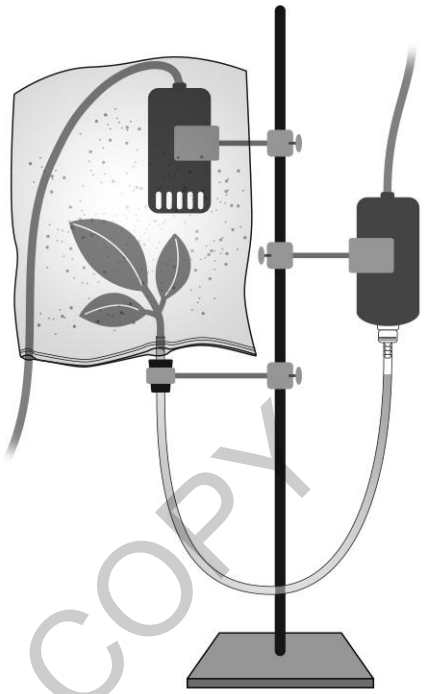
   a. Through which structures does water evaporate from a plant? Be as specific as you can in your answer.

   b. Do you expect that these structures are the same regarding their location and density in the leaves of different plant species or do they vary? Explain your reasoning.

13. After data collection stops, keep the plant sample, sensors, and other potometer components in place. Reset the pressure in the tubing by carefully twisting the quick-release connector to remove it from the sensor. Then reconnect it to the sensor so the tubing and sensor are once again connected.

COLLECT DATA: ALTERED CONDITION

14. Cover the plant sample and the weather sensor with a large, clear plastic bag. Mist the inside of the bag with water from a spray bottle and seal the plastic bag. Leave the system undisturbed for one minute and then begin data collection. Answer the following questions while the system records data for 10 minutes.



15. What environmental condition(s) that can be measured by the weather sensor do you expect to change in the plant's microclimate as a result of the plant being covered with the misted bag?

16. The amount of water vapor in the air affects the water potential of the air. The rate of transpiration is directly related to the water potential difference between the leaves and the atmosphere.

    a. Based on your knowledge of water potential, how should the water potential of the air in the "normal condition" test compare to the water potential of the air surrounding the plant covered with the bag?

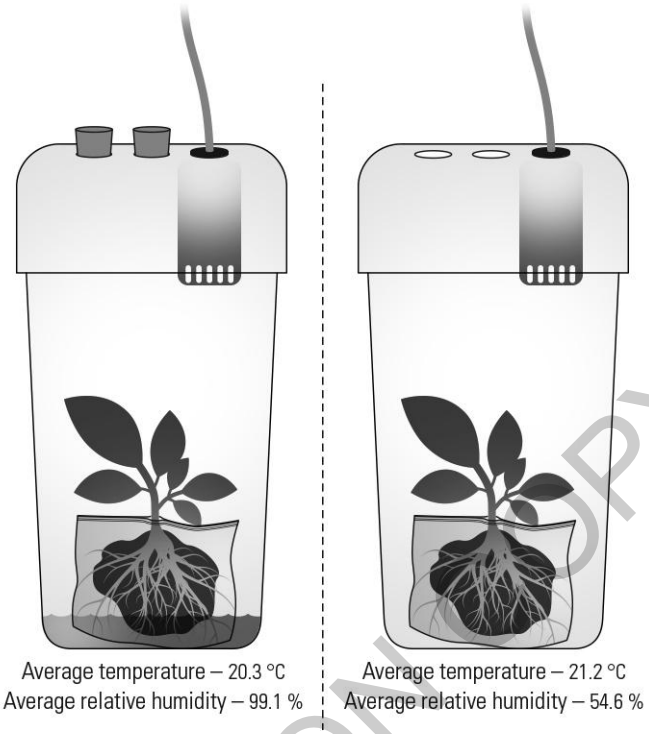
    b. How does water potential relate to transpiration? Predict whether or not you expect the rate of transpiration to be the same in both conditions? Explain your prediction.

17. End data collection after 10 minutes. Print or sketch a graph of Barometric pressure versus Time to preserve a record of your data. Clearly label each run of data. Also, for each run, record the minimum, maximum, and average relative humidity (%) and temperature during each 10 minute period.

18. Create a data table to summarize the results for the two conditions and compare the rates of transpiration using the change in pressure/minute.

19. Was your prediction correct? Explain any differences between the control data (normal conditions) and the data collected for the covered, misted plant (altered condition). Use data from both sensors to support your explanation.

20. Refer to the table below, which shows data for a whole-plant transpiration experiment. The diagram illustrates the setup for the experiment.



A weather sensor monitored the microclimate in each chamber. One chamber contained only a plant. The plant's root ball was sealed in plastic so that evaporation could only occur through its leaves. The other chamber was set up in the same way—using a plant of the same size and species as the first chamber, but it also contained a small amount of water in the bottom of the chamber and the holes of the chamber were sealed. The mass of each plant was measured on the day of set up and again 72 hours later. External factors, such as the amount of daylight the plants received, were kept constant by placing the chambers side-by-side in the same location for the 72 hours.

Table 1: Whole-plant—Comparing the rate of transpiration under normal and humid conditions

Environment	Change in Mass (g)	Change in Mass (%)	Total Leaf Surface Area (cm^2)
Chamber 1	–0.69	–0.7	183
Chamber 2	–22.42	–21	175

a. Why does the mass of the plants decrease during the 72-hour time period?

b. Does the data from this whole-plant method support or contradict the results of the potometer investigation? Explain your answer.

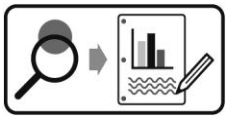
21. To investigate additional factors that may affect transpiration, either the potometer method or whole-plant method can be used.

a. Identify one or more advantages to the potometer method of measuring transpiration. What might be some possible sources of error with this method?

b. What are some advantages to the whole-plant method for measuring transpiration? Are there possible sources of error with this method?

Design and Conduct an Experiment

Humidity is only one of a number of environmental factors that can affect transpiration in plants. Transpiration rates also depend on the structural and physiological adaptations plants have to help them survive in the myriad of habitats on earth. Consider an aspect of transpiration or plant anatomy that you can explore further through an independent investigation.



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

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

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

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

Synthesis Questions

1. Explain how prolonged drought contributes to a decrease in productivity of crop plants.

2. Why is transpiration needed, even though the plant loses water in the process?

3. Name some of the structural or physiological adaptations that have evolved in plants that help limit water loss from their leaves. Explain how each adaptation is beneficial to the plant.

4. Stomatal densities are shown in the table below for three plant species. The initial stomatal density was determined when plants were exposed to normal carbon dioxide (CO_2) levels. Plants were then exposed to CO_2 enriched environments and the final stomatal density corresponds to this enriched environment. The study included observations from 100 different plant species of various types (trees, shrubs, and herbs) and found the same trend occurred in 74% of plants observed.[6]

   Table 2: Effect of CO_2 level on stomatal density

Species	Stomatal Density, Normal CO_2 Level (stomata/mm^2)	Stomatal Density, Enriched CO_2 Level (stomata/mm^2)
Anthyllis vulneraria	154	137
Cynodon dactylon	321	249
Hypoestes sanguinolenta	110	61

   a. What effect did higher carbon dioxide levels have on stomatal density?

[6] Woodward, F. I. and Kelly, C. K. (1995), The influence of CO_2 concentration on stomatal density. *New Phytologist*, 131: 311–327. doi: 10.1111/j.1469-8137.1995. tb03067.x

b. Propose an explanation for the change in stomatal density in response to higher carbon dioxide levels.

5. The table below summarizes data from a study of various species of *Eucalyptus* trees. During 24-hour time periods, a portable photosynthesis system was used to measure net carbon dioxide exchange for a sample of leaves from three or four trees of each species. Photosynthetic rate and stomatal conductance were calculated from data collected with the portable system. Stomatal conductance relates to the proportion of open stomata; the greater the number of open stomata, the greater the stomatal conductance.[7]

Table 3: Effect of time of day on photosynthetic rate and stomatal conductance in *Eucalyptus* trees

Species	Time of Day and Photosynthetic Rate (μmol CO_2/m^2)/s)		Stomatal Conductance (mol CO_2/m^2)/s)
E. argophloia	06:00	–1.5	0.02
	09:00	1	0.04
	11:00	7.5	0.10
	13:00	7	0.08
	16:00	4	0.02
	19:00	2	0.01
E. dunnii	06:00	–1	0.01
	09:00	3	0.03
	11:00	9.5	0.12
	13:00	5	0.05
	16:00	3	0.02
	19:00	1	0.01
E. sideroxylon	06:00	–4	0.02
	09:00	2.5	0.03
	11:00	13	0.28
	13:00	12.5	0.28
	16:00	8	0.11
	19:00	4.5	0.02

a. Describe the trend in stomata opening and closing over the course of 24 hours. Describe the environmental factors that are likely to be the cause of this trend.

b. Create an appropriately labeled scatter plot graph of Photosynthetic rate versus Stomatal conductance. Use a different symbol for each species.

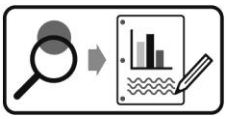
c. Describe the relationship between photosynthetic rate and the proportion of open stomata in leaves.

[7] Lewis, J.; Phillips, N.; Logan, B.; Hricko, C.; Tissue, D. Leaf photosynthesis, respiration and stomatal conductance in six *Eucalyptus* species native to mesic and xeric environments growing in a common garden. *Tree Physiol* (2011) 31 (9): 997-1006. doi: 10.1093/treephys/tpr 087

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

Humidity is only one of a number of environmental factors that can affect transpiration in plants. Transpiration rates also depend on the structural and physiological adaptations plants have to help them survive in the myriad of habitats on earth. Consider an aspect of transpiration or plant anatomy that you can explore further through an independent investigation.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of plants and transpiration, what environmental factors (abiotic or biotic) could affect this process?

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

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

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

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

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

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

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

_____

_____

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

_____

_____

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

_____

_____

_____

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

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

12. MITOSIS

Background

Eukaryotes carry out mitosis to produce identical cells. In most circumstances the new cells are needed for repair or growth of tissue. Mitosis is a complicated process involving many significant cellular changes, such as the disappearance of the nuclear membrane to allow chromosome movement and separation. The phases of mitosis are carefully regulated to ensure that heritable information is transmitted correctly to new cells, and to limit cell division to times when it is needed. Unregulated cell division can lead to cancer.

Onion roots are commonly used as a source of cells undergoing mitosis. As roots grow, mitosis occurs in the apical meristem to add cells to the tip of the root. Cells from the root tip can be stained and viewed under a microscope. The stain darkens condensed chromosomes, which helps to distinguish interphase cells from cells in mitosis. Many science research studies involve determination of the *mitotic index*, that is, determining the proportion of cells in interphase compared to mitosis. To understand normal growth and development, as well as cancer, biologists need to identify factors that affect mitosis and cell cycle regulation in organisms.

Driving Question

Under normal conditions, what proportion of cells in an onion root tip will be in mitosis?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Dissection scissors
- Forceps
- Razor blade or scalpel
- Glass test tube
- Glass microscope slides (3)
- Cover slips (2)
- Compound microscope with 400× magnification
- Disposable pipets (2), 1-mL
- Plastic cup, 16-oz
- Personal protective equipment: Disposable gloves and chemical apron
- Spot plate
- Carbol fuchsin solution, 1 mL
- 1 M Warm hydrochloric acid (HCl), 1 mL
- Onion bulb
- Paper towel
- Large toothpicks (4)
- Pencil with eraser
- Plastic wrap
- Disposable plastic gloves
- Permanent marker
- Distilled water

Safety

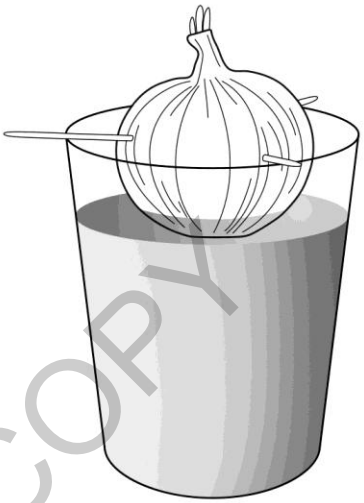
Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Wear disposable gloves and a chemical apron while performing the staining and microscope slide preparation steps.
- Use caution when cutting with the razor blade or scalpel. Cut away from the body and away from other students, and do not use excessive force when cutting.
- Wear disposable plastic gloves when handling treated onion bulbs.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

Part 1 – Growing root tips



1. Obtain a plastic cup and fill it two-thirds full with water. Label the cup with today's date and your initials.

2. Obtain a small onion. Remove any dry outer skin and any green leaves.

3. Use a razor blade to carefully cut off dry roots.

   NOTE: Make a shallow cut, such that the existing roots are cut back to the base of the bulb, but take care not to cut into the bottom of the bulb.

4. Stick long toothpicks into the sides of the onion to suspend it in the water of the cup. The area with the cut roots should be submerged in the water.

5. Loosely cover the cup with plastic wrap and place it in a dark location for 48 hours.

6. Following the growth period, you will squash and stain some of the root tips and observe the cells under the microscope. Answer the following questions in your lab notebook.

   a. Considering the purpose of mitosis, why are root tips a good source of tissue for observing cells undergoing this type of cell division?

   b. It will be critical that you can accurately determine if cells are in interphase or in mitosis. What cell features will you use to determine that a cell is in interphase?

   c. What cell features will you use to make a determination that a cell is in mitosis?

7. After 48 hours, observe the onion roots and record your observations. If the roots are at least 2 cm in length, continue to Part 2. Otherwise, place the onions back into the dark location for another 24 hours.

Part 2 – Staining Root Tips for Observation

NOTE: Wear safety goggles, disposable gloves, and chemical aprons during this activity. Work in a well-ventilated area—ideally a chemical hood.

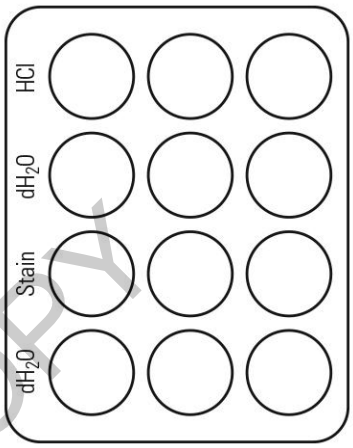
8. After the onion has been 2–3 days in the dark, remove it from the water and harvest the root tips for microscope analysis:

   a. Obtain a plastic spot plate.

   b. Use scissors to remove at least 4 or 5 roots from the onion. Trim the roots to approximately 1 cm in length. Discard the remainder of the root, being sure to keep the root tip.

   c. Use forceps to place the root tips into the first well of the spot plate.

   d. Use a disposable pipet to fill the spot containing root tips with 1 mL of warm (50–60 °C) 1 M hydrochloric acid (HCl). Soak the tips for 5 minutes.



9. While you wait, fill the remaining wells of the spot plate with the following solutions in the order shown in the diagram. Use a separate pipet for each solution.

10. After soaking the root tips for 5 minutes in the HCl, transfer them to the next well, containing distilled water (dH_2O). Rinse the tips in water for 1 minute.

11. Transfer the root tips to the stain (carbol fuchsin solution). Stain the tips for 2–3 minutes.

12. Transfer the root tips to distilled water and rinse them for 1 minute. Change the water during the rinse process until the rinse water is light pink.

    - Change the water by pipetting the colored water into a waste container and adding new water (with the pipet used for water).

    - Alternatively, if you have extra wells in the spot plate, you can fill them with water and transfer the roots from one spot to another.

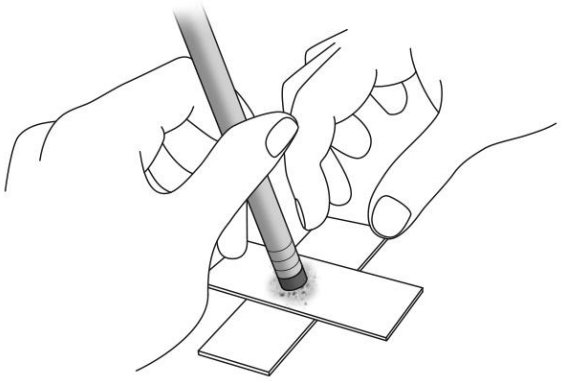
13. Place the stained roots on a paper towel. Use a scalpel or razor blade to trim the roots so that you keep only 3–5 mm of the root tip (the tapered end).

    NOTE: You can hold the root with forceps and cut across it with a razor blade. Be sure to discard the root cut away from the tip and keep only the tapered end.

14. Using a permanent marker, label one of the glass microscope slides "A" and the other "B".

15. Place at least three stained root tips on the slide, close together but not overlapping. Place a cover slip over the tips.

16. Place the second glass slide over the coverslip, perpendicular to the slide with the roots. Using the eraser of a pencil, press down firmly on the top slide to squash the root tips. Move the eraser and push down on each root tip. Do not twist the eraser. Remove the top slide.



17. Repeat the process to prepare the second slide of squashed root tips.

18. Observe Slide A on high magnification (400×).

    NOTE: Observe areas where cells can be seen clearly, that is, areas where there is a single layer of cells. Do not focus on areas where there is more than one cell layer.

19. Some cells will appear elongated and rectangular, other cells will appear small and square in shape. In regions with small, square cells, look for cells in metaphase or anaphase. These phases will be the easiest to recognize and can help you locate a good field of view (FOV) to count.

20. When you find a FOV that has at least two cells showing evidence of mitosis, determine a systematic way of viewing and counting all of the cells in that FOV. Every well-stained, distinct cell in the field of view should be counted.

21. For each cell in the FOV, determine if the cell is in interphase or mitosis. Put a tally mark in the appropriate column of Table 1 (copied to your notebook). Continue to tally the cells until you have counted all cells in the FOV. Repeat this procedure for two additional fields of view.

    NOTE: You do not need to track the number of cells in specific phases, just note whether a cell is in interphase or in mitosis.

Table 1: Comparing the number of cells in interphase and in mitosis in root tissue

Slide	Cells in Interphase				Cells in Mitosis				Total Cells Counted
	FOV 1	FOV 2	FOV 3	Total	FOV 1	FOV 2	FOV 3	Total	
A									
B									

22. Observe and tally the number of interphase and mitotic cells in three fields of view for the second root tip slide (Slide B).

23. For each field of view observed for Slide A, determine the percentage of cells in interphase. Then determine the mean and standard deviation for the 3 FOVs. Do the same for Slide B.

$$\text{Mean: } \bar{x} = \frac{1}{N}\sum_{i=1}^{N} x_i \qquad \text{Standard deviation: } s = \sqrt{\frac{\sum(x - \bar{x})}{n - 1}}$$

24. Consider all six fields of view as a single sample and report the mean percentage of cells in interphase to the class.

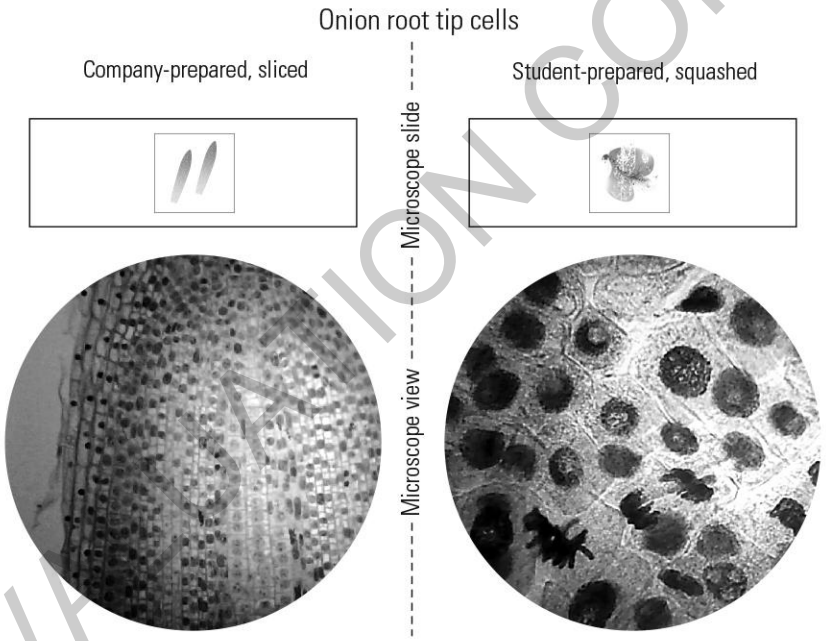
Data Analysis

1. Find the mean and standard deviation for the data provided by all groups.

2. Given the standard deviation for your group data and class data, what can you conclude regarding the amount of variation in the samples?

Chi-square analysis

Science education supply companies offer prepared slides of onion root tips for purchase. You have probably viewed such slides before. Preparation of these slides differs from the technique you used in the Initial Investigation. Using special equipment, supply companies are able to create very thin slices (cross-sections) of the root rather than squashing the root tips. Their technique results in slides that make it easy to view the actively dividing region of the root tip and find cells in the stages of mitosis. Since the purpose of professionally prepared slides is to provide students with experience finding dividing cells, as opposed to cells in interphase, it is plausible that companies treat the roots with a compound that promotes mitosis—ensuring that a slide will have a large number of dividing cells for a student to observe.



A chi-square test can be used to determine if prepared cross-section slides are significantly different than squash slides (such as those in the Initial Investigation) in terms of the proportion of cells that are in interphase or mitosis. The table below provides data for such a comparison.

Table 2: Observed values *o*

Source	Number of Cells		
	Interphase	Mitosis	Total
Student-prepared root tip squash	310	32	342
Prepared slide from a company	315	35	350
Total	625	67	$N = 692$

The formula used to calculate a chi-square value is:

$$X^2 = \sum \frac{(o - e)^2}{e}$$

Typically, a chi-square test is a *goodness-of-fit* test. This test is applicable in situations where expected results can be calculated from theory, such as a predicted phenotypic ratio in the F_1 generation of fruit flies based on the principles of Mendelian inheritance. The null hypothesis for a goodness-of-fit test states there is no difference between the observed *o* and expected *e* (theoretical) values.

However, in treatment studies, a different type of chi-square test is applied. This *test of independence* operates under the null hypothesis that there is no association between two groups (or two variables)—the two groups are independent. **For the provided data, the null hypothesis is that the probability of a cell being in interphase or mitosis is independent of its source: a prepared slide from a company or a student-prepared squashed root tip.** The same chi-square formula is used for goodness-of-fit tests and tests of independence. However, in the test of independence expected frequencies are derived from observed frequencies, rather than from theory. The observations for the two groups for each category are recorded in a *contingency table*. Table 2 is an example of a contingency table (also known as a *2 × 2 contingency table).*

NOTE: The degrees of freedom *(*df*) for this type of chi-square test is one.*

In the chi-square test of independence, to calculate the expected values from observed values, apply the following Law of Probability:

If A and B are independent, then the probability *P* of A and B both occurring is:

$$P(\text{A and B}) = P(\text{A}) \times P(\text{B})$$

Considering the total number of cells observed, the probability of A and B both occurring in a sample size of *N* would be:

$$[\text{P(A)} \times \text{P(B)}]/\text{N}$$

3. Copy Table 3 into your lab notebook.

Table 3: Calculation of chi square

Source	Observed	Expected	$(o - e)$	$(o - e)^2$	$[(o - e)^2]/e$
Root tip squash Interphase	310				
Root tip squash Mitosis	32	RECORD ANSWERS & DATA IN YOUR NOTEBOOK.			
Prepared slide Interphase	315				
Prepared slide Mitosis	35				
				$X^2 = \Sigma[(o - e)^2]/e =$	

From the observed values *o* and the total number of cells counted *N*, calculate each of the following:

a. What is the probability that a cell will be in interphase and sourced from the student-prepared squash? Record the value in the "Expected" column of Table 3.

b. What is the probability that a cell will be in mitosis and sourced from the student-prepared squash? Record the value in the "Expected" column of Table 3.

4. Calculate and record the remaining expected values to complete the Expected column of Table 3.

5. Perform the calculations necessary to complete the remainder of Table 3. Find the sum of the values in the last column to determine the chi-square value.

6. With regard to the null hypothesis, what can you conclude from the calculated chi square?

Table 4: Chi-square distribution

Degrees of Freedom	Probability *p* Value					
	0.75	0.50	0.25	0.10	0.05	0.01
1	0.10	0.46	1.32	2.71	3.84	6.64
2	0.58	1.30	2.77	4.60	5.99	9.21
3	1.21	2.37	4.11	6.25	7.82	11.34
4	1.92	3.36	5.39	7.78	9.49	13.28

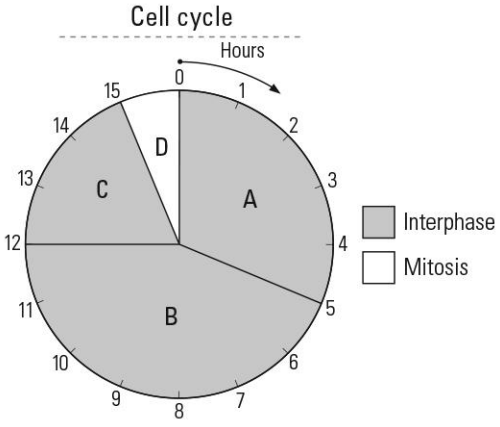
df = (number of groups – 1) × (number of categories – 1)

7. Do the chi-square results support the conjecture that supply companies treat roots with a compound to increase the rate of mitosis? Does the data provide conclusive evidence? Explain your answer.

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

Synthesis Questions

1. The diagram summarizes the cell cycle of an organism.

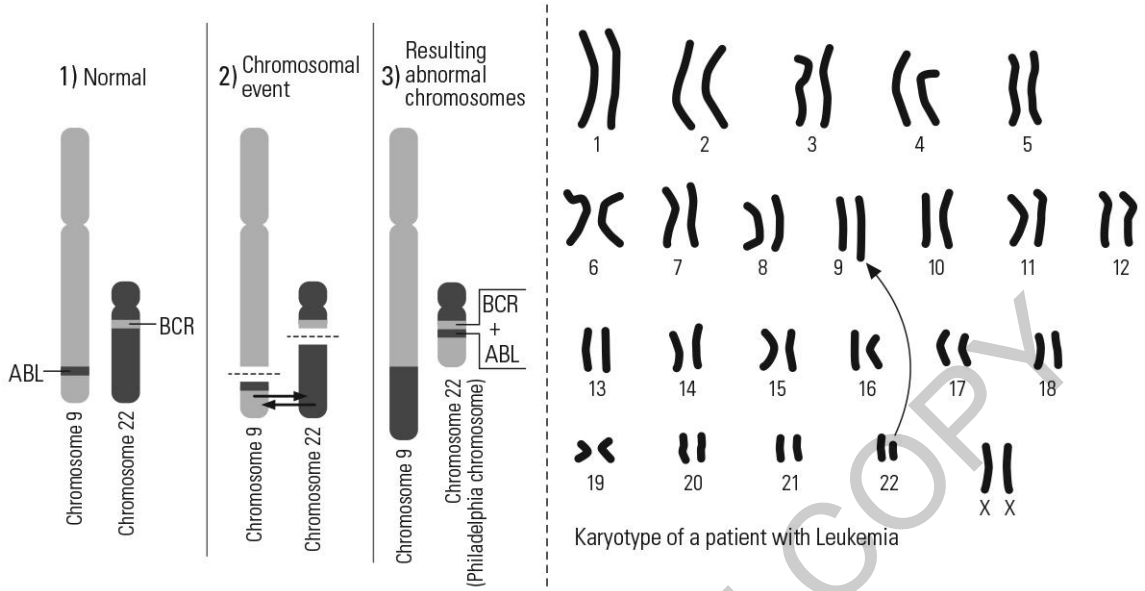


   a. Is the relative length of interphase and mitosis in the diagram consistent with the proportion of these stages observed in the root tips you harvested? Explain your answer.

   b. Compare and contrast the amount and organization of DNA in a cell at the following stages of interphase: A and C.

2. Mitosis, followed by cytokinesis, produces two genetically identical daughter cells. Draw a diagram that summarizes the transmittance of heritable information during the process of mitosis.

3. Cancer is characterized by a high mitotic rate that leads to formation of tumors. Research has shown that mutations or alterations to DNA can cause changes in the regulation of the cell cycle. An example of this is the *Philadelphia chromosome* associated with many forms of leukemia. The development of leukemia is related to a genetic change on chromosomes 9 and 22.



a. What is the name for the chromosomal event that occurs between chromosomes 9 and 22, creating the *Philadelphia chromosome*? Describe what happens during this event.

b. The cell cycle has a G_2 "checkpoint." The cell checks that DNA replication is complete and checks for DNA damage or mutations before moving into the mitotic phase. There are additional checkpoints in the cell cycle. Identify and describe one of these checkpoints and describe its importance.

4. A study was performed to determine the effects of 24-epibrassinolide (BL), a plant steroid hormone, on the mitotic index of onion root tips.[8] Investigators compared meristematic tissues of control onion bulbs to onion bulbs in experimental groups after 48 hours of root growth.

   The mitotic index is the percentage of cells in mitosis relative to the total number of cells examined. The mitotic index was calculated by counting 400 cells from five root tips obtained from each group (2000 cells total from each group). Data from this study is summarized in Table 4.

   Table 4: Effects of BL on mitosis in *Allium* roots

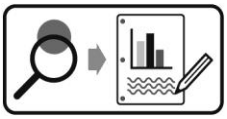
Concentration of BL	Number of Cells			Mitotic Index ± SD
	Interphase	Mitosis	Total	
Control (spring water)	1907	93	2000	4.65 ± 1.34
0.5 ppm BL	1918	82	2000	4.10 ± 1.34
0.05 ppm BL	1867	133	2000	6.65 ± 0.69

   a. What can be concluded from this study? Use chi-square analysis to provide evidence for your conclusions.

   b. Another study showed that hormones like BL induce transcription of a cyclin gene. Explain the role of cyclins in the cell cycle.

Design and Conduct an Experiment

If your teacher determines there is sufficient time and materials for you to carry out an experiment of your own design, explore factors that are likely to affect the rate of mitosis in organisms. For example, a number of studies involve testing compounds to see if they stimulate or inhibit mitosis.



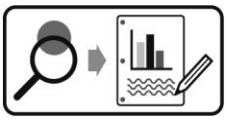
Design and carry out your experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

[8] Howell, W.M.; Keller III, G.E.; Kirkpatrick, J.D.; Jenkins, R.L.; Hunsinger, R.N.; McLaughlin, E.W. Effects of the plant steroidal hormone, 24-epibrassinolide, on the mitotic index and growth of onion (*Allium cepa*) root tips. *Genetics and Molecular Research*, Online Journal. Dept. of Biology, Samford University, Birmingham, AL. 2007 Retrieved April, 2014 from http://www.funpecrp.com.br/gmr/year2007/vol1-6/gmr0259_full_text.htm

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

A number of factors, internal and external, are likely to affect the rate of mitosis in organisms. Identify one of these factors and design an experiment to determine how that factor stimulates or inhibits mitosis.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of mitosis, what factors (abiotic or biotic) could affect this process?

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

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

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

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

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

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

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

_____

_____

9. It is beneficial to estimate at the beginning of the experiment the sample size needed to make statistically valid conclusions. The formula below can be used for this purpose. In the formula, n represents an adequate sample size, that is, the number of cells that need to be counted to compare the control and experimental groups. Solve for n in the equation. How many cells should you count in each of your groups?

   NOTE: The formula makes assumptions of other statistical values, such as margin of error and critical value.

$$0.03 = 2 \times \sqrt{\frac{0.9 \times 0.1}{n}}$$

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

_____

_____

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

_____

_____

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

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

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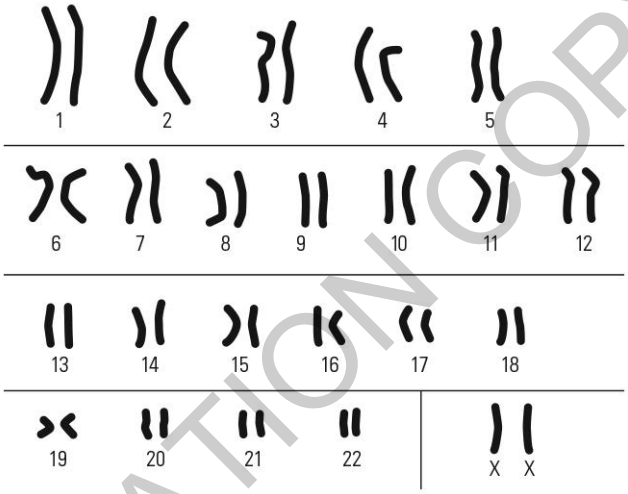
13. MEIOSIS

Background

Meiosis is a type of cell division that occurs in specialized germ cells. The events of meiosis produce genetically unique gametes—sperm and egg cells in animals. In sexually reproducing species, two gametes fuse during fertilization to produce an offspring organism. Each gamete contributes to the genetic composition and, therefore, the physical traits, of the offspring. The offspring inherits half of its chromosomes from the haploid gamete of one parent and the other half from the haploid gamete of the second parent. The cells of the offspring are diploid cells with homologous chromosomes.

The characterization of an organism's chromosomes, arranged in pairs, is called a *karyotype*.



A karyotype shows the diploid number of the species. In the illustration above, there are 46 chromosomes, so it is a human karyotype. The unique genome each offspring inherits results from the mix of chromosomes present in the two gametes that fused in fertilization to produce the offspring organism. Meiosis plays an important role in maintaining genetic diversity in a population.

Driving Questions

What is the role of meiosis in sexually reproducing species? How do the events of meiosis relate to its outcome?

How can crossover frequencies be used to determine the relative location of a gene on a chromosome?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- *Drosophila* Chromosome Sheet
- Karyotype of Offspring Fly Sheet
- Scissors
- Tape
- Pop bead chromosomes (4), 2 colors, 2 sizes
- String (2 pieces), approximately 1 m and 0.5 m
- Pop beads, 2 colors, enough to make sister chromatids
- Cards with images or photographs of *Sordaria* asci OR
- *Sordaria* crossing over kit

Drosophila Investigation

Record all observations, data, explanations, and answers in your lab notebook.

1. Obtain one copy each of the *Drosophila* Chromosome Sheet and Karyotype of Offspring Fly sheet.

2. In your lab notebook, answer the following questions before cutting and folding the chromosomes.

   NOTE: Refer to the Drosophila *Chromosome Sheet to help answer the questions. The letters on the chromosomes represent alleles, although, in the case of chromosome pair #1, the letter represents the sex chromosome: X or Y.*

   a. Each fly has four homologous pairs of chromosomes. What are homologous chromosomes?

   b. What is the diploid number for *Drosophila*? What is the haploid number?

   c. What cells in a fruit fly would be haploid? How are these cells produced?

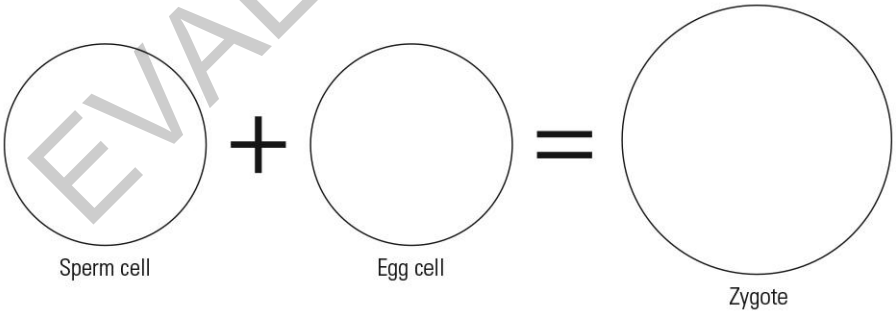
   d. With regard to sex, eyes, wings, and body color, describe the phenotypes of the two flies.

   e. If the male and female mate and produce offspring, can the offspring have normal wings? Can the offspring have vestigial wings? How do you know?

   f. To model the inheritance of chromosomes by an offspring organism, you will cut out each homologous pair from the female and male flies. The two chromosomes of a homologous pair will remain together when cut, but then will be folded in half. Why do you think the pair is being folded in half?

3. Follow the instructions on the *Drosophila* Chromosome Sheet to cut and fold the pairs of chromosomes. Keep the female fly's chromosomes separate from the male fly's chromosomes.

4. Simulate the segregation and independent assortment of chromosomes by tossing the female fly's chromosomes into the air. The chromosomes that land face up on the table represent those present in an egg cell in this female. In your notebook, copy the diagram below and draw the appropriate chromosomes in the "egg cell."



5. Toss the male fly's chromosomes into the air. Draw the appropriate chromosomes in the "sperm cell" on the diagram.

6. Draw the chromosomes that would be in the zygote, following fertilization.

7. Pair up the female and male flies' chromosomes on the Karyotype of Offspring Fly sheet. Put them onto the sheet with the side facing up the way the chromosome landed.

   a. Create a table in your notebook to organize the characteristics (genotype and phenotype) of five offspring.

   b. Record the genotype and phenotype in the table for the first offspring fly, based on the chromosomes placed on the Karyotype Sheet.

   c. Toss the male and female sets of chromosomes into the air again and pair them on the Karyotype page. Determine the genotype and phenotype of the second offspring fly. Repeat the procedures until five offspring are produced.

8. The offspring flies were all produced from the same two parents. Are the offspring identical? If so, explain why. If not, explain the variation.

9. Due to the *independent assortment* of chromosomes during meiosis, there are 2^n types of gametes that can be produced by an organism. That is, there are 2^n different assortments of chromosomes possible, where n is the haploid number of the organism. How many genetically unique egg cells can be produced by a female fruit fly? How does this compare to the number of different gametes a human female can produce?

10. The 2^n calculation does not take into account an event called *crossing over* that increases the genetic variation among gametes and therefore the variation in a population of a sexually-reproducing species. Describe how crossing over occurs during the formation of gametes and explain why it increases genetic variation.

Modeling Meiosis Investigation

Even a cell containing only 8 chromosomes, such as that of *Drosophila*, can be cumbersome to use for a model of meiosis. In this activity, you will use only four pop-bead chromosomes to simulate the events of meiosis.

1. Obtain four chromosomes. Each chromosome should be a single string of pop beads and there should be two homologous pairs. You should also have some loose pop beads for making additional chromosomes.

2. Before you begin the simulation, answer the following questions in your notebook.

   a. Why are two colors used for the pop-bead chromosomes to simulate meiosis?

   b. The chromosomes you are starting with are not "X-shaped," that is, there are no sister chromatids attached at a centromere. What happens to DNA to create X-shaped chromosomes? Summarize this event and identify when it occurs.

3. Build sister chromatids for each of the four chromosomes. Attach each sister chromatid to its replicate to create duplicated chromosomes.

4. Use string to make a large circle at your table to represent a cell. Use a smaller piece of string to make a nucleus. Place the chromosomes in the nucleus.

5. Work with your group to simulate and sketch the phases of meiosis I and meiosis II. Discuss the following questions with your group during the simulation activity. Answer the questions in your notebook and include sketches to help illustrate your explanations where appropriate.

   a. How can you show crossing over with the pop-bead chromosomes? Would the cross over frequency be the same for all genes? Why or why not?

   b. In this simulation of meiosis, a person is moving and arranging the chromosomes. What is the physical mechanism of chromosome movement and separation in an actual cell?

   c. At what point does the change from diploid to haploid occur? Why is it important that meiosis creates haploid cells?

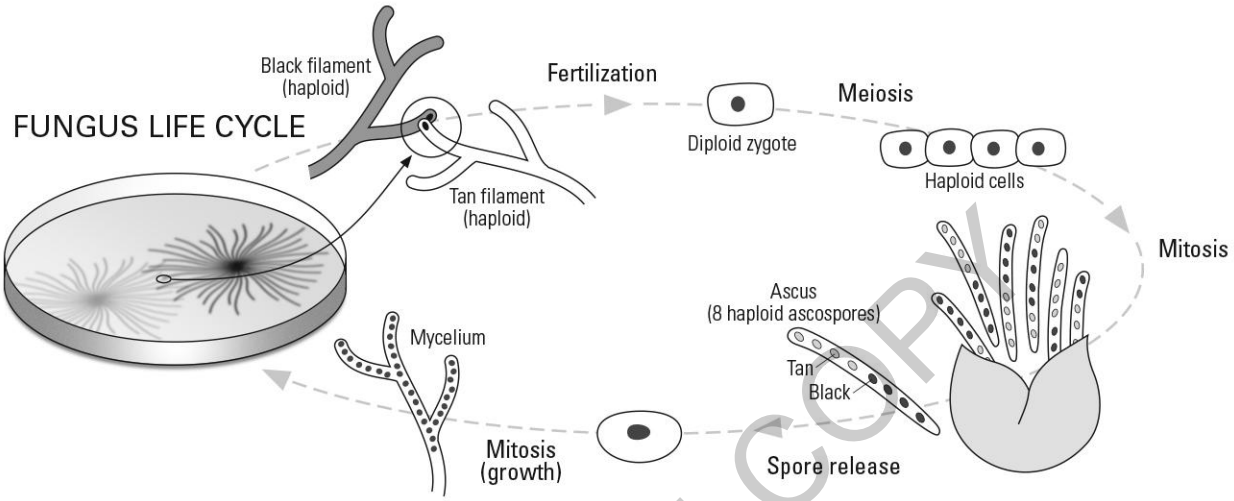
   d. What does *independent assortment* mean with regard to meiosis? How can you use the pop-bead chromosomes to explain this concept?

   e. What is the effect on gametes if homologous chromosomes fail to separate? How does nondisjunction affect the outcome of fertilization?

   f. How is the process and outcome of mitosis different from meiosis? What is the purpose of each type of cell division in the life cycle of animals?

Sordaria Investigation

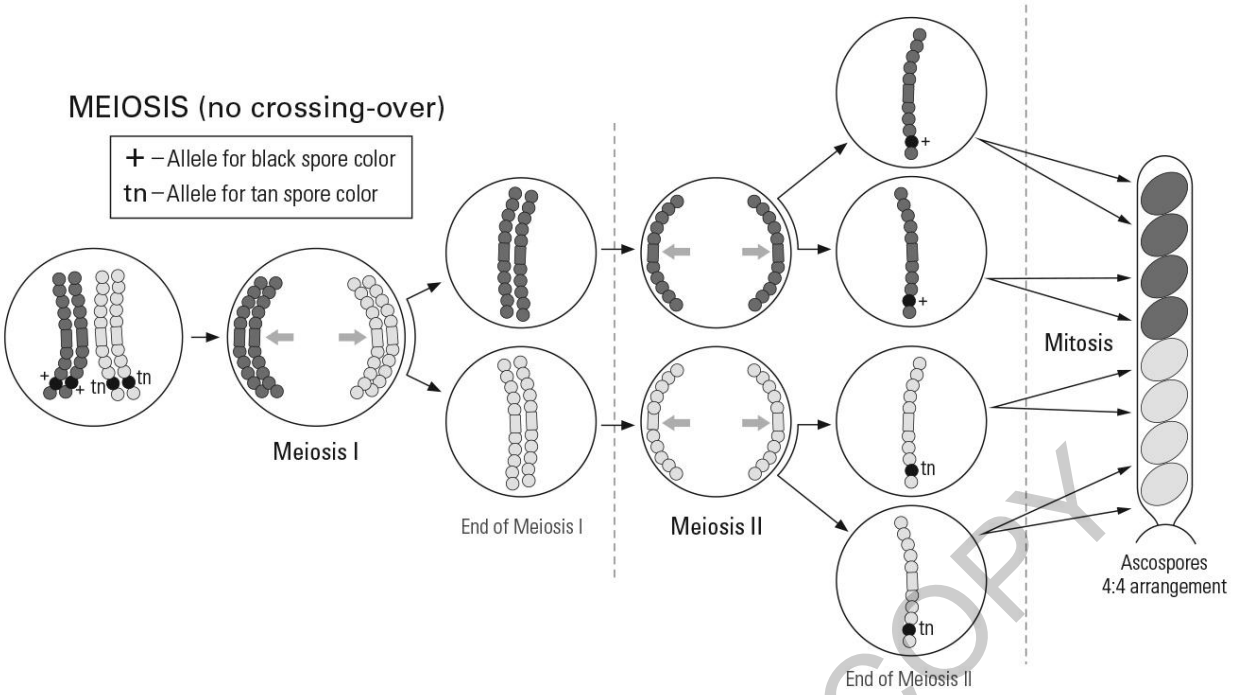
Mitosis does not always produce diploid cells from a diploid cell; sometimes haploid cells undergo mitosis to form more haploid cells. Such is the case in the life cycle of Sac Fungi (Ascomycota). The species investigated in this activity is the fungus *Sordaria fimicola*.



A mature fungus grows from an *ascospore*, a cell with a haploid nucleus. The cells of the fungus are all haploid, which is different from the cells of animals like fruit flies or humans, which are diploid. As the fungus grows, it produces many filaments (hyphae) which form the mycelium, the "fuzzy" structures you observe when you see fungus growing on bread.

If two strains of *Sordaria* are grown together, their filaments can overlap and fertilization can occur. Two haploid nuclei, one from each strain, fuse to form a diploid zygote. But rather than growing by mitosis, like an animal would, the fungal zygote undergoes meiosis, returning to the haploid state. The haploid cells undergo mitosis, forming more ascospores. The fungus keeps the ascospores in a structure called the ascus. The fungus produces fruiting bodies (the *perithecia*) which contain numerous asci. The ascospores in these asci are released into the environment and new fungi establish themselves and grow.

The *Sordaria* life cycle allows for a relatively straightforward investigation of gene mapping: determining the distance between a gene and the centromere of the chromosome it resides on. Spore color is a single-gene trait. By analyzing the colors and arrangement of spores within asci produced on a plate containing black and tan fungal strains, one can determine if crossing over occurred when the zygote underwent meiosis. If there is *no* crossing over during meiosis, the spores will be in a 4:4 arrangement within the ascus: four tan spores next to four black spores. (Refer to the diagram on the following page.) Other color arrangements are observed when crossing over has occurred. The frequency of crossover corresponds to a gene's location on the chromosome.



1. Obtain cards that show asci collected from a black × tan *Sordaria* cross plate. (Alternatively, follow your teacher's directions for preparing slides of asci to view with a microscope.)

2. Locate some asci that have the 4:4 spore color arrangement. Then find instances that differ from this arrangement. Sketch at least three arrangements that differ from the 4:4 arrangement.

3. How many cells are produced by meiosis? Why are there eight cells in each ascus?

4. Copy the table below into your notebook. Count at least 50 asci on one or more cards (or microscope slides) and indicate in the table if each ascus shows no crossover or if there is evidence of crossover. You can tally as you go and then calculate the total.

Table 1: Determining rate of crossover in *Sordaria*[1]

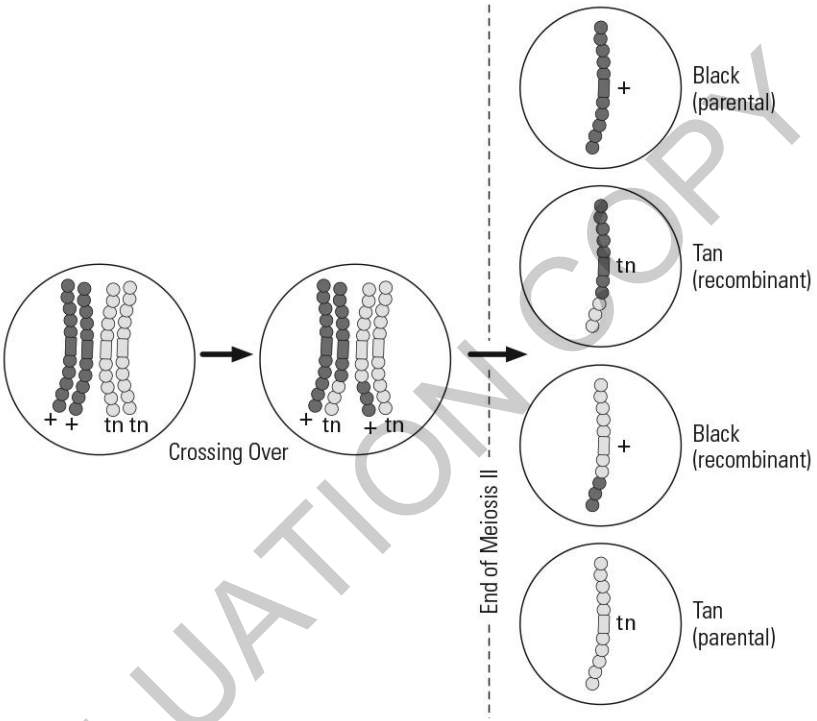
No Crossover (4:4 arrangement)	**Asci That Show Crossover**	**Total Asci Counted**	**Percentage of Asci Showing Crossover**
RECORD ANSWERS & DATA IN YOUR NOTEBOOK.			
Total:	Total:		

NOTE: If an ascus contains all black or all tan spores, do not count this ascus (it results from self-fertilization). Count only the asci that contain both black and tan spores.

Data Analysis

1. Determine the total number of asci you observed and calculate the percentage of asci that showed crossover.

2. When reporting gene-to-centromere distance, *map unit* (mu) is the unit associated with this distance. One map unit is equal to 1% recombination frequency. In most instances, the percentage of offspring with non-parental phenotypes is equal to the number of map units. In the *Sordaria* investigation, however, you observed asci not offspring. Each ascus you counted as recombinant—meaning the ascus showed crossover—actually contained both parental spores and recombinant spores. Only half of the spores in these asci were actually recombinant.



Therefore, the gene-to-centromere distance for *Sordaria* is calculated as:

Distance (mu) = % of crossover ⁄ 2

Calculate the gene-to-centromere distance from your data and compare your results to others in your class, and to the published map distance.

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

Synthesis Questions

1. Suppose pop beads of one color are used to represent a chromosome from the black *Sordaria* strain (carrying the "+" allele) and pop beads of a contrasting color are used to represent a chromosome from the tan *Sordaria* strain (carrying the "tn" allele). Use the pop bead chromosomes to model crossing over during meiosis and follow the chromosomes through meiosis II and the mitosis that follows. Draw pictures to show how a 2:2:2:2 and a 2:4:2 arrangement can be produced in asci.

2. A *map unit* (mu) is the same distance as a *centiMorgan* (cM), a unit named after the geneticist Thomas Hunt Morgan. Morgan's work was crucial to understanding that some genes are inherited as a unit, due to being on the same chromosome. In other words, they are "linked" genes. Morgan produced a number of mutant fruit flies that he used in breeding experiments to deduce gene locations. He first crossed true-breeding flies that differed in phenotype to obtain an F_1 generation.

   In some of Morgan's experiments, he performed a test cross between a wild type double heterozygous female fly (which was a fly from the F_1 generation) and a mutant fly with a double homozygous recessive genotype. The table below displays the results of an experiment similar to Morgan's.

   NOTE: The wild type traits ("+") are dominant to the mutant forms. The allele symbols used are those that Morgan used: "pr" for eyes and "vg" for wings.

   Table 2: Results of a cross between pr^+ pr vg^+ vg × pr pr vg vg

F2 Generation Phenotypes	Expected Number of Flies		Number of Flies Observed
	If Genes Sort Independently	If Genes Are Inherited as a Unit	
Wild type (red eyes, normal wings)			482
Purple eyes, vestigial wings			475
Red eyes, vestigial wings			56
Purple eyes, normal wings			59

   a. First suppose that the two genes, those for eye color and wing shape, are on different chromosomes. What would be the expected phenotypic ratio in the F_2 generation? Based on the number of flies observed, how many flies would be expected for each phenotype listed in the table? (Copy the table into your notebook and complete the second column based on your prediction.)

   b. Now suppose that the two genes are located on the same chromosome and are inherited as a unit. Complete the third column of the table, based on your prediction of the number of flies that should be observed exhibiting each phenotype if the genes are linked.

   c. Does the data support independent assortment of these genes, or linkage between the genes? Explain your answer.

   d. Assuming the genes are linked, how can the recombinant phenotypes observed be explained? What is the distance between the body color and eye color genes?

3. Explain the difference between *linked* genes and *sex-linked* genes. How did Thomas Hunt Morgan show that the white-eye mutation in *Drosophila* was located on the X chromosome?

4. Why does crossing over contribute more to genetic diversity in a sexually reproducing species than mutation does?

5. Mendel's studies of inheritance in pea plants occurred during the years 1856–1866, though the significance of his work was not realized until much later. During the years 1883–1901, enhanced microscopy allowed scientists to observe and describe the details of meiosis.

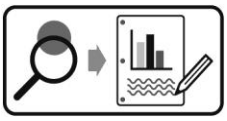
   a. Explain how the behavior of chromosomes during meiosis supports the conclusions Mendel arrived at from his studies of inheritance.

   b. If genes are located on different chromosomes and follow a Mendelian pattern of inheritance, then the rules of probability can be applied to predict or explain the outcome of a genetics experiment. Consider the male and female fruit flies in the initial *Drosophila* investigation. Calculate the probability that an offspring of these flies will be female, eyeless, and have vestigial wings and normal body color. Explain how the rules of probability are applied in your calculation.

Design and Conduct an Experiment

The Initial Investigation provides a comprehensive study of meiosis and introduces some procedural skills for observing the results of crossover in *Sordaria*. If time allows, culture *Sordaria* under different conditions to investigate whether environmental factors, such as pH or nutrient composition of the agar, affect the frequency of crossing over during meiosis.

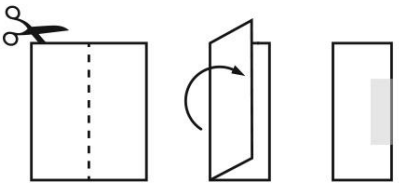
Alternatively, you can extend this investigation by researching further the connection between chromosome abnormalities and cancer (HeLa cells, for example), or by carrying out experiments with fruit flies to learn more about gene mapping.



Discuss the design of your experiment, or your plan for additional research, with your teacher.

Drosophila Chromosome Sheet

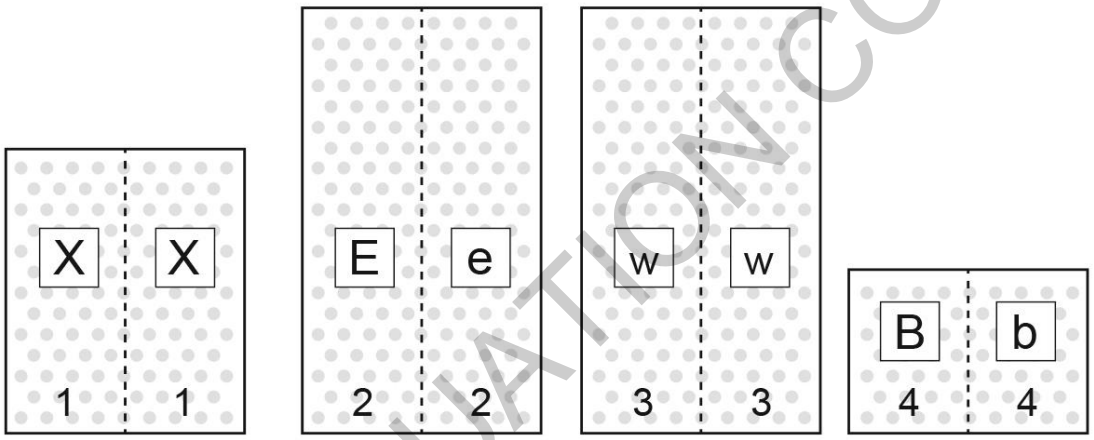
Instructions



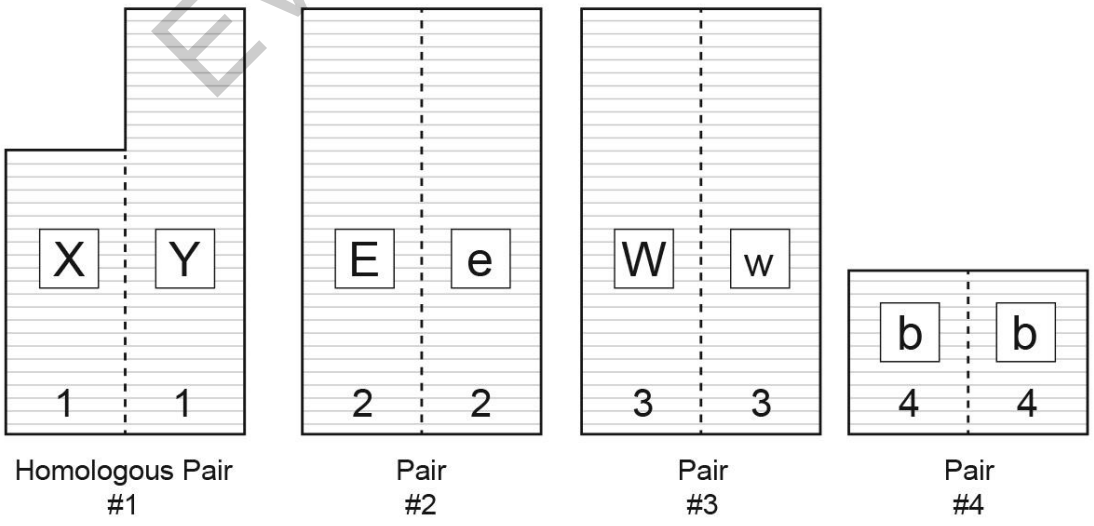
Legend

XX/XY = male / female
E/e = normal eyes / eyeless
W/w = normal wings / vestigal wings
B/b = normal body color / ebony body color

Female Fly

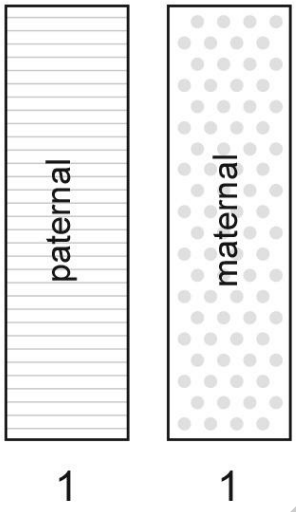


Male Fly

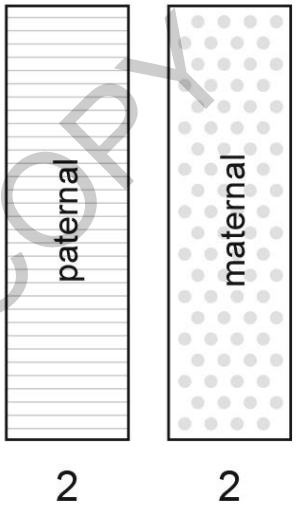


Karyotype of Offspring Fly

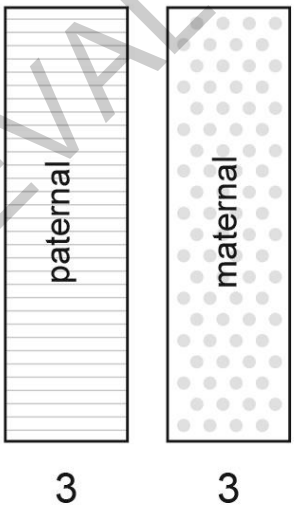
Paternal: inherited from the male (“father”)
Maternal: inherited from the female (“mother”)



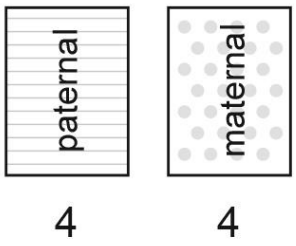
Sex _____



Eyes _____



Wings _____



Body Color _____

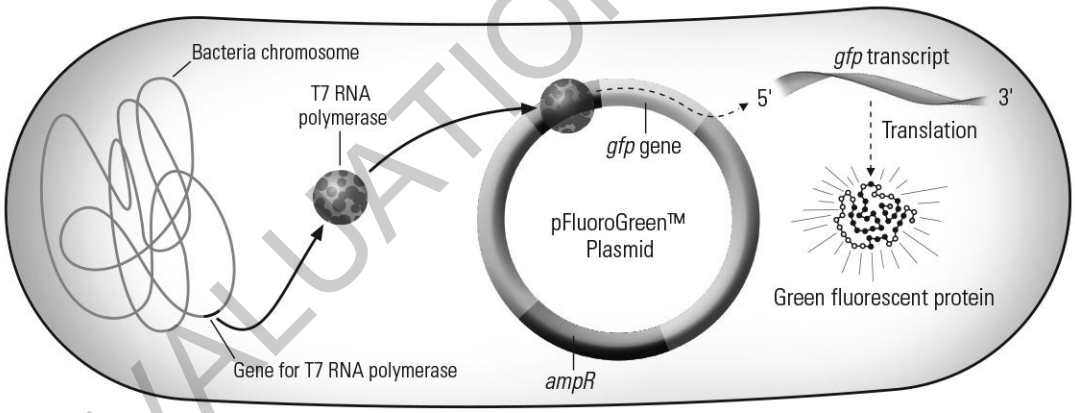
14. TRANSFORMATION

Background

Early in the 20th century there was great debate and uncertainty as to the identity of the molecule responsible for heredity. Some thought it was DNA, others thought it was protein. Experiments performed by Fred Griffith and Oswald Avery demonstrated that mixing cellular material, specifically DNA, from heat-killed pathogenic bacteria with nonpathogenic bacteria could transform the nonpathogenic type into the disease-causing type. This identified DNA as being the molecule responsible for heredity.

In nature, some bacteria can "transform" by taking in pieces of DNA from their environment. This is an important mechanism for maintaining genetic diversity in bacteria populations. Biologists have taken advantage of this characteristic of bacteria and use transformation in numerous biotechnology applications. For example, transformation is used to create bacteria that produce a protein of interest such as insulin. Transformation is also used to clone genes and make gene libraries (the library is "housed" in the genetically engineered organism).

This investigation provides you an opportunity to transform bacteria with the *gfp* gene for green fluorescent protein (GFP). This gene comes from the bioluminescent jellyfish, *Aquorea victoria*. If exposed to long-wave UV light, GFP emits a bright green light, a characteristic known as *fluorescence*.



Genetically engineered *E. coli* (elements not to scale proportionally)

The *gfp* gene is introduced into *E. coli* via a genetically engineered plasmid, pFluoroGreen. If the *gfp* gene is transcribed, the cell will produce GFP. As is commonly done in the biotechnology industry, this plasmid was engineered with a promoter sequence that helps ensure transcription of the gene. A special RNA polymerase, T7 RNA polymerase, recognizes this promoter and initiates transcription of *gfp*. The *E. coli* used in this investigation have been engineered to have the gene that codes for this polymerase in their chromosome.

Also on the plasmid is a *selectable marker*, an ampicillin resistance gene. This *ampR* gene codes for the enzyme β-lactamase, which destroys ampicillin. Culturing bacteria on agar that contains an antibiotic is a technique often used in industry to screen for bacteria that have been successfully transformed.

While research has led to the development of specific protocols for effective transformation, transformation is never 100% efficient. Not all bacteria cells exposed to plasmids will take in a plasmid. A transformation efficiency of 1×10^5 to 1×10^8 cells per microgram of plasmid DNA is expected in industrial applications.

Driving Question

How can foreign genes be added to bacteria? What is the efficiency of transformation, and do bacteria always express foreign genes?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- LB (Luria Broth) Petri plate
- LB/Amp Petri plate (2)
- LB/Amp/IPTG Petri plate
- Inoculating loops (2), sterile
- Transfer pipets (4), 1-mL, sterile
- Micropipet with a sterile tip
- Microcentrifuge tubes (2)
- Small cup or beaker, 100-mL, for ice
- Tube with 0.5 M Calcium chloride ($CaCl_2$), 1 mL[1]
- Tube with Recovery Broth, 1.5 mL
- Tube with pFluoroGreen™ (pGFP) plasmid, 12 µL[1]
- Toothpick, sterile
- Ice
- Permanent marker, fine
- Masking tape

[1]Keep these materials on ice.

For Class Use

- *E. coli* host cells (on 5 large Petri plates)
- Warm water baths (2), 37 °C and 42 °C
- Incubator (37 °C)
- Long wave UV light source
- Disinfectant

Safety

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

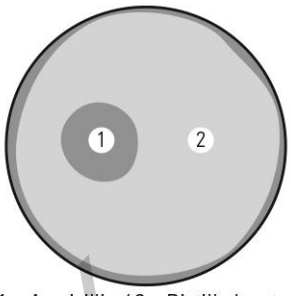
- Wear safety goggles at all times.
- Students with an allergy to antibiotics such as penicillin, ampicillin, kanamycin, or tetracycline should not participate in this experiment.
- Follow proper sterile technique while carrying out all procedures.
- Always wash hands thoroughly with soap and water after working with bacteria and use a 10% bleach solution to disinfect lab surfaces before and after the experiment.

  NOTE: wear gloves and goggles when working with bleach.
- Disinfect used materials (Petri plates, loops, pipets, and tubes) by placing them in an autoclavable, disposable bag and autoclaving at 121 °C for 20 minutes, or by soaking the materials in a 10% bleach solution overnight.
- Never look directly at a UV light. If available, wear UV safety goggles when using a long-wave UV light source.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

Before the transformation experiment



1 = Ampicillin / 2 = Distilled water

1. *E. coli* was grown on Luria Broth (LB) agar containing two paper discs. One disc was soaked in ampicillin and the other was soaked in distilled water before being placed on the surface of the agar. The dark area on the Petri plate indicates bacteria growth. What does the diagram suggest about the effect of ampicillin on *E. coli*? Explain your answer.

2. Some bacteria are resistant to antibiotics such as ampicillin. Bacteria that are sensitive to an antibiotic can become resistant if they acquire a resistance gene. The ability to survive in the presence of the antibiotic results from the gene coding for a beneficial protein, for example, for an enzyme that degrades the antibiotic. In nature, how might bacteria acquire new genes? Identify two or more possibilities and give a description of each.

3. For successful transformation, you will need to work efficiently. You will not have time to figure out the steps along the way. To prepare for a successful experiment, read the procedures of the investigation carefully and create a flow chart to guide you through the steps of the transformation procedure.

4. By introducing a plasmid into the cells you are altering the genotype of the bacteria. How will this affect the phenotype of the bacteria? In other words, how will you know if the transformation is successful?

5. Identify the purpose of each of the following:

   a. The labels "+" and "–" on the microcentrifuge tubes.

   b. Suspending cells in $CaCl_2$.

   c. Incubation of cells in 42 °C water, followed by incubation on ice.

   d. Culturing cells on an agar plate containing ampicillin ("LB/Amp").

Transformation experiment

6. Put on your safety goggles.

7. Add ice to half-fill the small beaker or cup and put the plasmid and calcium chloride tubes into it.

8. Use a sterile 1-mL pipet to transfer 0.5 mL (500 µL) of cold $CaCl_2$ solution to one of the microcentrifuge tubes. Place that tube into the ice.

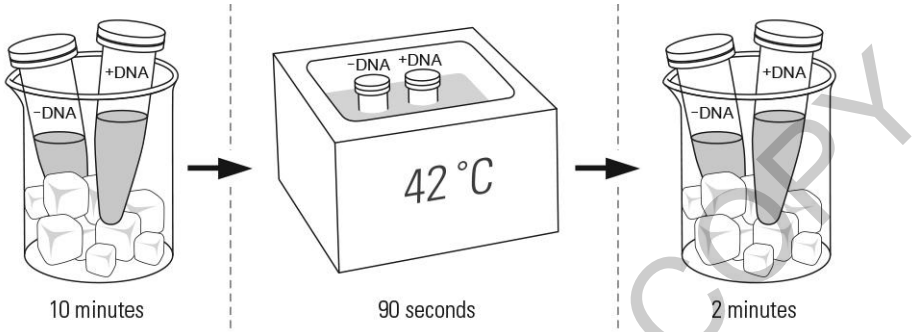
9. With a sterile toothpick, transfer the cells of 15 colonies from an *E. coli* source plate to the tube you just filled with the $CaCl_2$. Swirl the toothpick vigorously in the solution to dislodge the cells and flick the tube to fully suspend them. The suspension should look homogenous, without clumps.

10. Using the same pipet, transfer 0.25 mL (250 µL) of the cell suspension into a second microcentrifuge tube.

11. Use a permanent marker to mark one of the microcentrifuge tubes with the cell suspension with a "+" and the second one with a "–".

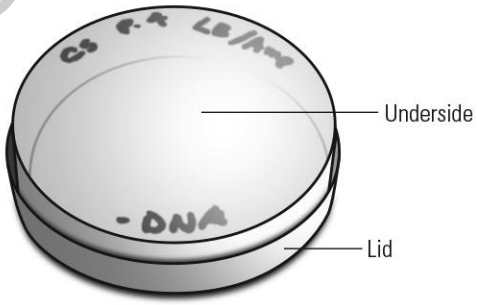
12. Place the "–" tube back in the ice. Use a micropipet with a sterile tip to add 10 µL of plasmid (pGFP) solution to the "+" tube and place it in ice. Leave both tubes in the ice for 10 minutes.

13. After the 10 minutes on ice, move the tubes to a 42 °C water bath for 90 seconds. Then return the tubes to ice for 2 minutes.



14. Use a fresh sterile pipet to add 250 µL of Recovery Broth to each of the tubes and mix them. Place the tubes in a 37 °C water bath for a 30-minute recovery period.

15. While you wait, label the four Petri plates with a permanent marker. Make all labels small and near the edge of the underside of the bottom of the plate (not on the removable lid) so as not to obscure the view of bacteria colonies that will form.



    a. Write your group's initials, class period, and the date on each plate.

    b. On the LB plate, write "–DNA".

    c. On one LB/Amp plate write "–DNA". Write "+DNA" on the second LB/Amp plate.

    d. On the LB/Amp/IPTG plate write "+DNA."

16. When the recovery period is over, bring the tubes back to your lab station. Use a sterile pipet to transfer 0.25 mL (250 µL) of cell suspension from the "–" tube to each of the two plates labeled "–DNA." Use a fresh sterile inoculating loop to spread the cell suspension over the entire plate (for both plates).

17. Use a fresh pipet to add 0.25 mL of cell suspension from the "+" tube to the plates labeled "+DNA" and use a fresh sterile inoculating loop to spread the cell suspension over the plates.

18. Leave the plates undisturbed for at least five minutes to allow the liquid to be absorbed into the agar in each plate.

? 19. On which plate(s) do you expect to observe growth of the transformed bacteria cells? Explain the reasoning for your prediction.

20. After the liquid has been absorbed, stack the set of plates and tape them together. Write your group's initials and class period on the tape. Invert the plates and place them in a 37 °C incubator for 16–20 hours.

21. Thoroughly disinfect your work space, place the used materials in the location designated by your teacher for disinfection and disposal, and wash your hands with soap.

Results of the transformation experiment

NOTE: Do NOT remove the lid of the Petri plates. Colonies are easily observed by keeping the plates inverted and viewing them through the bottom of the plate.

22. After the incubation period, obtain your stack of Petri plates. Record detailed observations and sketch the appearance of each of the plates.

23. Darken the room and use a long wave UV light to determine if any transformed colonies fluoresce, due to production of GFP. Record your observations.

24. Did you get any surprising or unexpected results? Explain your answer.

Data Analysis

1. Which plate has the greatest bacteria growth? Explain why this is the case.

2. Which plate has no bacteria growth? Explain why this is the case.

3. Control groups are an important part of good experimental design.

   a. Which plate or plates represent a control group for the transformation experiment? Explain your answer.

   b. Why are control groups essential?

Transformation efficiency

Transformation efficiency is an indicator of the success of the experiment and is obtained by determining the number of cells transformed per 1 µg of plasmid DNA.

4. To calculate the transformation efficiency:

   a. Begin by counting the number of colonies on the LB/Amp/IPTG plate. It may be helpful to mark counted colonies with a dry erase marker on the outside of the plate. Record the total number of colonies in your lab notebook.

   b. Use the colony count to calculate the transformation efficiency using the formula below and the accompanying information:

   Total DNA used: 0.050 µg

   Recovery volume: 0.50 mL

   Volume plated: 0.25 mL

$$\text{Transformation Efficiency} = \frac{\text{Number of transformants/plate}}{\text{µg DNA/plate}}$$

$$\text{where the number of µg DNA/plate} = \text{Total DNA (µg)} \times \frac{\text{Volume plated}}{\text{Recovery volume}}$$

5. In research laboratories, transformation efficiency ranges from 1×10^5 to 1×10^8 cells per microgram of plasmid DNA. How does the transformation efficiency of this investigation compare to that of a research laboratory?

6. How do the transformation efficiencies acquired by different student groups compare? What might account for differences in efficiency?

7. Count the colonies on the plate labeled LB/Amp (+DNA) and calculate the transformation efficiency. Record the data in your notebook. Is the efficiency similar between the two experimental group plates (LB/Amp and LB/Amp/IPTG)?

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

Synthesis Questions

The *E. coli* used in this experiment have been genetically engineered to be able to produce a special RNA polymerase, called *T7 RNA Polymerase*. The gene for this polymerase is under the control of the inducible promoter of the *lac* operon. IPTG is a substance that binds to the *lac* repressor, thereby inducing transcription of the T7 RNA polymerase gene. The pGFP plasmid used in the investigation was engineered to have a T7-specific promoter just upstream of the *gfp* gene. You may find it helpful to refer to the image in the Background section.

1. Think about how gene expression is regulated by an inducible promoter.

   a. What is the role of a repressor protein in gene regulation?

   b. What effect does IPTG in the growth medium have on the expression of the T7 RNA polymerase gene within the *E. coli* bacteria cells?

2. IPTG affects the expression of the *gfp* gene, and therefore affects whether *E. coli* colonies fluoresce. Why is the expression of the *gfp* gene dependent upon IPTG?

3. Explain how the transformation experiment demonstrates:

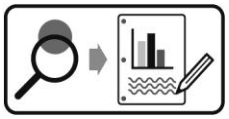
   a. the relationship between genotype and phenotype.

   b. the relationship between phenotype and the environment.

4. The *ampR* gene codes for a digestive enzyme that degrades ampicillin, thereby allowing *E. coli* cells to grow and reproduce in the presence of the antibiotic. Describe in detail the molecular processes involved in gene expression. That is, how does the DNA sequence of a gene result in the synthesis of a particular protein within cells?

Design and Conduct an Experiment

If your teacher determines there is sufficient time and materials for you to carry out an experiment of your own design, explore other aspects of transformation or antibiotic resistance.

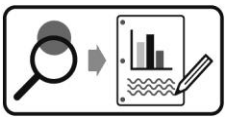


Design and carry out your experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

Explore other aspects of transformation or antibiotic resistance.



Develop and conduct your experiment using the following guide.

1. Create a driving question: develop a testable question for your experiment.

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

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

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

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

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

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

_____

_____

_____

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

_____

_____

_____

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

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

15. UNDERSTANDING INHERITED MITOCHONDRIAL DISORDERS

Background

Two patients are suspected of having MELAS, a genetic disorder caused by a mutation within mitochondrial DNA (mtDNA). (Refer to the Background Information supplement to help you understand this condition and its pattern of inheritance.) Use pedigree analysis and molecular diagnostics to confirm or refute the preliminary diagnosis. The following table summarizes each patient's case history.

Patient 1	Patient 2
Description	**Description**
Seven year old female with a history of normal development until age two. At that point she developed episodic vomiting, acidosis, epilepsy, general weakness, ataxia (stiff, unsteady gait), and dystonia (involuntary muscle contractions).	Fifty-two year old male with sudden onset headaches and seizures. Patient has a history of diabetes and deafness. MRI detected bi-temporal lesions.
Family History	**Family History**
A patient history was taken going back to the patient's grandparents on her mother's side. No similar symptoms have occurred in the patient's siblings (patient has twin older brothers.) No symptoms appear in the patient's parents nor in the mother's parents (maternal grandparents). The patient's grandfather (mother's father) has adult-onset diabetes (Type II diabetes).	A patient history was taken going back to the patient's grandparents on his mother's side. The patient's brother was found to have asymptomatic mild lactic acidosis. The patient's mother had diabetes, exercise intolerance, and ptosis (drooping eyelids). The patient's maternal uncle died of a stroke at age 37 and had multiple health issues (poorly defined). The patient's maternal grandmother had diabetes and possibly other symptoms and the patient's father has rheumatoid arthritis, but no history of diabetes or neurological problems.

DNA Samples

After reviewing the patients' histories, their physicians decided that a genetic test should be performed. Blood was drawn from both patients and the polymerase chain reaction (PCR) was used to isolate and amplify a 4300 bp region of the mitochondrial genome. This region includes the Leu (UUR) gene (our gene of interest). The patients' PCR products were then digested with restriction enzyme "ApaL1" for 2 hours at 37 °C. This enzyme (in conjunction with gel electrophoresis) can be used to distinguish between the normal and mutated forms of the gene since the enzyme will cut the normal DNA into two fragments but will leave the mutated DNA uncut. The restriction site for ApaL1, GTGCAC, is present in normal mtDNA, but the mutated mtDNA has the sequence GTGCGC, due to a substitution mutation (3243A>G). This altered sequence is not recognized by the enzyme.

The digested DNA samples from the patients are ready for analysis. Control samples of amplified normal mtDNA and mutated mtDNA were also digested with ApaL1 and are included for comparison to the patients' samples.

Driving Question

Do the pedigrees, case histories, and molecular diagnostic results of two patients generate enough evidence to support or refute the diagnosis of a mitochondrial disease (MELAS)?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Horizontal gel electrophoresis apparatus
- DC power supply
- Automatic micropipet, 5 to 50 µL, with 5 tips
- Tray with 0.8% agarose gel
- QuickStrip™ DNA samples
- InstaStain® Blue card
- Plastic tray for gel staining
- Plastic wrap
- Graduated cylinder, 100-mL
- Waste receptacles (for used tips)
- Disposable gloves
- Distilled water or buffer, 75–100 mL, for staining
- **OPTIONAL** (for preserving a record of the result)
- Camera (USB or other)
- Permanent marker
- Transparency film (for tracing the results)

One per Class

- DNA visualization system (white light)[1]
- Spatula (for handling the gel)

[1]A visualization system is not required, but if it is available, it will allow you to optimize the view of the gel.

Safety

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

- Wear safety goggles at all times
- Make sure that all liquid reagents are safely stored and that areas are dry before plugging in and turning on electrophoresis equipment.
- Wear gloves when working with stain.

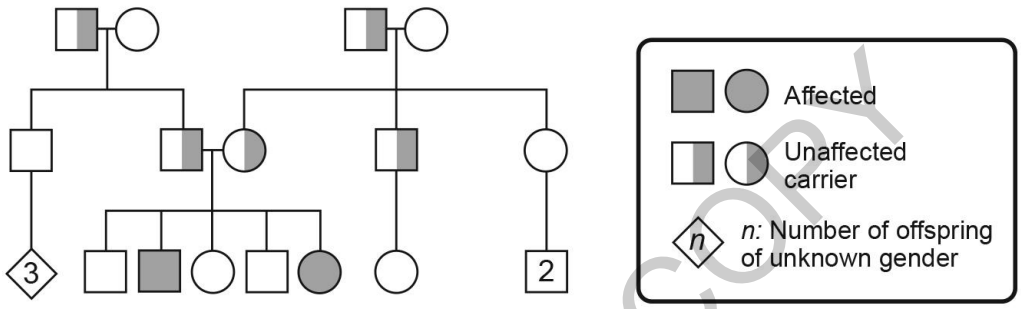
Pedigree Investigation

Record all observations, data, explanations, and answers in your lab notebook.

Pedigree analysis is a powerful tool used by geneticists, physicians, and genetic counselors to understand the *pattern of inheritance* (PoI) of certain traits. Before constructing pedigrees for the two patients, review the following three pedigrees.

1. Work with a partner and analyze Pedigree 1. Look for patterns emerging from the pedigree (for example, how the trait passed from one generation to the next).

Pedigree 1



2. Create a chart in your lab notebook, like the one below, that includes space for the PoI claim and for the evidence you are asked to provide. Sketch a portion, or all, of Pedigree 1 into your notebook as well.

Analysis of Pedigree 1

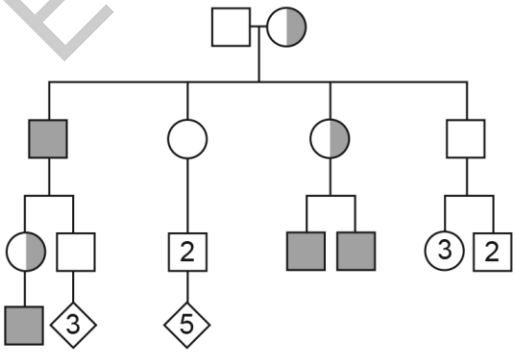
PoI Claim	Evidence

   a. Make a claim stating the PoI you observe in Pedigree 1.

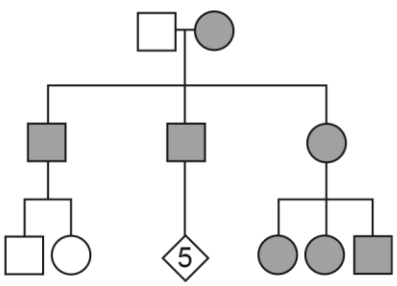
   b. Support your claim with evidence. Describe specific aspects of Pedigree 1 that indicate the PoI you identified.

3. Create charts for Pedigrees 2 and 3. For each of these pedigrees, make a claim regarding the PoI and support each claim with specific evidence from the pedigree.

Pedigree 2



Pedigree 3



4. Discuss with your teacher and classmates the unusual pattern of inheritance seen in Pedigree 3. What types of genetic diseases would exhibit the PoI seen in Pedigree 3? Why would that happen?

5. Construct a pedigree for Patient 1 based on the patient's family history (in the table in the Background section). Include all three generations. Shade any circles or squares that represent an individual who may have a mitochondrial disorder like MELAS.

6. Construct a pedigree for Patient 2 based on the patient's family history (in the table in the Background section).

Data Analysis

1. Which patient(s), if any, has a family pedigree consistent with the pattern of inheritance expected for the mitochondrial disorder MELAS? Use evidence from each pedigree to support your answer.

2. If a patient's family pedigree does not indicate a maternal pattern of inheritance, can MELAS be ruled out as a diagnosis? Explain your answer.

DNA Investigation

Record all observations, data, explanations, and answers in your lab notebook.

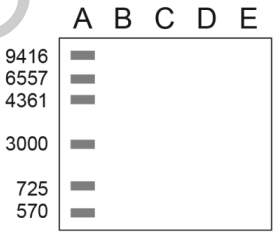
DNA samples from Patient 1 and Patient 2 were amplified via PCR to produce many copies of a 4,300 bp region of the mitochondrial genome. This PCR product contains the gene of interest, Leu 1 (UUR) as well as several neighboring genes. Because the Leu 1 gene is so small (44 bp) it is necessary to include more than just the gene of interest during DNA amplification. PCR was followed by digestion with restriction enzyme ApaL1, which recognizes the sequence GTGCAC within the normal gene, but not the sequence GTGCGC present in the mutant form of the gene. These DNA samples, as well as control samples, have been shipped to you for analysis.

Predict

1. Table 1 identifies the QuickStrip DNA samples. Copy this table and the gel diagram next to it into your lab notebook. Predict whether the restriction enzyme did or did not cut the DNA, and predict the number of bands you will see on the electrophoresis gel. Sketch your prediction of the results of the electrophoresis for samples B–E.

Table 1: Prediction of electrophoresis results

Sample	Contents	Restriction Enzyme Results	Number of Bands Expected
A	DNA Marker		
B	Normal DNA	RECORD THE DATA IN YOUR	
C	Mutated DNA	NOTEBOOK.	
D	Patient 1		
E	Patient 2		



Load the Gel

2. Obtain a tray with the 0.8% agarose gel and take it to an electrophoresis chamber. Identify the *positive* (red cord) and *negative* (black cord) sides of the chamber. Place the gel, with the tray, in the chamber, positioning the wells on the negative side of the chamber (the side with the black cord).

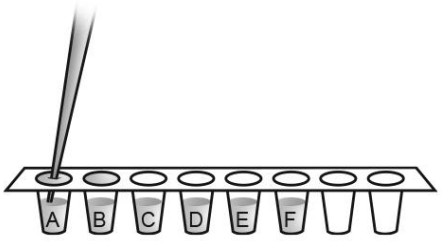
   NOTE: During electrophoresis, the DNA samples migrate through the gel towards the positive electrode. It is critical that you have the wells closest to the negative side of the chamber!

3. The gel should rest level and centered on the platform of the electrophoresis chamber and be submerged under the surface of dilute electrophoresis buffer. Add buffer to the chamber if the gel is not submerged.

4. Obtain one strip of QuickStrip DNA samples from your teacher. Tap the tubes gently on the table to ensure that the sample is at the bottom of the tubes.

5. Obtain an automatic micropipet and sterile tip. Place the sterile tip on the end of the micropipet. Set the pipet to 30 µL.

6. Pierce the protective overlay of the QuickStrip DNA samples container with the pipet tip, and draw 30 µL of sample A into the tip. Make sure there are no bubbles in the tip of the pipet after you have extracted your sample.



7. Carefully place the tip of the pipet halfway into the first well of the gel. *Slowly* press the plunger of the micropipet to expel the sample into the well.

   NOTE: You should see the DNA and loading dye drop into the bottom of the well. Do not push through the "soft stop" on the pipet. Leave your thumb at the soft stop, remove the pipet tip from the well, and eject the tip.

8. Using a clean pipet tip each time, load samples B–E into the wells in consecutive order.

Run the Gel

9. Place the lid securely on the electrophoresis chamber and connect the apparatus to the DC power supply.

10. Set the power source to the required voltage. Ask your teacher what voltage is recommended for your equipment.

11. Turn on the power supply. Check that the current is flowing properly: you should see bubbles forming on the two platinum electrodes.

12. Conduct the electrophoresis for the length of time instructed by your teacher. When instructed, turn off the power supply to stop the electrophoresis process.

Stain the Gel

13. Slowly pour 75 mL of water or electrophoresis buffer into the plastic tray for gel staining.

14. Put on disposable gloves. Carefully remove the gel from the electrophoresis chamber and place it in the plastic tray. (Slide the gel off the tray it was cast in.) The gel should be completely submerged in the liquid (add more liquid if necessary).

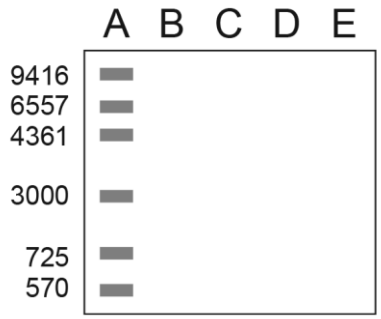
15. Place the blue dye side of the InstaStain card face down on the surface of the liquid, directly over the gel.

16. After 60 seconds, remove the card from the staining tray.

17. Cover the tray with plastic wrap and leave it undisturbed for at least 3 hours. (You can leave the gel in the tray overnight.)

18. Wearing the disposable gloves, remove the gel from the staining tray. If a light box visualization system is available, place the gel on the light box for an optimum view of the DNA bands.

19. Sketch a diagram in your lab notebook like the one shown below. Draw the banding patterns observed in your gel for each lane (sample).



20. Use the *molecular ruler* (the DNA Marker bands on the diagram), and the background information, to determine the sizes of the DNA bands in lanes 2 through 5 (samples B–E). Create a table in your lab notebook, or expand Table 1, to summarize the predictions and results of the DNA electrophoresis.

Data Analysis

1. Based on the evidence from the gel, which patient, if any, has the A>G point mutation in the Leu (UUR) gene—the mutation that is the most common cause of MELAS? Cite specific evidence from the gel to support your claim.

2. Based on the evidence from the gel, does either patient appear to have a normal (non-mutated) Leu (UUR) gene? Support your claim with evidence.

3. If the result of the mtDNA genetic test shows heteroplasmy, is the individual a carrier for the mitochondrial genetic disorder? Explain your answer.

4. If the genetic test is negative for the A>G mutation within the Leu (UUR) gene, can a diagnosis of MELAS (or other mitochondrial disorders) be ruled out? Explain your answer.

Synthesis Questions

1. Does the evidence from the case history, family pedigree, and DNA analysis for Patient 1 support or refute the original diagnosis of MELAS? Make a claim and then justify the claim with multiple lines of evidence.

2. Does the evidence from the case history, family pedigree, and DNA analysis for Patient 2 support or refute the original diagnosis of MELAS? Make a claim and then justify the claim with multiple lines of evidence.

3. If the diagnosis of MELAS cannot be justified for one or both of the patients, what is an alternate explanation for the symptoms present in the patient?

4. a. What does the tRNA Leu (UUR) gene do?

   b. How would a mutation in the Leu (UUR) gene affect the synthesis of protein complexes in the electron transport chain (ETC)?

   c. Why would one or more missing or nonfunctional proteins of the ETC result in insufficient ATP production?

5. MELAS is an acronym for "mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes." Copy the table below, provide the definition of each term, and identify the organ system(s) affected in each condition. Pick out two other symptoms exhibited by either patient (or the patient's family) and describe them also.

Table 2: MELAS symptoms

Condition	Definition	Affected Organ System
Myopathy		
Encephalopathy	RECORD THE ANSWERS IN YOUR NOTEBOOK.	
Lactic acidosis		
Stroke-like episodes		
Other symptom 1		
Other symptom 2		

6. Explain why cells and tissues that have high metabolic demands would be sensitive to mutations in protein complexes of the ETC.

7. What is heteroplasmy? Why might symptoms be more severe for one MELAS patient compared to another?

Background Information Supplement – Understanding Inherited Mitochondrial Disorders

Most genetic disorders are caused by mutations in nuclear DNA (nDNA). Each disorder has a *pattern of inheritance* (PoI); this pattern describes how the mutation is passed down through a number of generations. Common patterns of inheritance are *autosomal recessive* and *X-linked* inheritance. A less common pattern of inheritance is known as *maternal inheritance*. Family pedigrees (which convey the pattern of inheritance) help scientists identify and understand how a disorder is inherited.

During human reproduction, each parent provides an equal contribution of nDNA; 23 chromosomes from the egg and 23 chromosomes from the sperm provide the zygote with its diploid genome. The genes (and mutations) located on these chromosomes follow common patterns of inheritance. However, nDNA is not the only DNA within a cell. During reproduction, the mother provides additional DNA to offspring in the form of mitochondrial DNA (mtDNA). The cytoplasm of the zygote is derived from the egg; therefore all of the mitochondria present in the offspring are maternal, as is the mtDNA contained within those organelles. If a mutation is present in mtDNA, a disorder caused by the mutation will display a maternal pattern of inheritance.

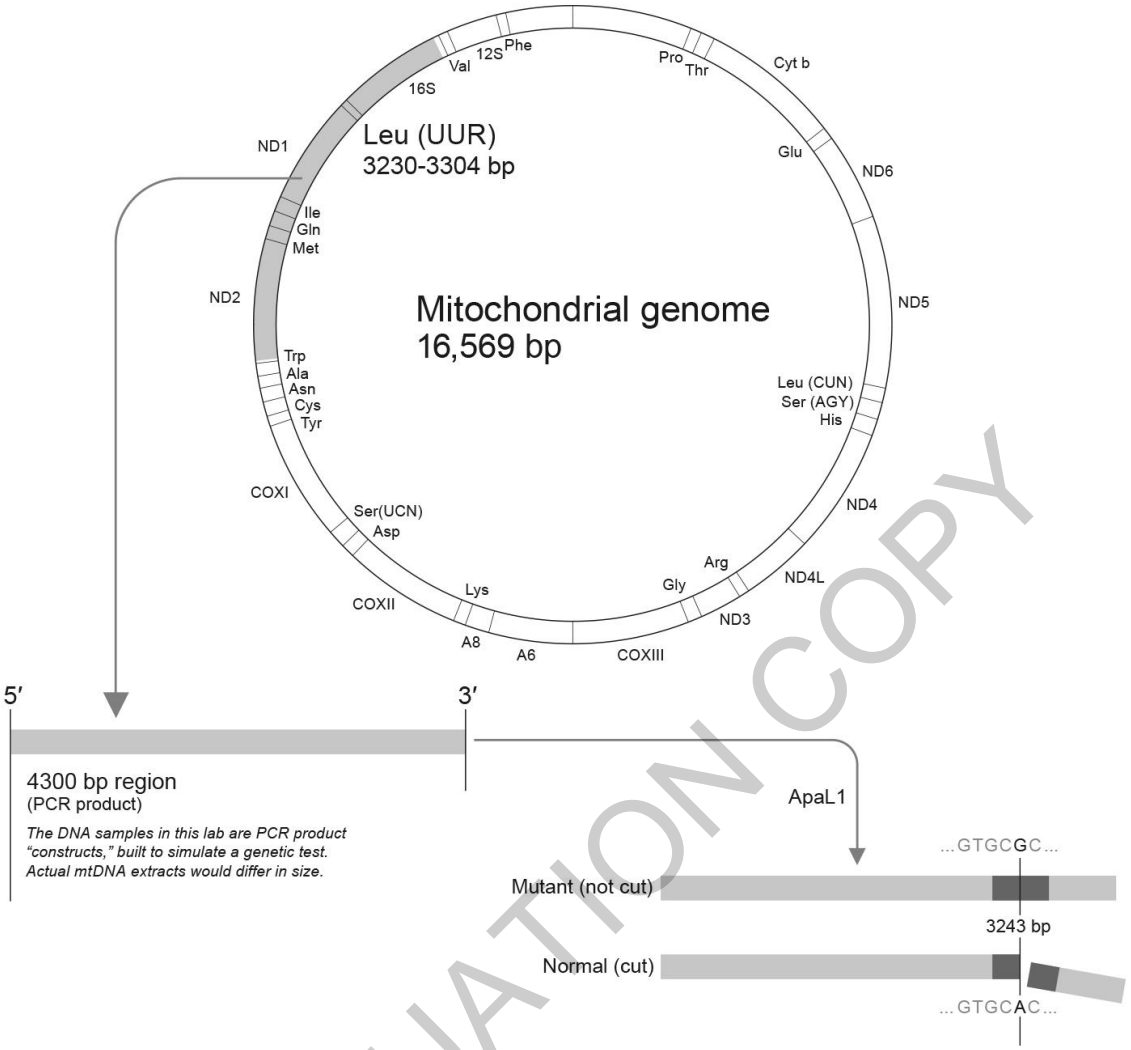
Mitochondrial DNA is evidence of the organelle's origin as a free-living, heterotrophic prokaryote. The genome is small, consisting of just 37 genes within a 16,569 bp loop of DNA. (Refer to the diagram of the mtDNA genome.) Thirteen of the genes code for proteins that form the complexes of the electron transport chain (ETC). (Additional ETC proteins are coded for by genes within nDNA.) The remaining mitochondrial genes code for rRNA and tRNA molecules that participate in the process of translation to build the proteins of the ETC. Mutations in these genes disable the protein synthesis process and cause the proteins of the ETC to be nonfunctional or not synthesized.

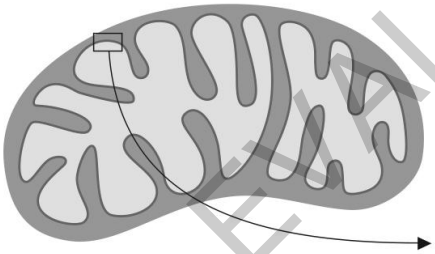
The electron transport chains within mitochondria are the critical structures that enable mitochondria to provide usable energy (ATP) to a cell. Mutations in the mtDNA genome can severely affect the functioning of the ETC, resulting in metabolic disorders due to insufficient production of ATP. Over 100 point mutations in the mtDNA genome have been identified and linked to genetic disorders. One of the most common is *MELAS* (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes). A point mutation in the Leu (UUR) gene is the cause of most MELAS cases. At the 3243 bp locus, adenine is substituted with guanine (symbolized by: 3243A>G). The Leu (UUR) gene is not a protein-coding gene, rather it codes for the tRNA molecule that brings leucine to a ribosome during protein synthesis.

Most cells are packed with hundreds, maybe thousands, of mitochondria. Often, most of these mitochondria have normal DNA; only some have the mutation. The condition of having both normal and abnormal mtDNA is known as *heteroplasmy* and results in differences in the severity of symptoms in affected individuals. Individuals with a small number of mitochondria containing mutated genomes may be asymptomatic or have low-level symptoms that are never officially linked to a diagnosis of MELAS. Individuals with a greater number of abnormal mitochondria are more likely to suffer symptoms that are debilitating or life-threatening, since the cells with “diseased” mitochondria are likely to function improperly or die.

Tissues that have high metabolic demands, needing a large quantity of ATP, have a low threshold for mitochondrial dysfunction and are more likely to show symptoms of mitochondrial disease. These tissues include neural, muscle, and renal tissues. Symptoms commonly associated with MELAS include: migraine-type headaches, brain lesions due to stroke-like events, vomiting, seizures, exercise intolerance, muscle weakness, hearing loss, diabetes, and short stature.

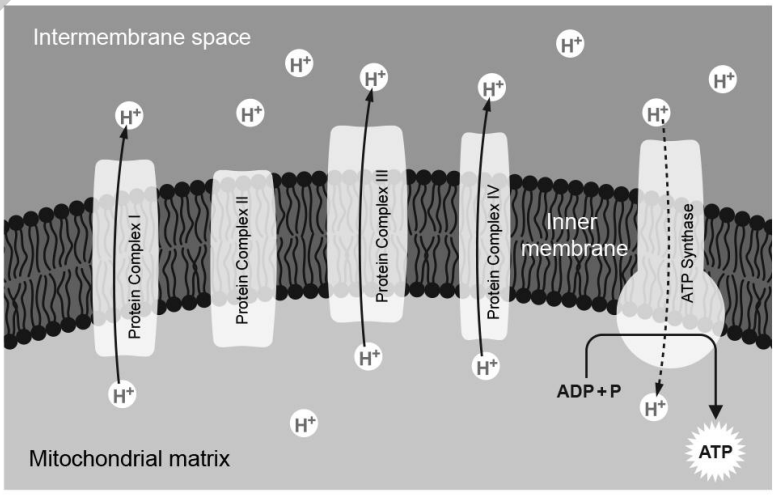
MELAS and other mitochondrial disorders can be difficult to diagnose. Typically, a doctor will evaluate the patient's family history and use the results of many tests, including a clinical exam, muscle biopsy, blood tests, and genetic tests to make a final diagnosis.





Mutations in coding regions of the mitochondrial genome can severely affect the functioning of the electron transport chain. Protein complexes are paramount to electron transfer and adequate ATP production. A mutation in the genetic "recipe" for a tRNA, such as Leu (UUR), is especially detrimental. Such a mutation affects the overall protein synthesis mechanism, and therefore affects the assembly of many proteins within these intermembrane complexes.

Electron transport chain



16. SICKLE CELL GENE DETECTION

Background

Sickle cell anemia, a common form of sickle cell disease, is a genetic disorder that results from a mutation in the hemoglobin B gene (*HBB*) that provides instructions for making hemoglobin B (Hb A), also known as beta-globin. This is one of two types of molecules comprising the hemoglobin protein. Hemoglobin is a globular protein that contains two alpha-globin chains and two beta-globin chains. The four-polypeptide complex contains *heme* groups that bind oxygen molecules. Thus, hemoglobin plays a critical role in delivering oxygen to cells throughout the body.

While sickle cell anemia, an autosomal recessive disorder, is relatively rare, it is estimated that 8–12% of African Americans are carriers of the disease. Diagnosis of sickle cell anemia, or identification of carrier status, can be accomplished using molecular biology techniques.

The polymerase chain reaction (PCR) produces many copies of the hemoglobin B gene, and restriction enzymes are used to differentially cut the normal and mutant alleles. Separation of DNA fragments on a gel using electrophoresis allows a technician to detect the alleles present in each DNA sample.

In this investigation, a mother, father, and their child will be analyzed for their genetic status with regard to sickle cell anemia. The mother is a 37 year-old African American woman who is pregnant with her first child. Her doctor recommended amniocentesis due to her advanced maternal age. In addition to the typical screening for Down syndrome and other chromosomal disorders more common in children born to older women, the doctor suggested a sickle cell genetic test. DNA samples from the mother, her husband, and cells obtained from the fetus have been prepared for your analysis.

Driving Questions

Does the child have sickle cell anemia? What is the genetic status of the child's parents?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

For Each Student Station

- Horizontal gel electrophoresis apparatus
- DC power supply
- Automatic micropipet, 5 to 50 µL, with tips
- Tray with 0.8% agarose gel
- QuickStrip™ DNA samples
- InstaStain® Blue card
- Plastic tray for gel staining
- Plastic wrap
- Graduated cylinder, 100-mL
- Waste receptacles (for used tips)
- Disposable gloves
- Distilled water or buffer, 75–100 mL, for staining
- **OPTIONAL** (for preserving a record of the result)
- Camera (USB or other)
- Permanent marker
- Transparency film (for tracing the results)

One per Class

- DNA visualization system (white light) [1]
- Spatula (for handling the gel)

[1]A visualization system is not required, but if it is available, it will allow you to optimize the view of the gel.

Safety

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

- Wear safety goggles at all times
- Make sure that all liquid reagents are safely stored and that areas are dry before plugging in and turning on electrophoresis equipment.
- Wear gloves when working with stain.

Investigation

Record all observations, data, explanations, and answers in your lab notebook.

1. Compare the DNA sequence for normal hemoglobin B (*Hb A*) to the mutant DNA sequence for abnormal hemoglobin B (*Hb S*), described in Table 1.

   a. Identify the type of mutation that resulted in the Hb S variant.

   b. Describe the effect the mutation has on the primary structure of the hemoglobin B polypeptide.

Table 1: Comparing the normal and mutant hemoglobin B alleles

Hemoglobin B	DNA Nucleotide Sequence (template strand for transcription) of the gene for Hemoglobin B (*HBB*)
Normal (*Hb A*)	C T G A C T C C T G A G G A G A A G T C T
Abnormal (*Hb S*)	C T G A C T C C T G T G G A G A A G T C T

2. Analyze each of the DNA sequences in Table 1 for the presence of one or more of the restriction site sequences listed in Table 2.

Table 2: Recognition sequences for various restriction enzymes

Enzyme	Restriction Site Sequence[1]
*Bam*HI	GGATCC
*Mst*II	CCTNAGG
*Sac*I	GAGCTC
*Hinf*I	GANTC

[1]"N" can be any of the four nitrogen bases.

   a. Which enzyme or enzymes would cut within the normal *HBB* sequence? How does this compare to the effect the enzyme or enzymes would have on the abnormal *HBB* sequence?

   b. In performing a diagnostic test for sickle cell disease, which enzyme would you choose to use in the digestion stage? Explain your reasoning.

3. A diagnostic test for sickle cell disease will be performed using samples from a mother, father, and their child. To accurately determine the genotype of each person for the *HBB* locus, what control DNA samples are needed?

4. Put on your safety goggles.

5. Obtain the QuickStrip DNA samples A–F and a micropipet. Set the micropipet volume to 30 µL.

Load the Gel

6. Obtain a tray with the 0.8% agarose gel and take it to an electrophoresis chamber. Identify the *positive* (red cord) and *negative* (black cord) sides of the chamber. Place the gel, with the tray, in the chamber, positioning the wells on the negative side of the chamber (the side with the black cord).

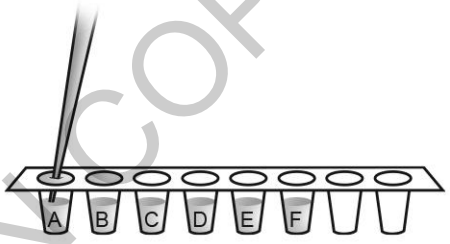
   NOTE: During electrophoresis, the DNA samples migrate through the gel towards the positive electrode. It is critical that you have the wells closest to the negative side of the chamber!

7. The gel should rest level and centered on the platform of the electrophoresis chamber and be submerged under the surface of dilute electrophoresis buffer. Add buffer to the chamber if the gel is not submerged.

8. Tap the QuickStrip DNA sample tubes gently on the table to ensure that the sample is at the bottom of the tubes.

9. Place a sterile tip on the end of the micropipet.

10. Pierce the protective overlay of the QuickStrip DNA sample container with the pipet tip and draw 30 µL of sample A into the tip. Make sure there are no bubbles in the tip of the pipet after you have extracted your sample.



11. Carefully place the tip of the pipet halfway into the first well of the gel. *Slowly* press the plunger of the micropipet to expel the sample into the well.

    NOTE: You should see the DNA and loading dye drop into the bottom of the well. Do not push through the "soft stop" on the pipet. Leave your thumb at the soft stop, remove the pipet tip from the well, and eject the tip.

12. Using a clean pipet tip each time, load samples B–F into the wells in consecutive order.

Run the Gel

13. Place the lid securely on the electrophoresis chamber and connect the apparatus to the DC power supply.

14. Set the power source to the required voltage. Ask your teacher what voltage is recommended for your equipment.

15. Turn on the power supply. Check that the current is flowing properly—you should see bubbles forming on the two platinum electrodes.

16. While you wait for results, copy Table 3 into your lab notebook and complete the "Expected Gel Pattern" section.

17. Conduct the electrophoresis for the length of time instructed by your teacher. When that time is up, turn off the power supply to stop the electrophoresis process.

Stain the Gel

18. Slowly pour 75 mL of water or electrophoresis buffer into the plastic tray for gel staining.

19. Put on disposable gloves. Carefully remove the gel from the electrophoresis chamber and place it into the plastic tray. (Slide the gel off the tray it was cast in.) The gel should be completely submerged in the liquid (add more liquid if necessary).

20. Place the blue dye side of the InstaStain® Blue card face down on the surface of the liquid, directly over the gel.

21. After 60 seconds, remove the card from the staining tray.

22. Cover the tray with plastic wrap and leave it undisturbed for at least 3 hours. (You can leave the gel in the tray overnight.)

23. Wearing the disposable gloves, remove the gel from the staining tray. If a light box visualization system is available, place the gel on the light box for an optimum view of the DNA bands.

24. Sketch a diagram, like the one shown below, in your lab notebook. Draw the banding patterns observed in your gel for each lane (each sample). Complete the "Actual Gel Pattern" column of Table 3.

Table 3: Results of electrophoresis

DNA Sample	DNA Source	Expected Gel Pattern	Actual Gel Pattern	Genetic Status
A	Sickle cell control	RECORD THE DATA IN		
B	Carrier control	YOUR NOTEBOOK.		
C	Normal control			
D	Mother			
E	Child			
F	Father			

Data Analysis

1. What is the genetic status of a person whose hemoglobin B DNA sample produces three bands on a gel? Explain why three bands are produced.

2. What is the genetic status of a person whose hemoglobin B DNA sample produces one band on a gel? Explain why only one band is present.

3. Why do the two fragments resulting from digestion with *Mst*II travel to different locations on the gel?

4. Consider the genetic status of the mother and father tested in this investigation.

   a. If the couple decides to have another child, what is the probability that their second child will have sickle cell anemia?

   b. Is the probability of inheriting sickle cell anemia affected by the gender of the individual? Explain your reasoning.

Synthesis Questions

1. The most common cause of sickle cell anemia is a mutation that results in the amino acid valine replacing glutamic acid in the hemoglobin B gene (*HBB*). This single amino acid difference leads to significant consequences on red blood cell structure and body physiology.

   a. Valine is a neutral and non-polar amino acid. Glutamic acid is an acidic and polar amino acid. Why do different amino acids have different chemical properties?

   b. Explain why the change from glutamic acid to valine affects the structure of the hemoglobin protein.

2. Persons with sickle cell anemia are cautioned against participating in strenuous activity. The symptoms of the disease are most severe when the supply of oxygen is limited. Relate this characteristic of the disease to the location and role of hemoglobin in the body, and explain why exercise would amplify symptoms.

3. Scientists have discovered that a drug called *hydroxyurea* promotes the production of fetal hemoglobin, a form of hemoglobin present only in trace amounts after 1–2 years of age. Treatment with hydroxyurea causes many sickle cell patients to produce more fetal hemoglobin which, in turn, helps reduce polymerization of hemoglobin in red blood cells.

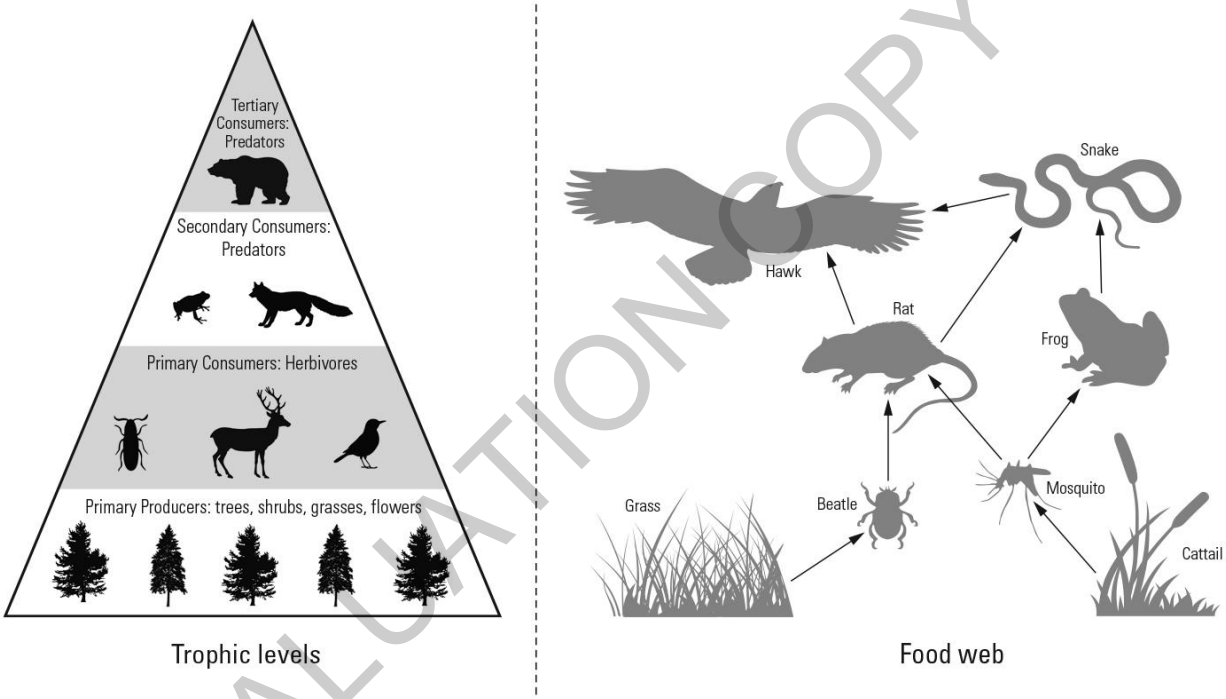
   a. Why would hydroxyurea be considered a treatment and not a cure for sickle cell anemia?

   b. To develop a cure for the disease, how might scientists induce the body to produce more fetal hemoglobin on its own, independent of a drug like hydroxyurea?

17. ENERGY DYNAMICS

Background

Energy flow and material cycling is a central theme in ecosystem science. Generally speaking, energy flows through ecosystems while material is cycled within ecosystems. Energy is first captured by producers that carry out photosynthesis, converting light energy to chemical potential energy stored in organic compounds. Producers use much of the energy for their own needs, but some of the energy captured by producers is transferred through successive trophic levels as herbivores consume producers and carnivores consume herbivores. Food webs and trophic level diagrams summarize these energy transfers.



What is not typically shown in these diagrams is that much of the energy from the trophic levels is transferred to the detrital pool rather than to a higher trophic level. Organisms often die and decompose, instead of being consumed. Additionally, wastes excreted by animals in an ecosystem contain organic material. The energy contained within the detritus of an ecosystem is an important food source for decomposers and detritivores.

As with other organisms, decomposers and detritivores use cellular respiration to break down these compounds and extract the energy contained within them. Energy transfers are not 100% efficient however, and eventually the majority of the energy that was initially present in the organic compounds manufactured by an ecosystem's producers is lost to the environment as heat. Although it is almost impossible to measure heat loss from an ecosystem, ecologists can estimate energy dynamics by measuring the biomass of successive trophic levels or by measuring the change in mass of detritus over time.

Driving Question

How quickly does organic matter decompose in an ecosystem, and how much energy is transferred from detritus to the organisms that feed upon it?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- Carbon dioxide gas sensor
- Sensor extension cable
- EcoChamber™ container, with lid and stoppers
- Electronic balance, centigram (at least 1 per class)
- Weigh boat
- Plastic pipet, 1-mL
- Disposable gloves
- Small knife (for cutting fruit)
- Filter paper or coffee filter (9 cm diameter)
- Yeast suspension or water, 5 mL
- Mealworms, 20
- Detritus (organic material such as apples and banana peels), approximately 60 g

Safety

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

- Wear safety goggles at all times
- Use caution while handling decomposing organic matter and detritivores. Wear disposable gloves while handling ecosystem components and wash your hands immediately following ecosystem setup and measuring the mass of materials.
- Handle living organisms with care.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

Part 1 – System setup and carbon dioxide gas concentration monitoring

1. Put on your safety goggles and disposable gloves.

2. Copy Table 1 into your lab notebook and determine which of the following systems your group is responsible for.

   NOTE: At the end of the four-day investigation, you will need to gather data from other groups and your teacher to complete the table and make comparisons between all of the systems.

Table 1: Gravimetric analysis of EcoChamber container components

System Components		Initial Mass (g) of Detritus Components and Detritivore Population			Final Mass (g) of Detritus Components and Detritivore Population		
		Apple	Banana Peels	*T. molitor* larvae	Apple	Banana Peels	*T. molitor* larvae
A	Detritus + yeast						
B	Detritus + T. molitor larvae						
C	Detritus + yeast + T. molitor larvae						
Ctrl 1	Unwrapped detritus						
Ctrl 2	Wrapped detritus						

3. Place a piece of filter paper flat on the bottom of an EcoChamber container. Soak the paper with 5 mL of water or yeast suspension depending on the setup your group has been assigned.

4. Obtain the detritus material for the EcoChamber container and measure the initial mass of each component (record the number of the balance you use and always make your measurements on this balance):

   a. Place a weigh boat on the electronic balance and tare the balance. Add apple pieces to the weigh boat until you have approximately 30 grams of material. Record the collective mass of the apple pieces in your lab notebook.

   b. Add the apple pieces to the EcoChamber container on top of the filter paper.

   c. Obtain approximately 30 grams of cut banana peels. Record the collective mass of the material in your notebook and add the banana peels to the EcoChamber container.

5. Obtain the detritivore population for the chamber—20 mealworms is an adequate population. Measure and record the collective mass of the detritivore population before placing the worms in the chamber.

   NOTE: Add mealworms only to systems B and C.

6. Connect a carbon dioxide gas sensor to the data collection system and calibrate the sensor.

7. Create a display of the sensor measurement in ppm versus Time in minutes, and adjust the sample rate to one sample every 5 minutes. If possible, set an auto-stop condition for four hours. Place the sensor into one of the openings of the lid of the EcoChamber container.

   NOTE: Since data collection will occur over many hours, connect the data collection system to an AC power adapter.

8. Secure the lid on the chamber and seal all openings with rubber stoppers. Place the chamber and data collection system in a location where it will not be disturbed and is away from direct sunlight. Begin recording data.

9. Describe aspects of the carbon cycle present within the model system. Name carbon storages as well as carbon fluxes (or movement) within the system. Do you expect the carbon dioxide gas concentration to change in the chamber? Why or why not?

10. After 4 hours, stop data recording and save the data. Remove the carbon dioxide gas sensor and place a stopper in the open hole. Alternatively, if an auto-stop condition has been set, the gas sensor can remain in the lid until you return to the classroom after 24 hours.

11. Draw or print a record of the change in carbon dioxide concentration within the EcoChamber container.

Part 2 – Gravimetric Analysis and Estimation of Energy Transfers

12. After 24 hours, record observations of the components of your EcoChamber container.

    NOTE: If you have not already done so, disconnect the carbon dioxide gas sensor from the data collection system. For the next part of the investigation, keep the lid off the chamber; the chamber will remain open to the air.

13. Leave the chamber in a location away from direct sunlight for three additional days. Re-wet the filter paper with 5 mL of water each day.

14. Ecologists differentiate between "fresh" mass (total mass of material) and *biomass* (the mass of tissue present in an organism). The biomass is the collective mass of the organic compounds found in an organism's tissues: carbohydrates, proteins, and fats. These molecules store a certain amount of energy (measured as kilocalories, or kcal) and ecologists attempt to determine energy transfers by monitoring changes in biomass and relating these changes to the energy contained in that biomass.

    a. In general, if the apple pieces have a mass of 30 grams, the biomass of the apple pieces is approximately 5 grams. Explain why the total mass of apple pieces is not the same as the biomass of the sample.

    b. Carbohydrates and proteins contain 4 kcal per gram and fats contain 9 kcal per gram. Which of these organic compounds is the primary component of apples? Estimate the energy, in kcal, contained in 30 g of apple pieces.

    c. Mealworm larvae contain more kcal per gram of biomass than apples. Why would larvae biomass contain more energy than apple biomass?

15. Copy Table 2 into your lab notebook.

Table 2: Determining the energy content of detritus materials and detritivores

System C Components	Dry Matter (DM)[1] (%)	Gross Energy[1] (MJ/kg DM)	Energy Content per Gram of Fresh Mass (kcal/g)[2]	Total Energy Content of the Initial Mass of Each Sample (kcal)
Apple pieces			0.52	
Banana peels	RECORD ANSWERS & DATA IN YOUR NOTEBOOK.			
Mealworms[3]				

[1]If the website www.feedipedia.org becomes unavailable, use a similar resource to obtain these values.

[2]There are 238 kilocalories in every megajoule (MJ).

[3]If you set up Chamber A, this sample is not applicable to your setup. However, you will use the Energy Content per Gram of Fresh Mass for mealworms in a later question, so you should calculate that value here.

a. Use the information provided in the table for apple pieces to determine the energy content of the apples placed in the EcoChamber container on the first day of the investigation.

b. The website www.feedipedia.org provides nutritional information for a number of animal feed samples, including banana peels and mealworms. The energy content, in megajoules per kilogram (MJ/kg), is reported for the biomass (the dry matter) of the sample. The biomass can be determined by knowing the percentage of dry matter (DM) in the fresh sample. Use the information from the website to complete the table and then use dimensional analysis to determine the energy content per gram (kcal per gram of fresh mass) and the total energy content of banana peels and mealworms.

16. On Day 4 of the investigation, record observations of the chamber and measure the final mass of the detritus components and mealworms (if applicable). Record the data in Table 1.

17. For each item measured, calculate the percent change in mass that occurred over 4 days. Share this data with the class.

18. Create a table to organize class data from the three different experimental systems (A–C), as well as the two controls set up by your teacher. Record the average percent change in mass for each component, and the rate of change in carbon dioxide during the first 4 hours of the investigation.

19. Which contributes more to decomposition, decomposers (such as yeast) or detritivores (such as mealworms)? What evidence do you have to support your claim?

20. What purpose do the controls serve in this investigation? What can be concluded from a comparison of the controls and the experimental setups?

21. Do the results of System C indicate that decomposers affect detritivores? What evidence supports your answer?

22. Use data from one group's setup of System C (or an average from multiple setups of System C) and the conversion factors established in Table 2 to calculate the energy transfer in the system over the four days.

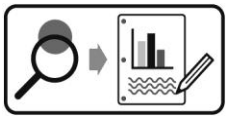
Table 3: Estimate of energy transfer in a model system containing detritus, decomposers, and detritivores

System C Ecosystem Component	Energy Content per Gram of Fresh Mass (kcal/g)	Change in Mass over 4 Days (g)	Change in Energy Content (kcal)
Detritus 1: Apple	0.52		
Detritus 2: Banana skin	RECORD ANSWERS & DATA IN YOUR NOTEBOOK.		
Detritivore: *T. monitor* larvae			

23. Calculate the ecological efficiency of the mealworms in System C during the Initial Investigation. Ecological efficiency can be calculated from the ratio of energy gained in the detritivore pool over energy lost from the detrital pool.

Design and Conduct an Experiment

The Initial Investigation offers insight into the processing of detritus within a simple ecosystem. In actual ecosystems, a significant amount of biomass and energy from primary production is transferred to the detrital pool. This detritus serves as a trophic base for both detritivores and decomposers. A number of biotic or abiotic factors can affect this trophic level. How can you change a component or condition of the model system to test factors that affect decomposition in ecosystems?



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

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

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

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

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

Synthesis Questions

1. How do the Laws of Thermodynamics relate to the model decomposition systems that were set up in the Initial Investigation?

2. With regard to measuring energy dynamics, describe at least three limitations of the model systems used in the Initial Investigation.

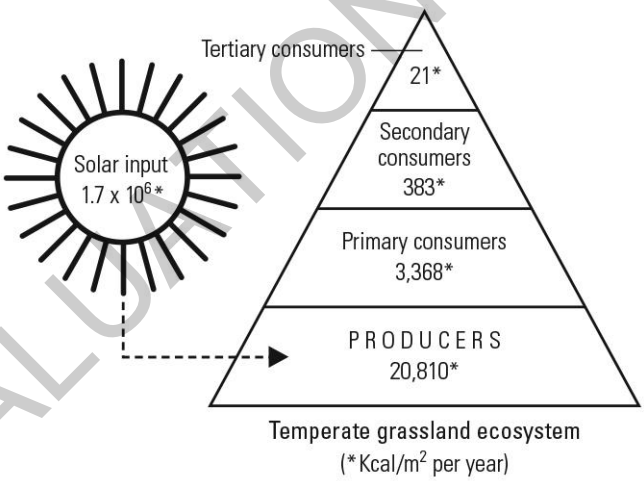
3. Movement of matter and energy occurs in all ecosystems. Often a model of this transfer is presented as a diagram, a food web for example.

   a. Describe the movement of matter and energy in ecosystems. Include in your description how different types of organisms acquire the free energy they need to sustain life, and why an ecosystem requires a constant input of free energy.

   b. Food webs and trophic level pyramids typically do not include decomposers or detritivores in the illustration. Propose an explanation for why this is the case.

4. The diagram below provides information about the amount of solar energy entering an ecosystem, the net primary productivity of the ecosystem's producers, and the amount of energy at each successive trophic level.

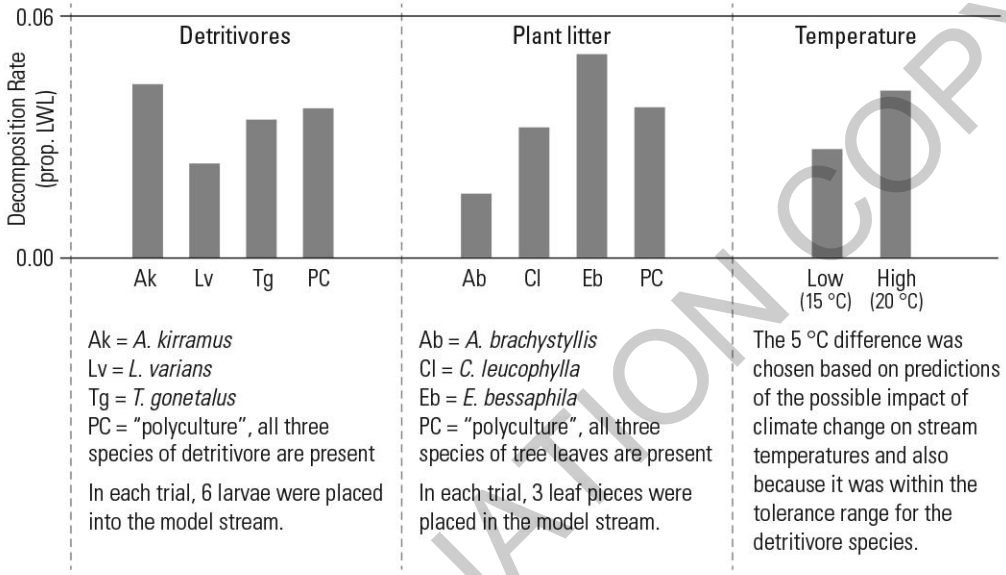
   a. Calculate the ecological efficiency of energy transfer between each successive trophic level and explain why ecological efficiency is often less than 10%.



   b. Identify a biome that would have a higher net primary productivity (NPP) than a grassland ecosystem and a biome that would have a lower NPP. For each of your choices, explain biotic or abiotic aspects of the biome that affect its NPP. How would decomposition rates compare for these biomes?

5. Researchers carried out numerous experiments designed to study decomposition rates under a variety of conditions.[9] The experiments were designed to model the decomposition that occurs in an Australian stream. Three different leaf-shredding detritivore species were taken from the stream for use in the study, leaves from three native riparian tree species were collected for use as plant litter, and the researchers carried out experiments in model stream habitats at two different temperatures (typical stream temperature and 5 °C warmer). In total, almost 200 experiments were performed by the researchers.

   The following diagram illustrates the results of these experiments. The bars show the mean detritivore-mediated decomposition rates measured as a proportion of leaf weight loss per detritivore (prop. LWL). Detritivores were present in all trials; the species identity or richness of detritivores was manipulated for some trials, as was the species identity or richness of plant litter. Each manipulation of detritivores or plant litter species was tested at two temperatures. Researchers performed a variety of statistical analyses to the data and the resulting probability values *p* are provided below each graph.



[9] Boyero, L.; Bradley, J.C.; Bastian, M; Pearson, R.G. Biotic vs. Abiotic Control of Decomposition: A Comparison of the Effects of Simulated Extinctions and Changes in Temperature. *PLoS ONE* (Impact Factor: 3.73). 01/2014; 9(1):e87426. DOI:10.1371/journal.pone.0087426 http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0087426 (accessed July 3, 2014).

Table 4: Statistical analysis of decomposition rates under different conditions of detritivore, leaf litter, and temperature

	Statistical Comparisons	
	Species Identity: ***compared decomposition rate by Ak, Lv, and Tg and compared rates of decomposition of Ab, Cl, and Eb***	**Species Richness:** ***compared the polyculture decomposition rates to the single-species decomposition rates***
Detritivores	$p < 0.001$	$p = 0.79$
Plant litter	$p < 0.001$	$p = 0.83$
	Compared decomposition rates in low temperature and high temperature stream water	
Temperature	$p < 0.001$	

a. Identify two scientific questions that the researchers were likely investigating with these experiments.

b. For one of the scientific questions you describe, propose an experimental design for the experiment used to test the question. Be sure to make clear the independent and dependent variables, as well as the constant variables for the proposed experimental design.

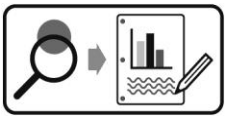
c. What can be concluded from the provided probability values?

d. For either detritivores or plant litter, identify a characteristic of the organism or leaves that may contribute to faster or slower decomposition.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

The Initial Investigation offers insight into the processing of detritus within a simple ecosystem. In actual ecosystems, a significant amount of biomass and energy from primary production is transferred to the detrital pool. This detritus serves as a trophic base for both detritivores and decomposers. A number of biotic or abiotic factors can affect this trophic level. How can you change a component or condition of the model system to test factors that affect decomposition in ecosystems?



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of energy dynamics and decomposition, what environmental factors (abiotic or biotic) could affect this process?

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

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

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

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

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

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

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

_____

_____

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

_____

_____

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

_____

_____

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

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

18. ARTIFICIAL SELECTION

Background

Artificial selection serves many practical purposes. Animal breeders for centuries have identified favorable traits in domesticated dogs, pigeons, and even mice, with the intention of carrying those traits on through subsequent generations. Furthermore, enhancement of certain characteristics such as size, speed, temperament, or coat color was obtained through deliberate breeding of animals selected for those desirable qualities. Underlying the practice is the understanding that in a population, extremes at each end of the distribution are likely to be found.

As with animals, plants too carry traits that are accessible to the selection process. In many ways, artificial selection draws parallels to natural selection, the process by which species change over time, as a mechanism for producing genetic change within a population.

Driving Question

Do heritable traits, once selected, lead to directional change in the gene pool?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Wisconsin Fast Plants® seeds (18), standard
- Seed-starting soil or germinating mix (such as Jiffy Mix®)
- Fertilizer, Osmocote™ pellets (24) or a water-soluble fertilizer
- Wicking material (3), #18 nylon mason twine
- Recycled plastic bottles (3), 0.5 L to 1 L
- Soda bottle cap with hole (3)
- Plant vermiculite
- Labeling tape and markers
- Black plastic to cover the water reservoir (3) (optional)
- Water in a rinse bottle
- Lighting system with fluorescent lights (shared by the class)
- Bee sticks or cotton applicators (3)
- Plastic plant labels (3)
- Scissors
- 12-inch ruler
- Stakes and holders, as needed (wooden splints and plastic straws)
- Dechlorinated water or nutrient solution (for the reservoir)
- Hand-held plastic magnifier
- Petri dish lid
- Paper envelope, small

Safety

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

- Wear safety goggles at all times.
- When handling scissors or other tools to cut plastic bottles, use them with care and work on a surface that can support sharp instruments.
- Keep water away from electrical outlets and all electronic equipment.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles.

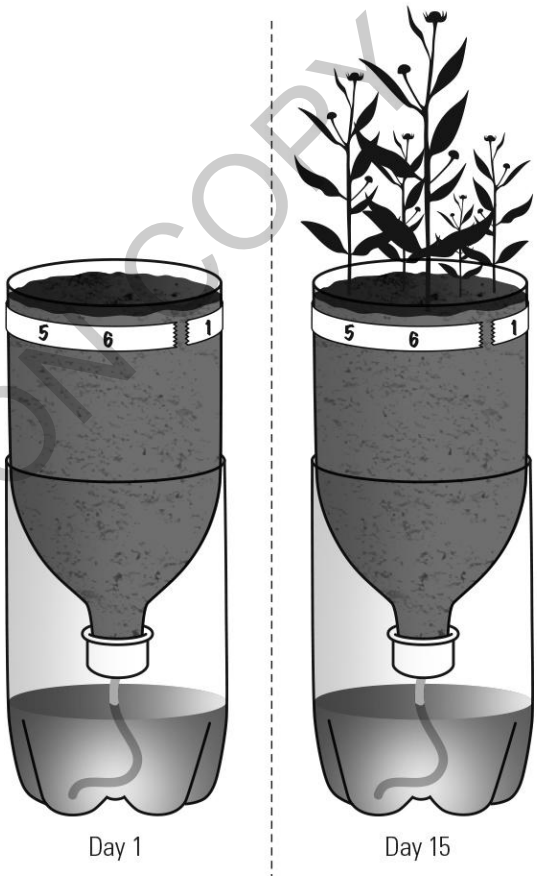
2. Before preparing the growing systems from reused plastic bottles, refer to the Wisconsin Fast Plants website (www.fastplants.org) for full instructions. A search of the website for "bottle growing system" will direct you to numerous relevant resources. The digital resource library also offers resources, such as this video: https://www.youtube.com/watch?v=eEOCRz0j6iA&feature=youtu.be.

3. Prepare the three growing systems, using recycled bottles that have been cut:

   a. Tie a knot in the wicking material, thoroughly wet the wick, and thread it through the hole of the bottle top. Screw the top onto the bottle and check that the wick will touch the bottom of the reservoir when the top piece of the growing system is placed (inverted) into the reservoir.

   b. Following instructions from the Wisconsin Fast Plants website resources—or your teacher's instructions, add starting soil, vermiculite, fertilizer (if not added to the reservoir), water, and seeds to each growing system. As you add the contents, be sure the wick remains in the center of the soil and does not extend above the soil surface.

      NOTE: **Before planting seeds** *in the growing system, wrap a piece of labeling tape around the cut edge of the top piece of each growing system. For the first one, write the numbers 1–6 on the tape, equally spacing the numbers around the circumference of the bottle. The numbers will be used to identify the seeds (plants) throughout the investigation.*



      Repeat the process, labeling the top piece of the second bottle with the numbers 7–12 and of the third bottle with 13–18.

   c. After adding the seeds, cover them with vermiculite and use the rinse bottle to wet the contents until water begins to drip from the wick.

   d. Add dechlorinated tap water or nutrient solution (water with fertilizer) to the water reservoir of the growing system. Place the top piece of the growing system into the reservoir to complete the system.

   e. Label three plastic plant labels with your group identification and the planting date; insert one label into the soil of each system.

4. Place your growing systems under the lighting system set up by your teacher.

   NOTE: The distance between the light source and the plants will need to be adjusted as the plants grow. Also, top off the water reservoir as needed due to water loss through evaporation.

5. Copy Tables 1 and 2 into your lab notebook to organize your observations (which are *not* limited to the "expected events") of the plants over the next several weeks of data collection. Detailed observations and careful measurement are an important aspect of this lab, and a full page or more of your notebook should be devoted to each data table.

   Notable events are likely to occur daily in the first part of the plants' life cycle. In the later weeks you may only need to make observations once or twice a week. Be sure to record specific seed (plant) ID numbers for certain observations.

Table 1: Observing growth and milestones in the Fast Plant life cycle

Week	Expected Events	Observation Date	Observations
1	Opening of cotyledons Emergence of true leaves		
2	Significant growth Development of flowers		
3	Select traits and plants for breeding Cross-pollination		
4–5	Appearance of seed pods		
6	Plant drying and seed harvesting		

Table 2: Quantitative trait measurement and breeding chart

Seed/Plant Number	Day 15 Height (cm)	Additional Quantitative Trait[1]: _____	Selected Trait: _____ Cross-pollinated?	Number of Pods	Number of Seeds Harvested
1					
2					
3					
4					
.					
.					
.					
16					
17					
18					

[1]Additional quantitative traits can include: germination time, size of cotyledons, time of appearance of true leaves, flowers, or seed pods, trichome density, number of leaves or distance between leaves (internode length), plant height at first flower or plant height at first seed pods, length of pods, and number of seeds per pod.

6. After observing the growth and development of the plants for two weeks, describe the variation you observe in the traits of the plants in your growing systems.

7. Measure the height of each of the individual plants on Day 15.

8. Create a histogram (frequency graph) that shows the height distribution at Day 15 for your population of Fast Plants®.

9. In your lab notebook, calculate and organize in a table the appropriate descriptive statistics about plant height for the first generation: mean, median, range, standard deviation, and standard error.

10. Plotting the means of two populations along with ±2 standard errors of the mean (SEM) is a good starting point to determine if any difference in the means is significant. Create such a graph in your lab notebook. Include the mean of your group's population of plants and the mean of a population of plants grown by another group in class.

? 11. Are the means of the two populations the same? If not, is the difference significant? Provide evidence to support your claim.

12. By Day 15 you need to make a selection decision, that is, you need to determine which trait to use as a criterion for selecting certain plants for cross-pollination. These selected plants will serve as the parents for the second generation. For example, one might choose to cross-pollinate plants that are 21 cm or taller to see if breeding the tallest plants changes the mean plant height in the second generation.

    Once you determine the trait you will select for, record this, as well as which plants you will keep to use for cross-pollination, in Table 2. To prevent unwanted pollination, remove and discard the non-selected plants from the growing systems.

13. When several flowers are present on each of the selected plants, cross-pollinate the plants with a single bee stick or a cotton applicator. Transfer pollen from the anthers of one plant to the stigma of another plant. Collect and distribute pollen from every flower on every selected plant. Repeat the cross-pollination procedure for 3 consecutive days.

    NOTE: Once the seed pods start to develop, trim away any additional flowers that grow on the plants. This will allow resources to be directed to the developing seeds. Replenish the water reservoirs as the seeds develop.

14. At Day 35, pour out the water from the reservoirs and allow the plants to dry for 3–5 days.

15. After the plants and seedpods have dried, harvest the seeds by breaking open the seedpods into the lid of a small Petri dish. Store the seeds in a small paper bag or envelope, labeled with your group identification and the date of harvest.

    NOTE: Remove the material from the growing systems and rinse the bottles. Keep the bottles to use for growing the second generation plants.

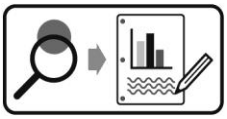
16. You now have a population of second-generation seeds. Plant the seeds as you did before, using fresh starting soil and other materials, and monitor the growth of the 2nd generation, keeping detailed records of your observations and measurements. Create an appropriate histogram for the data and calculate the appropriate descriptive statistics regarding the selected trait in the 2nd generation.

? 17. Did the selection process result in a significant change in phenotype distribution in the second generation compared to the first generation? Provide evidence to support your claim.

? 18. What factors were controlled over the duration of the 6-week experiment?

Design and Conduct an Experiment

Once you are experienced in the process of growing, pollinating, and harvesting seeds from Fast Plants, you may want to continue the selection experiment over multiple generations. Alternatively, you may want to test whether environmental conditions, such as acid rain, affect the results of the selection experiment. Yet another option is to explore if there is a relationship between two plant traits, such seedpod size and number of seeds.



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

1. From your observations and your data:
   a. Is your hypothesis for the driving question of your experiment supported? Justify your claim with evidence from your experiment.
   b. Based on the evidence you collected, explain why the results occurred.
2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?
3. Identify any new questions that have arisen as a result of your research.

Synthesis Questions

1. What do descriptive statistics such as the mean, median, range, standard deviation, and standard error tell the experimenter?
2. Refer to the graphs below. The data is from a study during which the investigators used artificial selection, selecting fish for breeding on the basis of brain size to create two distinctly different F_2 generations.[10] Figure 1 displays the change in brain size as a result of artificial selection, and the average brain size of four populations created for the purpose of the study: smaller-brained and larger-brained females, and smaller-brained and larger-brained males. The researchers developed a "learning test" to measure the cognitive ability of the fish and compared females of different brain sizes and males of different brain sizes.

Figure 1 / Artificial selection for small and large brain size

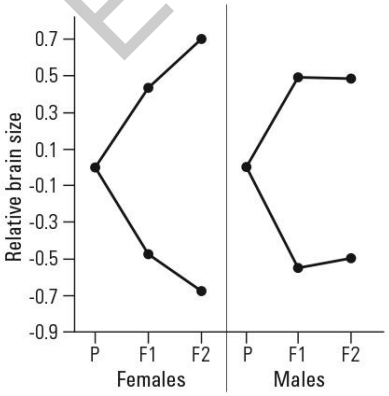


Figure 2 / Cognitive ability test

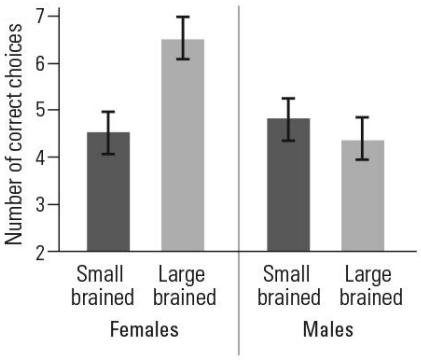
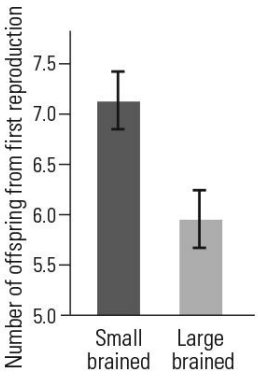


Figure 3 / Female fecundity



[10] Kotrschal et al., Artificial Selection on Relative Brain Size in the Guppy Reveals Costs and Benefits of Evolving a Larger Brain, Current Biology (2013), http://dx.doi.org/10.1016/j.cub.2012.11.058.

a. The scientists concluded that there is a correlation between brain size and cognitive ability for female guppies but that the correlation does not hold true for male guppies. Describe evidence from Figure 2 that supports the scientists' conclusion.

b. The scientists also compared the mean number of offspring produced by each category of female at first reproduction. The data are shown in Figure 3. The scientists used artificial selection in the laboratory to increase brain size in females. Do you predict natural selection will favor large-brained females? Provide evidence to support your prediction.

3. Quantitative traits in Wisconsin Fast Plants include plant height, number of seeds produced per seedpod, and time to flower.

   a. For humans, which is a quantitative trait, blood type or blood cholesterol level? Explain your reasoning for your answer.

   b. Many quantitative traits are polygenic. Explain the concept of a polygenic trait.

4. Since the advent of agriculture in ancient civilizations, humans have modified crops and domesticated animals through selective breeding, or more recently through biotechnology (genetic modification).

   a. Compare and contrast artificial selection and recombinant DNA technology.

   b. Considering the advantages and disadvantages of each technique, identify two instances in which artificial selection would be advantageous and two instances in which genetic modification technology would be advantageous for producing certain desired traits in organisms.

5. Natural selection works much like artificial selection.

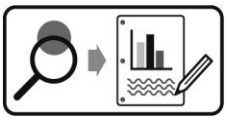
   a. Explain how Darwin's observations of artificial selection influenced his proposed theory of natural selection.

   b. Identify 3 abiotic factors and 3 biotic factors that influence natural selection. State a hypothesis for how EACH abiotic or biotic factor would have an effect on natural selection.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

Once you are experienced in the process of growing, pollinating, and harvesting seeds from Fast Plants, you may want to continue the selection experiment over multiple generations. Alternatively, you may want to test whether environmental conditions, such as acid rain, affect the results of the selection experiment. Yet another option is to explore if there is a relationship between two plant traits, such seedpod size and number of seeds.



Develop and conduct your experiment using the following guide.

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

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

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

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

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

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

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

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

_____

_____

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

_____

_____

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

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

19. BLAST BIOINFORMATICS

Background

Hemoglobin is an important protein found in the red blood cells of many species. Its heme groups bind to oxygen molecules, delivering oxygen to cells and removing carbon dioxide from the body. Hemoglobin is a protein that exhibits quaternary structure: it consists of two alpha chains and two beta chains. In this investigation you will analyze the DNA sequence of the gene that codes for the beta chain of hemoglobin—also known as *beta globin*. The gene for this protein is symbolized by "*HBB*." You will compare the *HBB* gene of five mammalian species to determine their evolutionary relatedness.

Much of today's biological research involves DNA sequencing. Sometimes scientists sequence the entire genome of an organism, other times they are interested in specific genes and variations in these genes. Also of importance is knowledge of the amino acid sequences of proteins in organisms to develop a better understanding of the structures and functions of these proteins. DNA and protein sequences are uploaded to a database that can be accessed by other scientists (and by non-scientists). The National Library of Medicine (NLM), part of the National Center for Biotechnology Information (NCBI), maintains this database, which currently contains thousands of DNA sequences and corresponding protein sequences for thousands of species.

For example, the database contains more than 2000 sequences for beta globin. Scientists employ a computer program called BLAST® (Basic Local Alignment Search Tool) to search NCBI's database to match a nucleotide or amino acid sequence of interest to a specific species. They also use BLAST to align two or more sequences to determine the amount of similarity between them. The NCBI database and BLAST have become invaluable tools for evolutionary biologists.

Driving Question

What species are most closely related and least closely related to the chimpanzee?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Computer with Internet access
- DNA Sequences Worksheet
- ABI BLAST Sequences.docx
- Highlighter
- Scissors (optional)
- Ruler or large index cards

Safety

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

- Never eat or drink around computer equipment.

Initial Investigation

Complete the following lab procedure and analysis before designing your own experiment. Record observations, data, and explanations in your lab notebook.

Manual comparison of mammalian DNA sequences of the HBB gene

1. Obtain a copy of the DNA Sequences Worksheet. This worksheet contains nucleotide sequences for the beta globin gene (*HBB*) of five mammalian species. Compare Species A, the chimpanzee (*Pan troglodytes*), to four other species, as follows.

   To complete the comparison of Species A to the other species, first copy the following data table into your lab notebook.

Table 1: Manual and computer database gene and protein comparison of chimpanzees to other mammals

Species	Common Name	Scientific Name	Number of Nucleotide Differences	BLAST Ident[4] (%): *HBB* Gene Comparison[1]	BLAST Ident[4] (%): Beta Globin Protein Comparison[2]
A	Chimpanzee				
B					not available[3]
C					90
D					85
E					82

[1]Gene accession number: FJ788228.1

[2]Protein accession number: P68873.2

[3]The full beta globin sequence has not been published for this species.

[4]"Ident" refers to the percentage of similarity of aspects of the sequences (nucleotide or protein).

2. Determine the number of nucleotide differences between Species A and Species B.

   a. Use a ruler or index card to move along the sequences of the two species one letter (one nucleotide) at a time, or one codon at a time.

   b. If Species B has a nucleotide that differs from A, highlight that letter in the sequence of Species B.

   c. Continue to compare sequences for each row of nucleotides, until you reach the final "A" (adenine), the 100th nucleotide). Then count and record the total number of differences present in the DNA sequences.

      NOTE: A dash instead of a letter in a species' sequence indicates an unknown base and should ***not*** *be counted as a difference.*

3. Repeat the steps above to compare Species A to Species C. Then continue with the remaining comparisons: A to D, and A to E. You may fold the paper or cut out sequence A to make the comparisons easier.

4. Confirm the number of differences you found with your classmates and reconcile any variations in the counts. Adjust the numbers recorded in the data table if necessary.

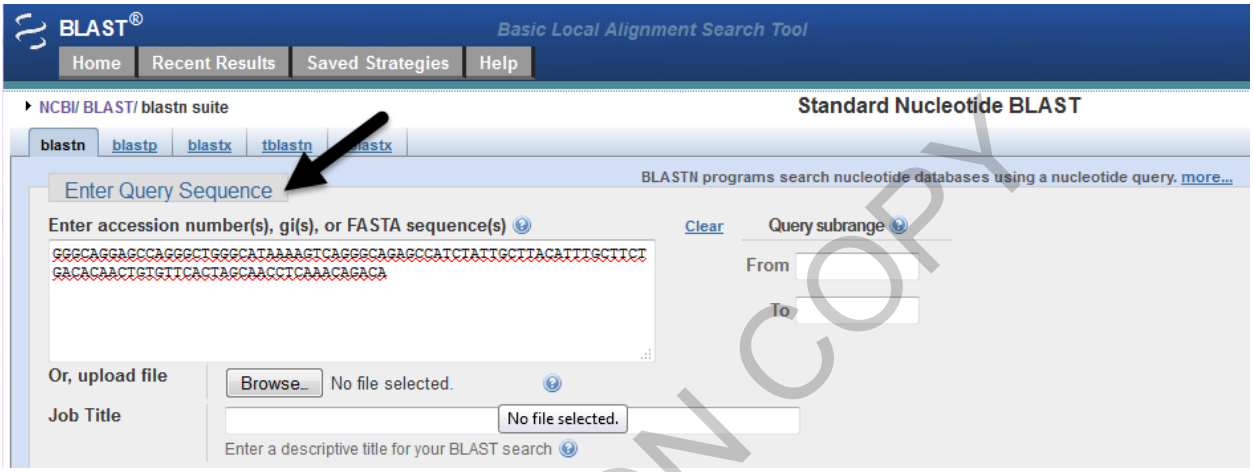
? 5. The four "unknown" species (B–E) on the worksheet are, in no particular order: cow, Norway rat, pig-tailed macaque, and dog. Which species do you predict has the least number of differences in the *HBB* gene compared to chimpanzees? Which species do you predict has the greatest number of differences? Provide an explanation for each of your predictions.

BLAST comparison of mammalian DNA sequences of the HBB gene

6. Go to the BLAST website: http://blast.ncbi.nlm.nih.gov/Blast.cgi. Select "nucleotide blast" from the "Basic BLAST" menu in the middle of the page.

IDENTIFYING SPECIES A

7. Open the digital copy of the BLAST Sequences Worksheet (ABI BLAST Sequences.docx). Copy the *HBB* sequence for Species A and paste the sequence into the query box of the nucleotide BLAST page.



8. Scroll down to "Program Selection" and select the option to optimize for "Somewhat similar sequences." Then click the "BLAST" button.

   NOTE: The BLAST program searches through thousands of sequences contained in a database for the best species match for the partial HBB *sequence you entered into the query.*

? 9. In the BLAST report generated from the search, scroll past the graphic summary to the "Descriptions" table. Note that *Homo sapiens*, *Gorilla gorilla*, and *Pan troglodytes* all have alignments with a "100%" Ident value, meaning that these species have no nucleotide differences in their *HBB* genes. From an evolutionary perspective, provide an explanation for this fact.

10. Click on the accession number for the first *Pan troglodytes* link: FJ788228.1. This takes you to a page with a wealth of information regarding the gene of interest, such as the number of base pairs and the scientific article where the gene sequence was originally published.

11. Scroll down to "FEATURES."

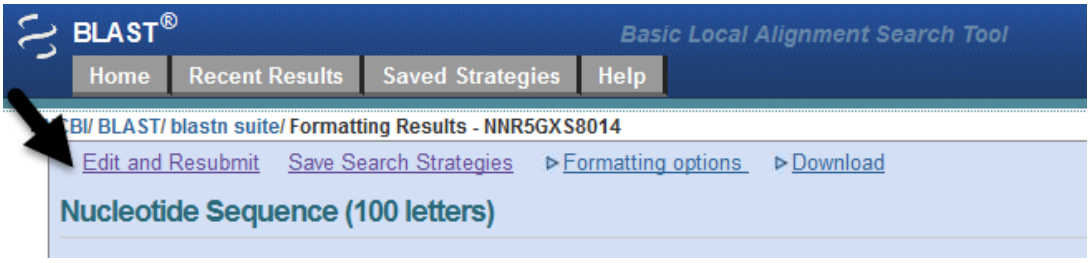
    - Click on "gene" and observe the section highlighted in the nucleotide sequence at the bottom of the page (under "ORIGIN").
    - Click on "mRNA" and observe the change in the nucleotide selection that is highlighted.
    - Click on the first "exon" link. Then click on the second "exon" link.

? 12. a. Why would the gene have a different number of nitrogen bases than mRNA?

    b. What do you observe when you compare the highlighted regions for the exons to the highlighted region for mRNA? What is the relationship between exons and mRNA?

IDENTIFYING SPECIES B THROUGH E AND COMPARISON WITH SPECIES A

13. The browser page for the nucleotide sequence for *Pan troglodytes* opened on either a new tab or a new window within the Internet browser. Return to the NCBI BLAST search report and click the "Edit and Resubmit" link at the top left of the page.



14. Copy and paste the nucleotide sequence for Species B from the BLAST Sequences Worksheet into the query box of the nucleotide BLAST page. Click "BLAST" to initiate a new search.

15. Scroll to the Descriptions table and click on the first accession number. Find the "SOURCE" line on the gene information page that opens after clicking the accession number. In Table 1 in your lab notebook, record the common name and scientific name of Species B.

16. On the gene information page for Species B, under "Analyze this sequence" (on the right side of the page), choose "Run BLAST." The query box will appear with the accession number of Species B. Now you can compare the entire *HBB* gene of Species A and Species B.

    Click the check box "Align two or more sequences" and type the accession number for the chimpanzee gene, FJ788228.1, into the "Enter Subject Sequence" area that appears. Click "BLAST" to generate the report.

17. Find the Ident value which indicates the amount of similarity in the two sequences being aligned. Record this value in Table 1.

18. To begin the comparison with the next species, click "Edit and Resubmit" and uncheck the "Align two or more sequences box." Copy and paste the nucleotide sequence of Species C from the BLAST Sequences Worksheet into the query box and initiate the BLAST search to find the identify of Species C.

19. Click on the first accession link and record the common name and scientific name of Species C. Then choose "Run BLAST" to align the sequences of Species A and C, as you did for the Species A and B comparison. Record the Ident value.

20. Repeat the process to identify Species D and E, and perform the alignments of each of these species with Species A.

    NOTE: For Species D, click on the 2nd accession number for the "adult beta globin gene."

21. In the comparison you did manually, you compared sequences 100 nucleotides in length; these sequences were just part of the *HBB* gene. The BLAST program compared over 1000 nucleotides of the *HBB* genes in these species. Do the results of your manual comparison agree with the results of the computer-generated alignment? Explain your answer.

22. What are the advantages of using a computer program over manual comparison of DNA sequences?

23. Now that you know the identities of Species B–E:

    a. Which of the four species is most closely related to the chimpanzee? Was your prediction correct? Do the results make sense based on other factors, such as morphology? Explain your answer.

    b. Which of the four species is least closely related to the chimpanzee? Was your prediction correct? Do the results make sense based on other factors? Explain your answer.

24. For the protein comparison Ident values provided in Table 1, BLAST was used to compare the amino acid sequence of chimpanzee beta globin to the amino acid sequences of beta globin proteins in the other species. Which have a greater similarity between two species: gene sequences or protein amino acid sequences? Explain why the percent similarity is not the same for genes and proteins.

COMPARING PROTEINS AND VISUALIZING EVOLUTIONARY RELATIONSHIPS

25. Use the NCBI website to find the beta globin sequences for two additional species: Atlantic salmon and minke whale, and then compare them to the sequence for the chimpanzee. For each of the additional species:

    a. Go to the NCBI homepage: http://www.ncbi.nlm.nih.gov/. In the search dropdown menu at the top, change "All Databases" to "Protein."

    b. In the search field, type "beta globin" and the species name and choose "Search."

    c. On the search results page, click on the FASTA link to see the amino acid sequence. Copy and paste the sequence into the space provided on page 2 of the digital copy of the BLAST Sequences Worksheet, making sure the letters are adjacent to each other, that is, they should not be separated by spaces or "returns" and are in a single paragraph.

    d. Find the "Run BLAST" option under "Analyze this sequence." Click on the "Align two or more sequences" box, as before, and enter this protein identification number for the chimpanzee into the Subject Sequence box: P68873.2. Click "BLAST." Record the Ident value for the comparison.

26. Which of the two species is least similar to chimpanzee, based on the beta globin comparison? Is this surprising? Explain your answer.

27. Ident values are useful for comparing species and inferring evolutionary relationships. However, *phylogenetic trees* or *cladograms* provide a more complete picture. Programs used to create these diagrams compare all selected species to one another, not just one species to others. To create a phylogenetic tree, go to www.phylogeny.fr.[11] Choose the "One Click" phylogenetic analysis option.

28. Copy and paste the entire text of beta globin sequences from the worksheet into the space provided on the phylogeny website. Then click "Submit." Save your results—the phylogenetic tree—as a PNG or PDF (select the "Download the tree" option just below the phylogenetic tree) and print a record for your lab notebook.

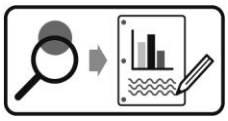
    NOTE: Be sure to include the " >[name]" *in addition to the letters symbolizing the amino acids when you copy and paste. Also, the sequence for each species must be separated by a blank line.*

29. What can you conclude regarding the evolutionary relationships between the Atlantic salmon, minke whale, and Species A–E?

[11] Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M., Gascuel O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*. **2008** Jul 1; *36* (Web Server Issue):W465-9. Epub 2008 Apr 19. PubMed: http://www.ncbi.nlm.nih.gov/pubmed?cmd=Retrieve&list_uids=18424797 (accessed May 28, 2014).

Design and Conduct an Experiment

Now that you are familiar with the tools available for comparing gene and protein sequences, you can investigate a question of your own related to the evolutionary relationships among species. Identify a set of species you are interested in investigating with regard to their evolutionary history.



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

Design and Conduct an Experiment: Data Analysis

1. Describe the evolutionary relationships between the species you investigated. Does the data support your hypothesis? Justify your claim with evidence.

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

Synthesis Questions

1. Differences in the nucleotide sequences of a gene in different species are the result of mutations that occur over time.

   a. Identify and describe three different types of mutations.

   b. Describe the possible consequence(s) of each type of mutation.

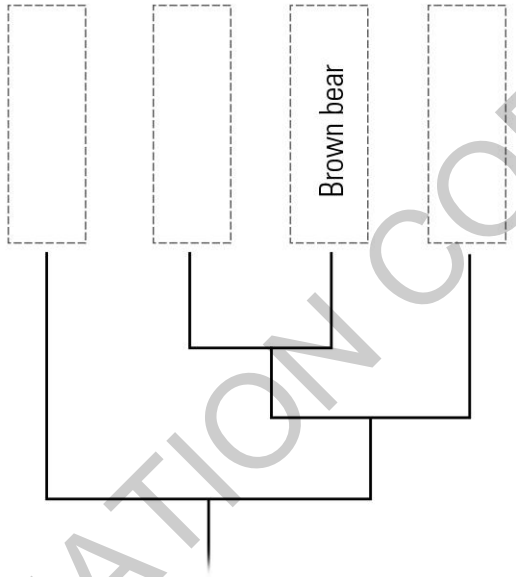
   c. Although genes from *Homo sapiens* (humans) and *Drosophila melanogaster* (fruit fly) differ significantly, there are "conserved" regions, that is, nucleotide sequences within genes that have not changed much over time. Why would conserved regions be of particular interest to scientists?

2. A student is interested in the evolutionary history of kingdom Fungi. For her investigation, she plans to use the NCBI protein database and BLAST to compare a number of fungal species to a variety of species from kingdom Plantae and kingdom Animalia. Of the following proteins, which one would you recommend the student use for her investigation: catalase, rubisco (RuBP), or hemoglobin? Provide an explanation for your choice.

3. The table below lists the first twenty four amino acids of the ATP synthase protein in each bear species. Analyze the data and complete the cladogram. Provide an explanation for your placement of each species on the cladogram.

Table 2: Comparing the ATP synthase proteins of bears

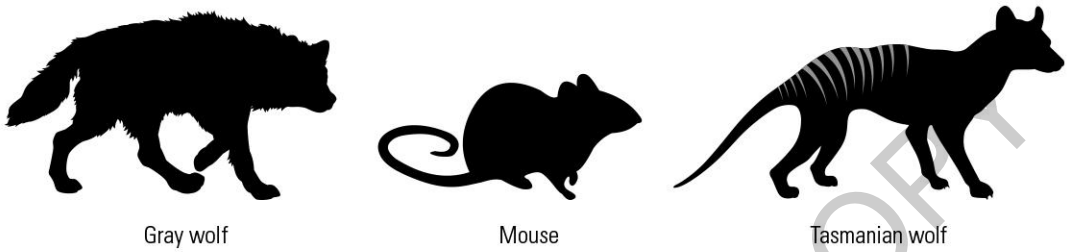
Common Name	Scientific Name	Amino Acid Sequence
Brown bear	*Ursus arctos*	MNENLFTSFITPTMVGIPIVLLII
American black bear	*Ursus americanus*	MNESLFTSFITPTMMGIPIVVLII
Giant panda	*Ailuropoda melanoleuca*	MNENLFASFTTPMMMGVPIVVLII
Polar bear	*Ursus maritimus*	MNENLFTSFITPTMVGIPIVPLII

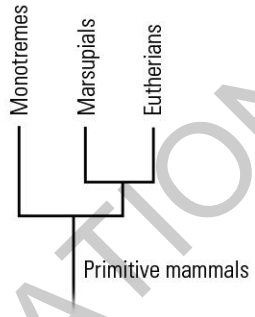


4. Table 3 shows the results from a BLAST comparison of the NADH dehydrogenase protein of the gray wolf with the common mouse and Tasmanian wolf (pictured below). The cladogram provides information regarding the evolution of three mammalian clades.

Table 3: Results from a BLAST comparison

Species	BLAST Ident (%): Similarity to the Gray wolf	Classification
Gray wolf		Eutherian
Mouse	55	Eutherian
Tasmanian wolf	47	Marsupial





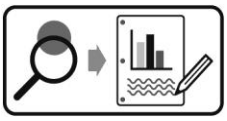
a. Provide an evolutionary explanation for the level of similarity between the gray wolf and the mouse.

b. Provide an evolutionary explanation for the level of similarity between the gray wolf and the Tasmanian wolf.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

Now that you are familiar with the tools available for comparing gene and protein sequences, you can investigate a question of your own related to the evolutionary relationships among species. Identify a set of species you are interested in investigating with regard to their evolutionary history.



Develop and conduct your experiment using the following guide.

1. List the species you are interested in comparing.

2. Create a driving question: develop a testable question for your experiment.

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

4. What gene or protein sequence(s) do you plan to use for the investigation? Describe the function of the gene or protein and indicate why you chose it.

5. What data will be collected, and how will it be collected, to build a phylogenetic tree for the species you're investigating?

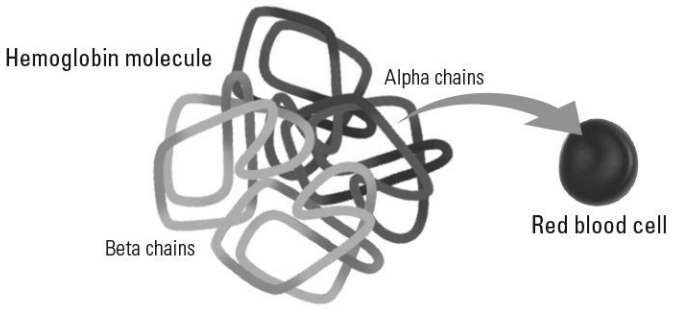
6. Write a testable hypothesis (If…then…).

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

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

DNA Sequences Worksheet

Beta Globin Gene Nucleotide Sequences



Species A	GGGCAGGAG GAGCCATCT TGTGTTCAC	CCAGGGCTG ATTGCTTAC TAGCAACCT	GGCATAAAA ATTTGCTTC CAAACAGAC	GTCAGGGCA TGACACAAC A
Species B	GGGCAGGAG GAGCCATCT TGTGTTCAC	CCAGGGCTG ATTGCTTAC GAGCAACCT	GGCATAAAA ACTTGCTTC CAAACAGAC	GTCAGGGCA TGACACAAC A
Species C	GGGCAAGAT GGGACAGCT CGTGTTCAC	C–AGGGCTG GCTGCTTAC TAGCAACCA	GGCATAAAA ATTTGCTTC CAAACAGAC	GGAAGAACA TGAAACAAC A
Species D	GGGCAGGAG GGGCCAGCT CGTGTTCAC	GCAGGGCTG GCTGCTTAC TAGCA––CA	GGCATAAAA ACTTGCTTC CAAACAGAC	GGAAGAGCT TGACACAAC A
Species E	––GCAGGAG GGATCAGTC TGTGTTGAC	CCAGG–CAG GCTCCTCAC TCACAAAC–	AGCATAAAA ATTTGCTTC –AAACAGAC	GGTGGGGCG TGACATAGT A

19. POPULATION GENETICS

Background

In the early 1930's, Arthur L. Fox was working in his laboratory with a chemical named phenylthiocarbamide (PTC). The chemical was in powder form and as Fox added it to a beaker, some of the powder dispersed through the room to Fox's colleague who complained about the powder's bitter taste. Fox perceived it to be tasteless and debated with his colleague over the issue. The two scientists enlisted others to taste small amounts of the chemical and found that to most people it had a bitter taste but to others it had no taste at all. This began a study in human genetics that has continued into the 21st century.

The *taster* phenotype was discovered to be dominant to the *non-taster* phenotype. While threshold studies have shown that people who are heterozygous (Tt) for the trait have an intermediate phenotype—in other words, the compound may taste less bitter to them than to homozygous dominant individuals—this intermediacy is difficult to identify in the simple test used in this activity. You will be given a piece of paper with a small amount of PTC on it and you will simply determine if you are a taster or a non-taster. The number of homozygous and heterozygous taster genotypes will be derived using an equation commonly applied to studies in population genetics:

$$p^2 + 2pq + q^2 = 1.0$$

where p and q represent the allele frequencies of the dominant and recessive alleles for a particular trait, p^2 and q^2 represent the frequencies of homozygous dominant and homozygous recessive genotypes in a population, and $2pq$ represent the frequency of heterozygotes.

Related to this equation is the fact that $p + q = 1.0$, that is, the total allele frequency must equal 100%. This equation is known as the *Hardy–Weinberg* equation. It is often used by population geneticists to derive allele frequencies from the frequencies of different phenotypes in a population. By monitoring populations over time, scientists can determine if a population is evolving.

Driving Questions

What are the allele frequencies for the PTC tasting trait in the class population?

What causes allele frequencies to change or remain in equilibrium over time?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- PTC (phenylthiocarbamide) paper
- Control paper *(optional)*
- Calculator with square root function
- Allele cards from the gene pool (2 per person)
- Class data page (1 per class)

Safety

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

- PTC, or phenylthiocarbamide, is safe for consumption at the concentrations provided on the PTC paper.

Initial Investigation

Complete the following lab procedure and analysis before designing your own experiment. Record observations, data, and explanations in your lab notebook.

1. Obtain a piece of PTC paper and place it on your tongue. Record your phenotype for the PTC tasting trait as "taster" or "non-taster."

   NOTE: If it tastes bitter, you are a "taster" and if it is tasteless, you are a "non-taster." If control paper is available, you can use this paper to confirm your ability to taste or not taste the PTC. If the control paper and PTC paper taste the same, you are a non-taster.

2. Based on your phenotype, can you identify your genotype? Explain the reasoning for the answer you provide.

3. Follow your teacher's directions to report your phenotype to the class. Copy Tables 1 and 2 into your lab notebook and use the compiled class data and the directions below to complete Table 1.

Table 1: Determining the phenotypic and allelic frequencies for the PTC tasting trait

Phenotype	Class Population Phenotype and Allele Frequencies			
	Number of Students	Phenotype Frequency	Allele Frequencies	
Tasters	RECORD ANSWERS & DATA IN		p	
Non-taster	YOUR NOTEBOOK.		q	

Table 2: Determining the number of dominant and recessive alleles in the gene pool

Genotype	Class Population Gene Pool				
	Frequency of the Genotype		Number of Students of This Genotype	Number of T alleles	Number of t alleles
Homozygous dominant	p^2	RECORD ANSWERS & DATA IN YOUR NOTEBOOK.			
Heterozygous	$2pq$				
Homozygous recessive	q^2				
Total number in the gene pool					

a. Determine the frequency of each phenotype in the class population.

b. Use the frequency of non-tasters and the following equation to find the frequency q of the recessive allele for the population.

$p^2 + 2pq + q^2 = 1.0$, where q^2 is equal to the frequency of the recessive genotype.

c. Derive the frequency p of the dominant allele in the population.

4. Why did you determine the value for q first, rather than determining the value for p?

5. To model the gene pool of the class population, the number of homozygous dominant genotypes and heterozygous genotypes need to be determined. The frequencies of these genotypes can be derived from the Hardy–Weinberg equation and the size of the class population.

   a. Since the frequency of the T allele is known, p^2 can be easily calculated. Use p^2 to estimate how many students in the class population have the TT genotype. Record these values in Table 2.

   b. How many students are likely to have a Tt genotype? Record this number in Table 2.

   c. If you have not already done so, record the frequency and number of students in Table 2 for the homozygous recessive genotype.

6. The gene pool will be simulated using index cards with letters written on them to symbolize alleles. Each card represents a gamete and will therefore have only one allele written on it.

   a. Work together as a class to determine the total number of T alleles and the total number of t alleles that should be in the gene pool. Your teacher will then place the correct number of "T" and "t" cards in a designated area.

   b. Based on your genotype for the PTC tasting trait, pick up two appropriate cards from the designated area.

   NOTE: If you are a taster, your teacher will use the information from Table 2 to assign tasters to be either homozygous or heterozygous.

7. Follow your teacher's directions to find a "mate" at random. Hold the two cards behind your back and shuffle them. Pull out one gamete card and show it to your mate. Observe the combination of alleles present on your card and your mate's card and determine the phenotype inherited by the offspring.

8. On the class data page, record a tally mark next to the genotype and phenotype that describes your offspring and place the two gamete cards into the class "Offspring" container provided by your teacher.

9. Copy Table 3 and record the compiled data for the offspring produced in the simulation.

Table 3: Determining the number of dominant and recessive alleles in offspring generation

Phenotype	Number of Offspring	Genotype	Number of Offspring	Number of T alleles	Number of t alleles
Taster		TT			
		Tt			
Non-taster		tt			
Total number of alleles in the offspring					

10. What are p and q for the offspring population?

11. Did the allele frequencies change in the second generation? If yes, propose one or more reasons for the change. If no, propose one or more reasons for the frequencies remaining stable.

12. Studies have been performed which analyzed DNA from over 300 human populations and found the mean frequency for *q* for the PTC trait is 0.48.[12]

    a. How do these reported frequencies compare to your class population and the offspring population produced by the class?

    b. Why might the class or offspring population not be representative of an actual human population used for genetic research?

13. Imagine that a teacher devises a similar lab activity for her students, but has students use colored beads to represent the two alleles for the PTC tasting trait and has them start with a gene pool of 100 beads placed into a large cup. The number of dark-colored beads is equal to the frequency of the T allele in the class population; the number of light-colored beads is equal to the frequency of the t allele. The cup is shaken to mix the beads and one person blindly pulls out two beads at a time to randomly determine the alleles inherited by an offspring.

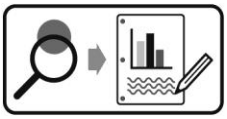
    For the first four offspring produced, the two beads inherited by the offspring are placed into a cup labeled "Survived." For the 5th offspring produced, a coin is flipped to determine if that offspring survives to reproductive age. If the coin lands heads up, the beads go into the "Survived" cup; if it lands tails up the beads go into a "Died" cup. The process is repeated, flipping the coin every 5th offspring produced. After all beads have been removed from the "gene pool," the beads from the "Survived" cup are poured back into the gene pool cup. After 10 generations of offspring have been produced, the data is analyzed to determine if the *p* and *q* frequencies changed over time.

    The activity you performed with cards and the activity described above with beads are both meant to model reproduction in a population and determine if a population evolves over time, that is, to determine if allele frequencies change over time. What are some advantages to the beads model described above?

[12] Wooding, S.; Kim, U.; Bamshad, M.J.; Larsen, J.; Jorde, L.B.; Drayna, D. Natural Selection and Molecular Evolution in PTC, a Bitter-Taste Receptor Gene . *American Journal of Human Genetics*. Apr 2004; 74(4): 637–646. doi: 10.1086/383092

Design and Conduct an Experiment

The Hardy–Weinberg theory states five conditions that must be met by populations for allele frequencies to remain in equilibrium over time. In actual populations, these conditions are rarely met. Devise a way to model the effect of violating one of the Hardy–Weinberg conditions in a simulated population and determine if the population remains in equilibrium or whether the population evolves under the conditions of your model.



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected allele frequencies in the population over time. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

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

   c. Describe a real-life population or situation in which a species has experienced or would experience the conditions modeled in your simulation.

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

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

Synthesis Questions

1. Phenylketonuria (PKU) is a severe form of mental retardation caused by a rare autosomal recessive genotype. Parents typically have no idea they carry an allele for PKU until they have an affected child. In North America, approximately 1 in 10,000 Caucasian babies are born with the disease. Estimate the number of persons who are carriers of the PKU allele in North America.

2. A population of 900 students was surveyed to determine the frequency of *positive* and *negative* blood types. This characteristic of red blood cells is called the *Rhesus factor* and the allele that codes for the factor is dominant over a mutated form of the gene that results in the factor being absent from red blood cells.

   Ninety-three percent of the students are Rh-positive, that is, they have the Rhesus factor. If the population is in Hardy–Weinberg equilibrium, how many students would have each of the following genotypes: homozygous dominant, heterozygous, and homozygous recessive?

3. Analyze the following situations and explain the evolutionary mechanism that results in significantly different allele frequencies in the populations.

   a. Sickle cell disease is an autosomal recessive disease caused by the inheritance of a mutated gene that results in abnormal hemoglobin. Northern European populations, living in an area with a low incidence of malaria, have a q between 0 and 0.005. In Africa where malaria is a cause of many deaths, q is much greater, between 0.020 and 0.181.

   b. Tay-Sachs is an autosomal recessive disease that results in death typically before age five. Affected individuals lack a vital enzyme and are unable to break down a fatty substance found in the brain. In Ashkenazi Jews (Jews of Eastern European Jewish decent), 1 in 27 people are carriers of the fatal recessive allele. In the general non-Jewish population, the carrier rate is 1 in 250.

4. DNA profiling has become an important crime-solving tool in the 21st century. The FBI maintains a Combined DNA Index System (CODIS) that contains over 10 million DNA profiles. A person's DNA profile is comprised of the person's genotypes for 13 STR (short tandem repeats) loci. Scientists have analyzed these STR loci in DNA from thousands of people in hundreds of populations to determine allele frequencies. The Hardy–Weinberg equation is then used to help determine the frequency with which a particular genotype is observed in a population. DNA analysis, combined with probability calculations, can provide compelling evidence of guilt or innocence.

   The diagram below illustrates part of a person's DNA profile. The table provides allele frequencies for the STR loci shown in the diagram as well as other STR loci.

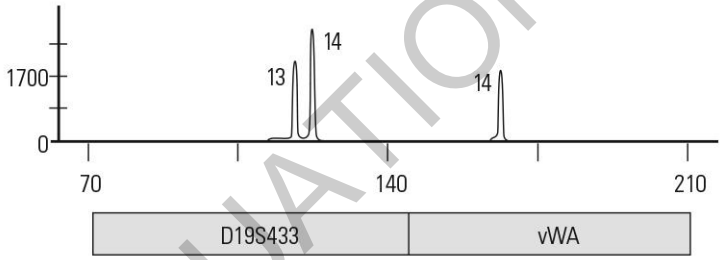


Table 4: Allele frequencies of several STR loci

STR Locus	STR Loci Data Compiled from Unrelated U.S. Population Samples[13]			
	Allele	Frequency	Allele	Frequency
D19S433	13	0.2471	14	0.3041
vWA	13	0.0034	14	0.0956
FGA	17	0.0014	20	0.0883
D21S11	28	0.1646	29	0.2042
THO1	6	0.1959	9.3	0.2056

   a. The person is heterozygous for the D19S433 locus, having alleles 13 and 14 at this site. Based on the information provided and the Hardy–Weinberg equation, what is the frequency of the (13, 14) heterozygous genotype for this locus?

   b. What is the frequency of the person's genotype for the vWA locus in the U.S. population?

[13] Hill, C.R.; Duewer, D.L.; Kline, M.C.; Coble, M.D.; Butler, J.M. U.S. population data for 29 autosomal STR loci. *Forensic Sci. Int. Genet.* 7(2013): e82-e83 (Supplemental Material Table 2).

c. How can the person's genotype be identified as homozygous or heterozygous in the DNA profile diagram?

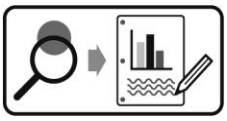
d. What is the frequency of the following genotype in the population: D19S433 (13, 14), vWA (14, 14), FGA (17, 20), D21S11 (28, 29), and THO1 (9.3, 9.3)?

e. Do the frequencies of the two alleles shown in the table for the various STR loci add up to 1.0? If yes, explain why this is the case. If no, propose a reason for this.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

The Hardy–Weinberg theory states five conditions that must be met by populations for allele frequencies to remain in equilibrium over time. In actual populations, these conditions are rarely met. Devise a way to model the effect of violating one of the Hardy–Weinberg conditions in a simulated population and determine if the population remains in equilibrium or whether the population evolves under the conditions of your model.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of evolution and the Hardy–Weinberg theory, what factors (abiotic or biotic) could affect gene frequencies in a population?

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

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

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

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

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

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

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

_____

_____

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

_____

_____

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

_____

_____

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

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

21. MATHEMATICAL MODELING OF EVOLUTION

Background

Evolution in a biological context refers specifically to changes in the genetic makeup of populations over time. It involves variation in the population, heredity, and differential survival. Thus, one way to study if a population is evolving is to monitor the frequencies of alleles in a population over time—that is, from generation to generation.

This leads to the question, "What are the inheritance patterns of alleles, not just from two parental organisms but also in a population?" and to the exploration of how allele frequencies change in populations and how these changes can be predicted in a population for future generations.

Due to the complexity of biological systems that make them very difficult to study, mathematical models and computer simulations are perfect tools for the exploration of the changing genetic makeup of a population from generation to generation. In this investigation, a spreadsheet programmed to model changes of a hypothetical gene pool from one generation to the next will be used to explore parameters that affect allele frequencies, such as selection, mutation, and migration.

Driving Question

How can mathematical models be used to investigate factors that affect changes in allele frequencies within a population? How do these factors contribute to evolutionary change in populations?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Computer
- Mathematical model spreadsheet file: ABI Mathematical Modeling Spreadsheet.xlsx
- Spreadsheet program (such as Microsoft Excel®, Numbers®[14], or Google Docs™[15])

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

[15] © 2012 Google Inc. All rights reserved. Google Docs is a trademark of Google Inc.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

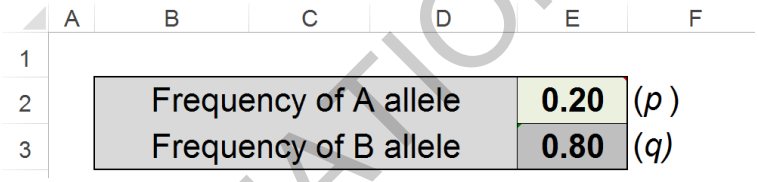
The spreadsheet for modeling evolution has four sheets: Small Population, Large Population, Selection, and Multiple Generations. This investigation starts on the "Small Population" sheet, which shows the initial gene frequencies in the population of 500 individuals. The alleles are represented by "A" and "B."

The Small Population model makes several assumptions, including:

- No mutations
- No selection
- No migration
- Sexual reproduction and alleles of each gamete are randomly selected
- Two-allele, simple-dominance pattern of inheritance
- All gametes form a viable zygote that lives to become an adult
- Population size remains constant

1. Open the mathematical model spreadsheet file. Save the file locally with a different filename. Select the Small Population sheet (click on the tab).

2. Enter some different frequencies for the A allele in cell E2.



   a. When the frequency of the A allele changes, what happens in cell E3? How does the spreadsheet determine the value for E3?

      NOTE: To see the formula for any part of the spreadsheet, simply select the cell of interest and look in the formula bar (f_x).

   b. If a number greater than 1.00 is entered in cell E2, the message box labeled "Allele Frequency Invalid" appears. Use the Hardy–Weinberg equation to explain why the model requires a value less than or equal to 1.00 entered in cell E2.

3. In the model population, a gamete can contain either an A allele or a B allele. Which of the two alleles is in any given gamete is random, so columns B and C have been set up using the spreadsheet's RANDOM function; this function generates a random number between 0 and 1.

   Observe how the RANDOM function works by entering the formula "=RAND()" into cell G2. Press the "*F9*" key on a Windows® computer or the "*command"* and "=" keys simultaneously on a Mac® computer to randomly generate a new number in this cell. Repeat this command three or more times.

4. Notice that the command not only generated a new value in G2, but data in other parts of the spreadsheet also changed. This is because some cells contain formulas that involve the RAND() function. For example, this function is part of the formula the spreadsheet uses to randomly determine if an A or a B allele is in a gamete. In this case, the function is not dependent on the *F9* or "*command* =" commands, though. Change the frequency of the A allele in cell E2 and press the *enter (*or *return)* key; the gamete columns change, as does the summary chart, and a new value appears in G2.

   NOTE: Delete the RANDOM function formula from cell G2 before continuing.

5. A formula using the RAND() function has been set up in cells B6 to B55 and C6 to C55 as follows:

   =IF(RAND()<=E$2,"A", "B")

   Click on cell B6 and look for the equation in the f_x window. This equation directs the spreadsheet program to first generate a random number between 0 and 1. Each time a random number is generated, the IF statement checks to see if it is less than or equal to the frequency of the A allele. If so, an "A" gamete is produced, whereas a random number that is greater than the frequency of the allele causes a "B" gamete to be produced.

   In the 50 rows of the table, gametes are randomly selected to be "A" or "B" and 50 zygotes are generated from the combination of these gametes.

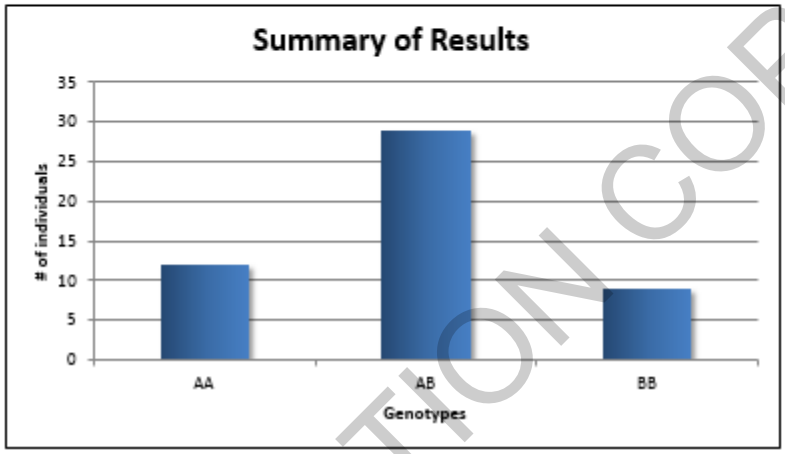
5	Gametes		Zygote	AA	AB	BB
6	B	B	BB	0	0	1
7	B	B	BB	0	0	1
8	A	B	AB	0	1	0

6. If the frequency of the A allele is 0.50 and the RAND() function generates a value of 0.49, the gamete will be assigned an A allele. Why does the model use the current allele frequency to determine which allele is assigned to a gamete?

7. Is the spreadsheet model an accurate representation of reproduction in real populations? In other words, does the random selection of alleles and random combining of gametes occur in real populations? Explain your answer.

Another part of the spreadsheet is the results summary—a summary of the population's gene pool. Formulas in cells N5, O5, and P5 determine the number of zygotes of each genotype and this information is used to determine the number and frequency of A and B alleles in the new generation. The bar graph provides a visual summary of the genetic makeup of the population.

Totals for each genotype:	**AA**	**AB**	**BB**
	12	29	9
Next generation—number of alleles:	**A**		**B**
	53		47
Next generation—allele frequency:	***p***		***q***
	0.53		0.47



8. Click on cell N8.

   a. What formula is the spreadsheet program using to calculate the value for this cell?

   b. Explain what this formula is doing and explain the reason for using this formula to determine the number of A alleles.

9. Set the starting A allele frequency in the model to 0.50. Create a data table to record the frequencies of the A and B alleles (*p* and *q*) for 5 generations, as instructed below.

   a. Record in the table the initial frequency of each allele and the next generation allele frequencies from the summary chart.

      NOTE: The next generation was automatically calculated when you pressed enter/return *after setting the initial* p *frequency to 0.50.*

   b. Enter the value from N11 (the frequency of *p* in the next generation) into E2. Record the A allele frequency of the 2nd generation into the data table. Recalculate the model until you have created five generations. Do not forget to enter the new allele frequency in E2 *each time*.

10. How did the allele frequencies change in the population over five generations? Are changes in allele frequency evidence that the population is evolving? Explain your answer.

11. Repeat the process to produce four more data sets showing the change in allele frequency over five generations. For each data set, start with initial frequencies of 0.50 as you did before. (Create additional data tables for the new data sets.)

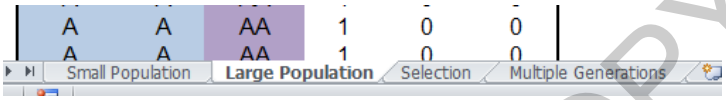
12. Are the results the same in all five data sets? If yes, why do you think this is the case? If no, what might be a reason for the differences? Do you detect any trends?

13. Repeat the above test showing the change of allele frequency, but set the initial *p* frequency to 0.90.

14. After reviewing your results from each scenario, what can you conclude about the effect of the initial allele frequency on the change in frequency over five generations?

15. Do the changes in frequencies generation-to-generation progress in one direction? Why might a high A allele frequency in a small population be detrimental to the survival of that population?

16. Move to the next tab in the spreadsheet, Large Population. The Large Population sheet is identical to the Small Population sheet except the population size has been increased to 5,000 individuals.



Set the starting frequency of the A allele to 0.90 and create three data sets of five generations each, as you did before. Create data tables to record the results.

17. Did the allele frequencies change similarly in the small and large populations over five generations? How do you explain the difference (if any) between the populations?

18. Move to the Selection sheet in the spreadsheet. On this sheet, the survival rate of each genotype can be manipulated and the gene frequencies are shown for a population of 500 individuals. Set the starting frequency to 0.50 for the A allele and set the survival rate of the BB genotype to 0%. The survival rate of AA and AB should be set at 100%.

    Run the model for five generations, again setting the allele frequency in E2 after each run. Record the new allele frequency of each generation in a table.

19. In some populations there is a *heterozygote advantage*. Change the spreadsheet in a way you think models heterozygote advantage. Describe what you change and record the results in a table for five generations. Record both the allele frequencies for each generation and the number of individuals with each genotype.

20. How did the selection against the BB genotype affect the allele frequencies over five generations? How do these results compare to those of the model reflecting heterozygote advantage?

21. Which of the four situations, if any, represented a population in Hardy–Weinberg equilibrium? Justify your answer with evidence. For any population that was not in equilibrium, identify the factor or factors that disrupted the equilibrium.

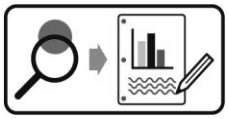
22. Move to the final sheet in the spreadsheet, Multiple Generations, which models a population of 500 organisms for 20 generations. In this sheet, the allele frequencies are shown in the table and on the graph. Set the initial frequency to 0.80 for the A allele and observe the population changes for 20 generations. Instead of creating a data table, graph your results.

23. Compare your results to those of several other classmates or groups. How do they differ? Do differences in the results indicate a flaw in the model? Explain your reasoning.

24. Computer modeling is a powerful tool for biologists but, as with all models, it has limitations. For the spreadsheet models you used to investigate population genetics and evolution, identify two useful aspects of computer modeling and two limitations.

Design and Conduct an Experiment

The Hardy–Weinberg equilibrium model states that both allele and genotype frequencies in a population remain constant from generation to generation unless specific disturbing influences are introduced into the population. Use the mathematical model provided for this investigation to simulate a population over multiple generations where a disturbance is introduced into the population.



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

Design and Conduct an Experiment: Data Analysis

1. Based on your model and data, describe how the independent variable in the models affected allele frequencies in the population.

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

Synthesis Questions

1. A great deal of genetic variation is found within healthy populations. This goes against the logic that natural selection works toward genetic uniformity in a population—the most fit genotypes produce the most offspring, thereby increasing the frequency of some alleles. Describe factors that could cause this genetic variation.

2. The assumptions of the Hardy–Weinberg equilibrium are stringent and are seldom observed or never completely met in real populations, so why do genotype frequencies of many populations not deviate significantly from Hardy–Weinberg expectations?

3. Changes in allele frequencies lead to an evolving population. In a population of 200 peppered moths where white coloration is dominant over dark coloration, there are 32 dark peppered moths.

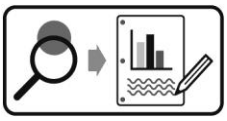
    a. What is the genotype breakdown of the population for the peppered moths?

    b. Environments by nature are consistently changing. Identify a particular change and describe how it would affect the allele frequency of the peppered moth. Explain how the Hardy–Weinberg principle would be affected.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

The Hardy–Weinberg equilibrium model states that both allele and genotype frequencies in a population remain constant from generation to generation unless specific disturbing influences are introduced into the population. Use the mathematical model provided for this investigation to simulate a population over multiple generations where a disturbance is introduced into the population.



Develop and conduct your experiment using the following guide.

1. For a population in Hardy–Weinberg equilibrium, what five factors or conditions must be taking place in order to maintain the equilibrium of the population?

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

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

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

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

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

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

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

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

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

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

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

22. ANIMAL BEHAVIOR

Background

The fruit fly *Drosophila melanogaster* is an organism commonly used in behavioral studies due to its short generation time and ease of culture. It is known to perform dozens of complex behaviors that can be quantified and explored, making it a perfect organism for studying animal behavior.

Animal behavior refers to the responses an animal makes and why it makes them. These behaviors are triggered by either internal or external stimuli and can be either an instinct (based on the organism's genes) or a learned behavior acquired by the interaction of the organism with its parents or surroundings.

Some of the simplest behaviors are those related to an organism's reaction to environmental factors such as light, sound, or moisture. If an organism changes its behavior in response to stimulus, but is not directed by the stimulus, it is called a *kinesis*. However if the organism responds positively (moves towards) or negatively (moves away from) to the stimulus, the movement is called a *taxis*. For example, an organism might sense the presence of a chemical substance and the organism may be attracted to the substance or repelled by it. The observed directional movements of the organism in response to the substance are referred to as *chemotaxis*.

In the lab, you will collect and analyze data from a choice chamber to identify whether fruit flies respond to an environmental stimulus and identify if taxis or kinesis behavior occurs.

Driving Question

How is the orientation behavior of fruit flies influenced by the presence of different stimuli in an environment?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Clear drinking straw
- Droppers (2)
- Cotton swabs (10)
- Timer
- Sheet of white paper
- Wingless fruit flies (10), or similar small organism
- Mashed ripe banana, 10 mL
- Mashed unripe banana, 10 mL
- Distilled water, 10 mL

Safety

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

- Wear safety goggles when working with chemicals.
- Students should not eat any food items in the lab.
- Treat all living organisms with care.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Place 5–10 drops of distilled water onto the end of a cotton swab and place the swab into one end of a clear drinking straw.

2. Using a paper funnel, place 10 wingless fruit flies into the drinking straw.

3. Place 5–10 drops of distilled water onto a second dry cotton swab and place it into the other end of the drinking straw.

4. Lay the choice chamber with the fruit flies onto a white sheet of paper. Label one end of the straw as side A and one as side B. Allow the fruit flies 5 minutes, with no disturbances, to acclimate to the choice chamber.

5. What is the purpose of collecting data for a situation in which fruit flies are given the same choice on either side of the choice chamber?

6. After the 5 minute acclimation period, start a 5 minute observation period. Count the number of flies on side A and the number of flies on side B every minute for 5 minutes. Record your observations in a data table arranged like Table 2.

7. For the following situations using the choice chamber, predict whether you expect fruit flies to exhibit a preference or whether you predict the null hypothesis to be supported (that the flies will have no preference).

   (a) ripe bananas vs distilled water

   (b) ripe bananas vs unripe bananas

8. Using the same choice chamber and fruit flies, repeat the above procedures to expose the flies to the two combinations of substances specified in the previous step. Record the data in your lab notebook for each one.

9. Calculate the average number of flies on each side of the chamber for each situation.

10. Complete a chi-square analysis of the results to determine if the flies' distribution in the choice chambers is significant. (Table 1 is provided for reference.)

Use this null hypothesis for all experiments: The fruit flies do not have a preference for either substance in the choice chamber.

Table 1: Chi-square distribution

Degrees of Freedom	Probability *p* Value					
	0.75	0.50	0.25	0.10	0.05	0.01
1	0.10	0.46	1.32	2.71	3.84	6.64
2	0.58	1.30	2.77	4.60	5.99	9.21
3	1.21	2.37	4.11	6.25	7.82	11.34
4	1.92	3.36	5.39	7.78	9.49	13.28

Table 2: Determination of significant trends

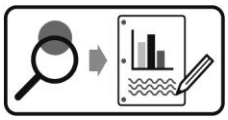
Time (minute)	Number of Fruit Flies					
	Water vs Water		Water vs Ripe Banana		Ripe vs Unripe Banana	
	A	B	A	B	A	B
1						
2						
3						
4						
5						
Total (Observed *o*)						
Average(Observed *o*)						
Expected *e*						
$(o - e)^2/e$						
$\chi^2 = \sum[(o - e)^2/e]$						

RECORD ANSWERS & DATA IN YOUR NOTEBOOK.

11. After reviewing the data and completing a chi-square test, what is your conclusion? Did the fruit flies demonstrate a chemotaxis to any of the substances that were tested? Was it a positive or negative taxis?

Design and Conduct an Experiment

Organisms exhibit a variety of behaviors that can be classified as taxis or kinesis behavior. Using a choice chamber and a small organism, select an environmental factor and conduct an experiment to determine if that factor produces a taxis or kinesis behavior in the organism.



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

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

   a. Describe how the independent variable you manipulated affected the behavior of the wingless fruit flies. Does chi-square analysis of your data indicate the null hypothesis can be rejected? Justify your claim with evidence from your experiment.

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

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

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

Synthesis Questions

1. Animal adaptive behaviors are crucial for survival. Two types of adaptive behaviors are *innate behaviors* and *learned behaviors*. Explain how these two behaviors can impact an individual organism.

2. Animals exhibit behaviors that are classified as either a *taxis* or *kinesis*. What is the difference between taxis and kinesis in relation to animal behavior? In addition to chemotaxis, what other types of taxis responses exist?

3. An experiment was performed to investigate aggressive behavior in olive fruit flies (*B. oleae*).[16] Aggressive behavior was observed in swarms of flies around olive trees—males fighting to occupy leaves to perform courtship displays and females fighting for sites for laying eggs. Additionally, both sexes could gain access to food sources by occupying leaves or fruits on the tree. Investigators recorded fly behavior with high-speed video cameras and determined three behaviors to categorize as aggressive: wing waving, fast running toward the opponent, and pouncing and boxing on the head and thorax of the foe. One of the driving questions of the investigation was: Do resident flies win more combats than non-resident flies?

   NOTE: A resident fly is a fly placed into the chamber first, allowing it to establish a territory ("residence") before other flies are added to the testing chamber. A "win" is awarded to a fly if it remains on an olive leaf for at least 30 seconds after an aggressive interaction that displaces another fly.

[16] Benelli, G. Aggressive Behavior and Territoriality in the Olive Fruit Fly, Bactrocera oleae (Rossi) (Diptera: Tephritidae): Role of Residence and Time of Day. *Journal of Insect Behavior* (2014) 27:145–161. doi 10.1007/s10905-013-9411-7.

Table 3: Observations of aggressive interactions in olive fruit flies

Sex	Initiator of an Aggressive Interaction		Winner of an Aggressive Interaction	
	Resident	Non-resident	Resident	Non-resident
Males	16	14	21	9
Females	19	11	22	8

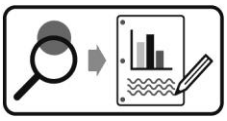
a. Write a null hypothesis for the driving question of the experiment and use chi-square analysis to determine if the null hypothesis should be accepted or rejected. Are the results different for different sexes?

b. Aggressive behavior has a genetic basis and has been conserved in insect evolution. In other words, the behavior is common in many insect taxa. Explain the relationship between natural selection and behavior in organisms.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

Organisms exhibit a variety of behaviors that can be classified as taxis or kinesis behavior. Using a choice chamber and a small organism, select an environmental factor and conduct an experiment to determine if that factor produces a taxis or kinesis behavior in the organism.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of animal behavior, what environmental factors (abiotic or biotic) could affect an organism's behavior?

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

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

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

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

6. Write a testable hypothesis (If…then…). Is this hypothesis the null hypothesis or alternate hypothesis for the chi-square analysis?

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

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

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

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

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