BIOL 101: GENERAL BIOLOGY L - LABORATORY MANUAL

Brad Basehore, Michelle A. Bucks, & Christine M. Mummert
Harrisburg Area Community College

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HACC General Biology l (Biol 101)
Laboratory Manual

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CHAPTER OVERVIEW

1: Labs

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1.1: Scientific Investigation

What is science and how do we “do” science?

Science is how we gain knowledge about the natural world. Typically, it pertains only to what we can investigate or observe using our senses – or instruments that extend the ability of our senses. As a science, **biology** concerns itself with understanding the **unity and diversity of living things** – the 2,300,00, or so, described (and millions of undescribed) species with which we share planet earth.

Ideally, the **SCIENTIFIC METHOD** is a **process** that describes *how* scientists perform investigations to provide a systematic and rational approach to answer questions about the natural world. One goal is to eliminate bias – and **be as objective as possible** in what we study. That being said, bias cannot ever be fully removed, but the goal is to recognize and minimize it as much as possible. **Ideas that can’t be tested, directly observed, or measured in some way should not be subjected to the scientific method.** There are certainly other ways to obtain knowledge (cultural, emotional, etc) they usually do not qualify as science because they do not follow the axioms of science. An axiom in a general sense, is a truth that is accepted without proof. It might seem that science does not assume anything but there are many assumptions that are often ignored such as the relationship between cause and effect, and that our senses and measurements accurately represent reality.

The goal of today’s lab is to familiarize you with the *idealized* steps of the **scientific method** but it's important to recognize that science rarely proceeds so linearly.

You will use these steps to determine the effects of caffeine and ethanol on the heart rate of a small aquatic organism known by the **Latin name** *Daphnia magna* (**common name**: “water flea”; Fig. 1).

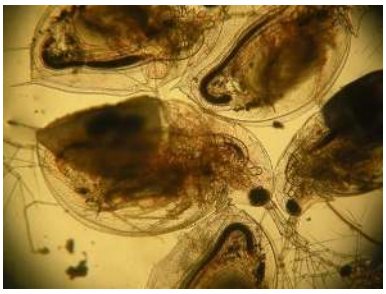


Figure 1.1.1: "Daphnies" by ComputerHotline *is licensed under* CC BY 2.0

This is an ideal **model organism** because its body is transparent, allowing its internal organs to be viewed with the help of a **dissecting microscope** (Fig. 2).

Figure 1.1.2: Dissecting Microscope (original photo)

Model organisms are non-human species used in research to investigate biological processes. Information learned in studies of model organisms can often be applied to other species, including humans. We use model organisms to learn about many different

processes, including genetics, cellular mechanisms, and growth and development. There are certain characteristics that make a species an ideal model organism. For example, it must be easy to manipulate for study, inexpensive and easy to cultivate, and produce lots of offspring. Some commonly used model organisms include *Drosophila melanogaster* (fruit fly), *Caenorhabditis elegans* (*C. elegans*, roundworm), and *Escherichia coli* (*E. coli*, bacteria). The best model organism to use for a study depends upon the question being investigated. In this study, *Daphnia magna* is a good model organism because of its transparent body, which allows for ease of measuring heart rate and gathering data on the effects of caffeine and ethanol.

Steps of the Scientific Method

The scientific method consists of the following steps:

1. Making an **observation**
2. **Asking a question** based on that observation
3. Forming a logical AND testable answer to that question (stated in terms of a **hypothesis**)
4. **Designing a controlled experiment** to see if the hypothesis is supported or rejected
5. **Collecting**, **analyzing**, and **interpreting the data** generated by the experiment

If the **conclusion of an experiment** is such that a hypothesis is *not* supported, then another hypothesis must be developed along with another experiment designed to test it. Ultimately, the results of experimentation are often **published in peer-reviewed journals** (along with detailed methods used to obtain them) so that other researchers can verify or replicate the experiment, and build on that work.

An **idealized version of the scientific method** is demonstrated in Figure 3. It is considered "idealized" because it is important to note that **chance** plays an important role in science. Often, the initial observations that result in important discoveries are stumbled upon by accident rather than sought out. Also remember that the scientific method does not apply to observational or discovery science, which is descriptive in nature.

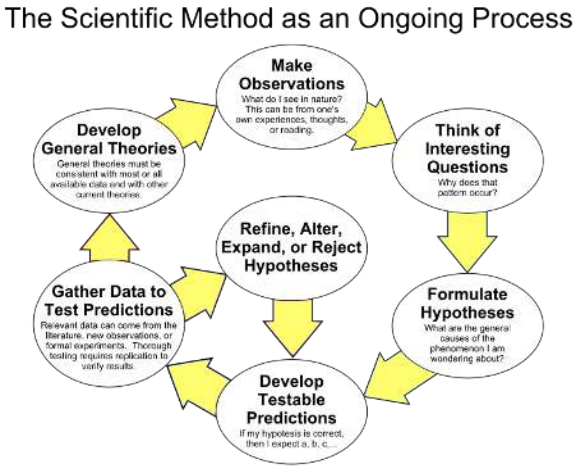


Figure 1.1.3: Idealized Steps of the Scientific Method (This image is an SVG adaptation of the original JPEG image by Whatiguana CC-BY)

MATERIALS AND METHODS

Materials and Supplies:

- Daphnia magna specimens
- Compound light microscope
- Concavity slides
- Disposable transfer pipettes
- Test solutions (water and caffeine and ethanol in varying concentrations)

- Paper towels / Kimwipes
- Stopwatch / clock (with second hand)
- Scissors
- Sharpie and plain paper
- Dissecting probe
- Diagram of Daphnia magna anatomy

PROCEDURE

Step 1: Making an Observation

Making and recording observations (often referred to as **DESCRIPTIVE SCIENCE**) is the first step in the scientific method. Start by making general observations of the Daphnia in a watch glass.

1. Remove a compound light scope from the storage cabinet as instructed and plug it in.
2. Obtain a concavity slide.
3. Obtain a transfer pipette. Cut off the tip with a pair of scissors. This will prevent the *Daphnia* from being crushed when forced through a tip that is too narrow.
4. Use the transfer pipette to remove the *Daphnia*, and some of the water it is in, from the specimen jar and place it on the concavity slide. Make sure that the *Daphnia* is totally covered by water.
5. Take the *Daphnia* specimen back to your lab bench and place the slide on the stage of your microscope. Make sure that the 4X objective lens is over the stage.
6. Use the dissecting probe to gently maneuver the *Daphnia* onto its side so that you can clearly view its heart.
7. View your *Daphnia* under the microscope. Refer to the anatomy chart and identify the animal's various parts.
8. Make a sketch of the *Daphnia* in the circle below. Label the following parts:

- head region
- antennae
- compound eye
- heart
- digestive tract (midgut)
- thoracic appendages (leg-like structures that function as gills)
- shell spine

9. Answer the following questions.
    1. Which body parts are moving?
2. Do you see any eggs or young?
10. Once you have found and observed your Daphnia's heart, count the number of heart beats in one minute. The heartbeat of a healthy specimen is about 2 to 5 beats per second. Because it is so fast, count the heartbeat for 15 seconds and then multiply that number by 4. If necessary, you can keep track of the heart beats by tapping a marker onto a blank sheet of paper and then counting up the number of tap marks.
11. Record the following information:
    1. Heart beats per 15 seconds: _____
    2. Heart beats per one minute: _____

Steps 2 and 3: Formulating a Question and Stating a Hypothesis

In science, observations often lead to the formulation of **questions** that generate hypotheses – and associated **predictions** that are testable. In today's lab, we are considering the following question: "**What is the effect of commonly consumed chemicals on *Daphnia* heart rate**"?

A **hypothesis** is a testable explanation of a set of observations based on available data. It is a **tentative answer to the question you are asking** based on **knowledge** about what you're observing and asking. This knowledge can be pre-existing or information from a published resource. For these reasons, it is NOT correct to say that a hypothesis is an educated guess.

In this lab, you need to formulate several hypotheses about how you believe various test solutions will affect the heart rate of a *Daphnia* based on your **prior knowledge** of how these solutions affect humans. After you formulate the hypotheses, you will test predictions based on these hypotheses. Hypotheses can be rephrased as predictions and can be written as "**If**…., **then**…."statements.

For example: "**If** I put *Daphnia* in ice water, **then** their heart rates will decrease since decreasing temperatures slow down the movement of molecules."

It's important to note that the "If…, then…" statement is not the hypothesis, it is a prediction made about the hypothesis.

Formulate a hypothesis to describe what you predict will happen to the *Daphnia* in each of the following test solutions.

1. **Water (from the Daphnia culture jar):**
2. **Ethyl alcohol (in increasing concentrations: 2%, 4%, 6%, and 8%):**

3. **Caffeine (in increasing concentrations: 1%, 2%, and 3%):**

Step 4: Designing a Controlled Experiment

The next step in the scientific method is to **test the predictions based on your hypotheses** by designing one or more **experiments** that allow you to collect the best **data** to answer your question.

Before doing this, it is necessary to determine the factors (or **variables**) you are interested in testing. There are several variables to consider when designing an experiment.

- **An independent variable is the condition or event under study. It is the predetermined condition *the investigator* sets (and can vary). Only one independent variable is tested at a time, so that an observed response is attributable to just that variable.**
- A **dependent variable** is the condition or event that occurs (the data collected) in response to the specified, predetermined, independent variables that are set.
- **Controlled variables** are any conditions or events that *could* potentially affect the outcome of an experiment. Consequently, they *must* be **held constant (controlled)** and **never varied**. In the case of our *Daphnia* experiment, an example of a controlled variable would be the temperature of the water in which the *Daphnia* are tested. This variable needs to be controlled because *Daphnia* hearts beat faster in warm water than they do in cold water.

In the spaces below, define the variables that will be considered in your experiment today:

1. What will be the **independent variables** in the *Daphnia* experiments? List all of them.
2. What will be the **dependent variables** in the Daphnia experiments? Be specific with your answers.
3. Apart from water temperature, what other **variables** should be **controlled**? List at least 3 controlled variables.

Importance of a Control Group

Most well-planned experiments contain a **control group** in addition to an **experimental group**. The experimental group is the group whose experience is manipulated – usually by only one variable at a time. The control group is the group used for a comparison; it serves as a baseline against which the effects of a treatment can be evaluated. A control group should be as much like the experimental group as possible. It should be treated in every way like the experimental group except for one manipulated factor (the independent variable).

Performing the Experiment

1. Your group will test the effects of:
    1. **plain water** (from the *Daphnia* culture jar)

and

2. **JUST ONE** of the test solutions (at all concentrations listed): Either -

- Ethyl alcohol (2%, 4%, 6%, and 8%)

or

- Caffeine (1%, 2%, 3%, 4%)

2. To test a solution, you will need to remove most of the existing water covering the *Daphnia* in your concavity slide. “Wick it away” with a Kimwipe at the same time you add your test solution with a transfer pipette. Determine the volume of water needed to fill the concavity slide and cover the *Daphnia*. Use this same volume of water for each treatment. Make sure to keep the *Daphnia* submerged in fluid! **If your *Daphnia* dies at any point, you need to re-start the experiment with a new specimen from the culture jar**.
3. You will subject the *Daphnia* to water for a replicate of 8 treatments. Add the first treatment (water from the *Daphnia* culture jar), wait 1 minute, and count the heartbeats for 15 seconds. Record your data in Table 1 (**Step 5**) and calculate the number of beats per minute.
4. Add the second treatment (more water from the Daphnia culture jar) by wicking the previous water sample away as described above. Wait 1 minute, then count the heartbeats for 15 seconds. Record your data in Table 1 and calculate the number of beats per minute.
5. Repeat these steps 6 more times. Use your data to calculate an average value for the effects of water on the heart rate of your *Daphnia*. This part of the experiment is the **control** for your experiment. It serves as the **baseline** against which you can compare the results from the *Daphnia* you subject to the ethyl alcohol or caffeine.
6. Next, test all the other solutions your group has been assigned (either ethyl alcohol or caffeine). Start with the lowest concentration of the test solution and progress to the highest concentration.
7. **Note**: Be sure to keep all the steps of your experimental protocols exactly the same (add the same volume of test solution, equivalent to the volume of water added in the control treatments). Always wait one minute before counting, and record the heartbeats for 15 seconds (just as performed in the control experiment). Due to time constraints, **do only one run (treatment) for each test solution**. Record your data in Table 2 (Step 5).
8. When your tests have been completed, use a pipe test to transfer your *Daphnia* to the recovery beaker (as indicated by the instructor).
9. Wash and dry all the glassware you used and put it back where you found it. Dispose of the used pipettes in the trash. Make sure the lids are placed back on all of your solution bottles. Clean up any mess you may have made and wipe down the lab benches with the paper towels.
10. Compile your class data as directed by the instructor.

Step 5: Collecting, Analyzing, and Interpreting the Data

Table 1: Baseline (Control) Group – *Daphnia* Heartbeats in Water

Water Treatment #	Beats per 15 seconds	Beats per 1 minute
Treatment #1		
Treatment #2		
Treatment #3		
Treatment #4		
Treatment #5		
Treatment #6		
Treatment #7		
Treatment #8		
Average of 8 treatments		

Substance tested by your group: _____

*Start with the lowest concentration of the test solution, followed by the next higher concentration (lowest to highest concentrations).

Table 2: Experimental Group - *Daphnia* Heartbeats in Test Solutions

Substance Concentration	Beats per 15 seconds	Beats per 1 minute

Table 3: Class Data

Drug Concentration	Group #1	Group #2	Group #3	Group #4	Group #5	Group #6	Class Average
2% Alcohol							
4% Alcohol							
6% Alcohol							
8% Alcohol							
1% Caffeine							
2% Caffeine							
3% Caffeine							
4% Caffeine							

Experimental data and results must be displayed in a clear logical manner. Tables, **charts**, and **graphs** are usually the most effective tools to provide a concise summary of the type of numerical data you collected today.

A graph is a **diagram showing the relationship between independent and dependent variables**.

When making graphs, the following rules should be observed:

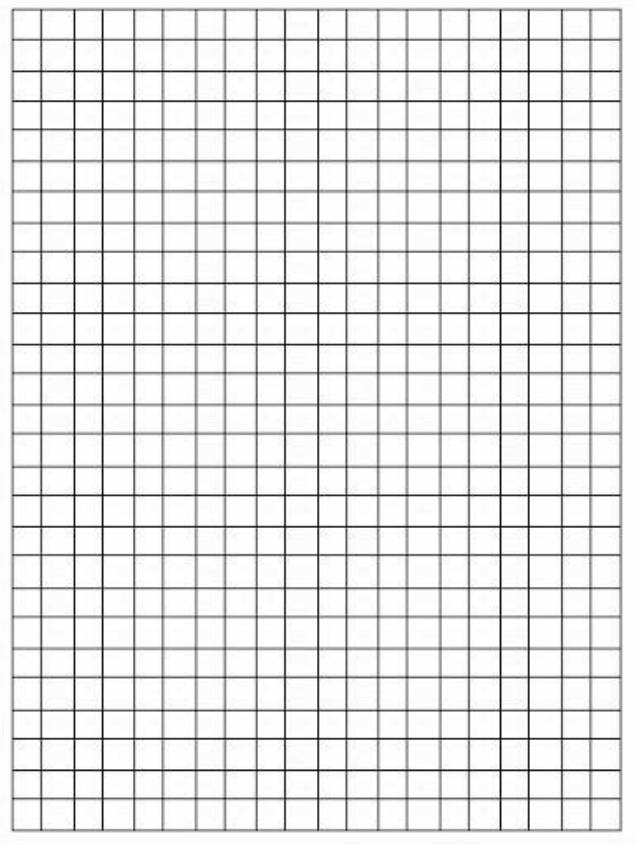
1. The **independent variable** is usually plotted on the **X-axis** (horizontal axis) and the **dependent variable** is plotted on the **Y-axis** (vertical axis).
2. Each axis should be **labeled properly** with the name of the variable and the units of measurement.
3. Data intervals must be evenly spaced across the axes, usually beginning with zero and increasing in consistent even increments.
4. All graphs should have a title or caption to describe the information presented. Capitalize the first word in the title and place a period at the end.
5. Choose a graph that best represents the type of data you collected:
    1. **Line graphs** show changes in the quantity of the chosen variable and emphasize the rise and fall of the values over their range.
    2. **Bar graphs** are used for data that represent separate or discontinuous groups or non-numerical categories, thus emphasizing the discrete differences between the groups.

*Refer to the Graphing Grading Rubric at the end of this lab.

Graph Your Results:

Discuss with your group how to design the graph so it best represents your data and ultimately the conclusions you draw.

Use the grid below to graph your group's results:



Title:

Interpret Your Results:

Once you have collected your data and summarized it as a graph, the last step is to **analyze and interpret** your results. Ultimately, you have reached the stage in the scientific method process where you need to determine whether the hypothesis you initially generated has been **supported** or **refuted** (not supported).

Questions for Review

1. What is the difference between a control group and a controlled variable?
2. What are some types of questions science can't answer?
3. Where would you find an independent variable on a line graph?
4. What are 'levels of treatment'?
5. Much like citing your sources, making a graph usually follows format guidelines. What is the correct way to format a graph in APA style?

Practical Challenge

1. Give an example of a well written hypothesis and a prediction based on this hypothesis.
2. What kind of data would be appropriate to use for a bar graph?
3. Did the results of your experiments support or refute your hypothesis?

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1.2: Microscopes

Many biological specimens such as cells and tissues are difficult or impossible to be seen with the naked eye so they must be magnified to be studied. Consequently, the basic operation and care of **microscopes** is an important skill in biology.

Two basic types of microscopes are used in our introductory biology labs: **compound light microscopes** and **stereo microscopes** (aka **dissecting microscopes).**

Compound light microscopes pass visible light through two sets of magnifying lenses (the ocular and the objective lenses) to magnify specimens mounted on a **glass slide** and placed on the flat surface (**stage**) of the microscope. The specimens must be sliced very thin in order for light coming from the light source to pass up through them so that they can be viewed. Specimens are sometimes stained with dyes to add contrast and to make structures more easily identifiable. Microscopic examination of cells and tissues allows students to observe the principle of complementarity - how cell and tissue structure determines function.

Exercise 1: Parts of the Microscope

In this exercise, you will identify and learn the functions of various microscope parts. Proper practice for handling and use of compound light microscopes is as follows:

Materials:

- Compound light microscope

Procedure:

1. Obtain a compound light microscope as directed by your instructor.
2. **Carry the microscope upright with one hand supporting the base and the other hand grasping the arm.** Care should be taken not to bump the microscope on the microscope cabinet, chairs, tables, or other obstacles. **Gently** place your microscope on your laboratory bench and remove its protective plastic cover (if present).
3. **Do not push or slide the microscope across the table.** This causes vibrations that can loosen screws or misalign microscope parts.
4. **Clean the lenses with lens paper (if needed).** The lenses on the microscope scratch easily. If you need to clean them, use ONLY lens paper (KIM Wipes are not lens paper).
5. Before you use the microscope, locate the parts of the microscope and label **Figure** 1.2.1. Additionally, Table 1 provides the function of each part.

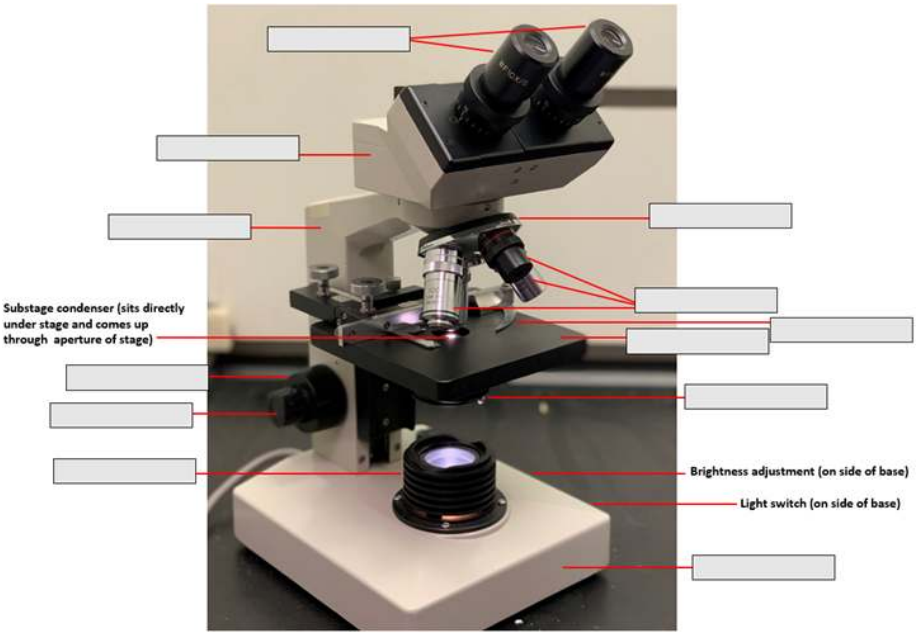

Figure 1.2.1: Compound Light Microscope (original photo)

Table 1.2.1

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Microscope Part	Function
Head	Upper part of microscope that extends from arm and contains the ocular lenses and revolving nosepiece with objective lenses.
Arm	Supports the body tube and lenses. Use the arm and base to carry the microscope.
Base	Supports the entire microscope.
Ocular (eyepiece)	The lens in the upper part of the microscope. **Monocular** microscopes have one ocular, while **binocular**_microscopes have two oculars. Ocular magnification is **10x**.
Body tube (turret)	Holds the ocular at one end and the nosepiece at the other. Conducts light rays.
Revolving nosepiece	Located at the lower end of the body tube. A revolving device that holds the objective lenses (*aka* objectives).
Objective lenses	Located on the revolving nosepiece. Each lens has a different magnifying power. The smallest objective (**scanning objective**) is the smallest magnification at 4x, followed by the **low power** objective at 10x, the **high power** objective at 40x, and the highest magnification **(immersion oil** objective) at 100x. Only one objective may be used at a time. The selected lens is rotated into position by turning the nosepiece.
Stage	The horizontal platform upon which the slide rests.
Condenser	Lens beneath the stage that concentrates and focuses the light before it passes through the specimen to be viewed.
Iris diaphragm lever	Small lever beneath the condenser which controls the amount of light passing through the condenser.
Light source	Provides a beam of visible light to be passed through the specimen.
Mechanical stage	Moveable part of the stage controlled by stage adjustment dials located below the stage. Allows the observer to move the stage forwards, backwards, left, and right.
Coarse adjustment knob	Located on either side of the arm. Moves the stage to bring the object into focus. **This knob should only be used when the scanning (4x) objective is positioned above the stage!**
Fine adjustment knob	Located within the coarse adjustment knob. Allows fine focusing of the specimen.

7. The head of the microscope supports the first set of magnifying lenses called the **ocular lenses**. These are located at the top of the microscope. These lenses are where we place our eyes and are also referred to as eyepieces. The ocular lenses magnify a specimen ten times (10x). If your microscope is binocular, the interpupillary distance (the distance between the eyepieces) can be adjusted to accommodate this distance.
8. The second set of four lenses is located on a **revolving nosepiece**. Collectively known as **objective lenses**, these lenses can magnify a specimen four times (4x), ten times (10x), forty times (40x), or 100 times (100x), respectively. Magnification values are etched on the sides of the metal casings that protect the lenses.
9. Closely related to the topic of magnification is resolution. Resolution (or **resolving power**) is the ability of a microscope to distinguish two adjacent structures as distinct, or separate. The higher the resolution, the better the clarity and detail of the image.
10. The bending of light through objective and ocular lenses is called **refraction.** This is what can give us our magnification power. At even higher magnifications, excessive refraction can cause distortion of the image but the **oil immersion objective lens** (100x) can remedy this problem by eliminating the gap between the specimen and the objective lens with a drop of oil. We will NOT be using the oil immersion lens in this class unless otherwise noted by your instructor.
11. The light source in these microscopes can be controlled at a variety of points on the microscope, which you will see as part of this lab. Adjusting the light source correctly can be as important to see your image as adjusting focus.
12. **Calculating Magnification:** When using a compound light microscope, both the **ocular** lens and **objective** lens help magnify the image. Therefore, to calculate the **total magnification** of an image, the contribution of each lens must be taken into consideration. Total magnification can be calculated using a simple formula:

$$\text{Total magnification} = \text{Ocular lens power} \times \text{Objective lens power} \tag{1.2.1}$$

Calculate total magnification values for your microscope and record the values in Table 2 below.

Table 2: Total magnification calculations using a compound light microscope

	Magnification	Total Magnification (Objective x Ocular)
Objective lens - Scanning Power		
Objective lens - Low Power		
Objective lens - High Power		
Ocular lens		

13. **Always begin viewing a slide using the scanning (4x) objective**. Never begin an observation with the higher-powered (10x, 40x, 100x) objectives. Doing so could result in broken slides or scratched lenses.
14. ***Never* use the *coarse* focus adjustment at high magnification.** Once a specimen is brought into focus using the lowest power, you can rotate to a higher power objective lens to increase the magnification. You will probably need to focus only slightly, as most light microscopes are **parfocal**, meaning that the image remains nearly in focus as you change lenses (from lowest to highest power).
15. **Replace the microscope properly.** When you are finished using the microscope, turn off the light, remove the last slide from the stage, and wipe any material from the stage. Lower the stage and move the lowest power (4x) objective into position. Bundle the electrical cord securely (not around the arm of the microscope), replace the plastic cover, and put the microscope back in the storage cabinet.

Exercise 2: Focusing & Image Inversion

The optics of a light microscope's lenses change the orientation of the image the user sees. A specimen that is ***right-side up*** and ***facing right*** on the microscope slide will appear ***upside-down*** and ***facing left*** when viewed through a microscope, and vice versa. Similarly, if the slide is moved left while looking through the microscope, it will appear to move right. If moved down, it will seem to move up. This occurs because microscopes use two sets of lenses to magnify the image. Because of the manner by which light travels through the lenses, this system of two lenses produces an inverted image.

Tip: Quick Focus

You can get a slide nearly into focus without ever looking into the ocular lens by following these simple steps:

- Turn the light on and adjust the objective lens to the lowest power scanning lens.
- With the light on, place your slide on the stage and center the specimen in the light using the stage adjustment knobs.
- Raise the stage the whole way up, then bring it down with half a rotation of the coarse adjustment knob.

- Compound light microscope
- Prepared slides: letter “e”

Procedure:

1. Obtain a slide of the letter “e.”
2. Look at the letter “e” and draw what you see in the circle provided.

Draw the letter “e” as seen on the slide while holding it in your hand.

3. Place the slide on the **stage**. Secure it with the **stage clips**. Make sure that the **scanning objective (4x)** is clicked into place. Use the **stage adjustment knobs** to center the letter “e” over the **light source** and directly in the center of the **field of view** (the lighted circular area you see as you look through the ocular lenses).
4. While looking into the **ocular lenses**, use the **coarse focus adjustment** to bring the letter “e” into focus. Use the **fine focus adjustment** to “fine tune” the image. Try adjusting the light with the **iris diaphragm lever.** Decreasing the aperture size decreases the amount of light on the specimen and increases contrast.
5. Observe the position of the letter “e” as it appears in the **field of view** (the circular area that can be seen when looking through the ocular). **Draw the letter as it appears through the microscope** in the circle provided.

Letter “e” as seen through microscope

6. Compare your drawing from step (b) to that of step (e). How do the two drawings differ?

   _____

7. While observing the letter “e” through the ocular lenses, describe the movement that you observe as you move the slide:

- to the left - _____
- to the right - _____
- forward - _____
- backward - _____

8. When you view the letter “e” using the **scanning objective**, what is the **total magnification**?

   _____

9. Center the letter “e” in the field of view. Look at your microscope from the side (not through the oculars) and rotate the **revolving nosepiece** so the **low power (10x) objective** clicks into place.
10. Focus the letter using the **fine focus adjustment.** (Do not use the coarse focus adjustment.) Most microscopes are **parfocal**. This means that when you focus the specimen under the lowest magnification, that the specimen is “almost” in focus at any higher magnification, such that you will only need to use the fine focus adjustment to focus the image.
11. Under **low power**, how many times has the letter “e” been magnified?

    _____

12. Compared to **scanning magnification**, does the letter appear larger?

    _____

13. Compared to scanning magnification, can you see more detail in the letter or the paper it is printed on?

    _____

Exercise 3: Diameter of the Field of View

Since the **scanning objective** is 4x and the **low power objective** is 10x, images will be magnified ***more*** with low power than with scanning power. Because objects will appear larger, the **low power “field of view”** will be *smaller* than the **scanning power field**. Therefore the relationship between the diameter of the field of view and the magnification is inversely proportional. Not only does

measuring the field of view at different magnifications demonstrate this property, but once you know the diameter of the field of view in millimeters (mm) at various magnifications, you will be able to estimate the size of the cells or other structures being viewed.

- Compound light microscope
- Clear metric ruler

Procedure:

1. Lower the stage, and bring the **scanning objective (4x)** back to the center position. Remove the letter "e" slide if you haven't already removed it.
2. Position a clear plastic ruler across the stage, and focus on its edge, so that the edge of the ruler, with millimeter markings, is visible in the field of view and equally bisects the field of view.
3. The distance between two lines on the ruler is 1mm. Draw what you see in the circle provided. Measure the diameter of the field of view (in mm). _____ mm.
4. Leaving the ruler in place, rotate the **low power (10x) objective** into position.
5. Use the **fine focus** if necessary to bring the ruler into focus.
6. Estimate the number of millimeters that you see in the field of view:

_____mm

7. Leaving the ruler in place, rotate the **high power (40x) objective** into position.
8. Use the **fine focus** if necessary to bring the ruler into focus.
9. Estimate the number of millimeters that you see in the field of view:

_____mm

10. What happens to the field of view when you increase magnification? Explain.

    _____
11. Do you see MORE or LESS of an object if you increase magnification? Explain.

    _____
12. Move the scanning (4x) objective lens back into place, lower the stage with the coarse adjustment knob, and remove the ruler.
13. Place the letter "e" slide back onto the stage and secure it with the stage clip. Using the scanning objective lens, estimate the diameter of the letter "e". If it occupies ½ of the field of view, then it is ½ times the measurement you recorded in step (c) above. Record the diameter of the letter "e". _____ mm

Exercise 4: Depth of field

Depth of field is the area (top to bottom) of an object that comes into focus while slowly moving the fine adjustment knob up and down. Because the depth of focus is very short in the compound microscope, you must focus up and down to clearly view all of the planes of a specimen.

- Compound light microscope
- Slide of crossed colored threads

Procedure:

1. Obtain a **slide with colored threads** mounted together. Place it on the stage and focus using the **scanning (4x) objective**. The center of your field should be the point where the three fibers cross each other.
2. Focus up and down using your **coarse focus adjustment**. Under scanning magnification, are all three fibers in focus at the same time? _____
3. Can you easily tell which fiber is on top and which is on the bottom?

    _____
4. Rotate the nosepiece so that the **low power (10x) objective** clicks into place.
5. Focus up and down using your **fine focus adjustment**. Under low magnification, are all three fibers in focus at the same time?

    _____

6. Can you easily tell which fiber is on top and which is on the bottom?

   _____

7. At which magnification is there a greater **depth of field**? _____
8. Use the iris diaphragm to change the amount of light passing across the fibers. Note how changing the position of the diaphragm helps to increase (or decrease) your ability to view objects.
9. Does the 4x or the 10x objective have a shorter depth of field?

   _____

10. Using the fine focus adjustment, slowly rotate the knob until all three threads are just out of focus. Slowly refocus using the fine focus adjustment.

- Which thread comes into focus first? _____
- Is this thread lying under or over the next thread? _____

11. Slowly rotate the high power (40x) objective into position and focus up and down using the fine focus adjustment only. Does the 10x or the 40x objective have a shorter depth of field? _____

Exercise 5: Wet Mount of Human Epithelial Cell (Cheek Cell)

According to the cell theory, the *cell* is the fundamental biological unit, the smallest and simplest biological structure which possesses all of the traits of something that is living. All living organisms are composed of one or more cells, and every activity taking place in any living organism is ultimately related to the metabolic processes that are taking place in cells. Therefore, to understand the processes of life, it is necessary to understand the structure and function of the cell. Cells that line the interior of the mouth and cheeks are situated very close together, similar to tiles on a floor. These thin cells form a thick layer that protects the underlying tissue from abrasion and foreign pathogens (e.g. viruses and bacteria). The cells comprising the most superficial layer are continually sloughed off and replaced by underlying cells. Gently scraping the lining of the cheek removes the superficial cells. In this activity, you will prepare a wet mount slide of cheek cells and observe them under the compound light microscope.

- Compound light microscope
- Clean microscope slides and coverslips
- Toothpicks
- Methylene blue dye
- Water with dropper

Procedure:

1. Obtain a clean microscope slide and coverslip. Add one drop of water to the slide.
2. Obtain a flat toothpick and obtain a sample of your cheek epithelial cells from the inside lining of the oral cavity. The goal is to gently scrape loose cells free, not to draw blood from your cheek.
3. Apply the cells to the slide, rotating the toothpick between your thumb and forefinger to dislodge the cells into the water. Dispose of the toothpick in the biohazard trash.
4. Obtain a small bottle of methylene blue dye. Make sure that the dropper does not actually touch the slide (*do not contaminate the dropper bottle with your cheek cells*). Let a VERY small drop of methylene blue dye fall onto the slide. (*Methylene blue will stain just about anything it touches a deep blue, so be careful not to get it on your skin or clothing.)

- What is the purpose of adding stain to the biological specimen? _____

5. Place a clear cover slip on top of the specimen. You have just prepared a

wet mount slide!

6. Bring the epithelial cells into focus using the **scanning (4x)** objective and the coarse focus adjustment. Zoom in on a few cells by switching to the **low power (10x) objective lens.**
7. Confirm with your instructor that you have viewed a cheek epithelial cell.
8. Switch the objective lens to **high power (40x)**. Sketch one cell in the circle below.

Total Magnification_____

9.
10. What is the genetic material found in the nucleus of these eukaryotic cells?

    _____

11. What is the approximate size of one cell? _____ mm
12. How do you know this? _____

Exercise 6: Observation of Plant Cells (Elodea Cells)

Leaves of Elodea, a common aquatic water plant, are great for observing the major characteristics of a typical plant cell. In this activity you will prepare a wet mount and examine one of the leaves from the Elodea under the compound light microscope.

- Compound light microscope
- Clean microscope slides and coverslips
- Elodea cells
- Water with dropper
- Optional: Onion epithelial cells or prepared slide of onion root tip
- Methylene blue dye (only if using onion epithelial cells as dye is not needed to see the cellular structures in the Elodea leaf)

Procedure for Elodea Cells:

1. Obtain a clean microscope slide and coverslip.
2. Using forceps, remove an Elodea leaf (smallest, youngest leaves are best) from the sprig of Elodea provided and place it on the slide. Make sure it is pressed down and flat.
3. Add a drop of water to cover the top of the leaf.
4. Place a clear cover slip on top of the specimen. You have just prepared a

wet mount slide!

5. Bring the plant cells into focus using the **scanning (4x)** objective and the coarse focus adjustment. Zoom in on a few cells by switching to the **low power (10x) objective lens.**
6. Switch the objective lens to **high power (40x)**. Sketch several Elodea cells in the circle below.

Total Magnification_____

7. Identify the following structures:

- **Cell wall** - the rigid framework consisting of the carbohydrate cellulose that surrounds the cell and lies outside the cell membrane. Its function is to give the cell a definite shape and provide support. Cell walls are not found in animal cells.
- **Protoplasm** - the organized contents of the cell, exclusive of the cell wall.
- **Central vacuole** - the membrane bound sac, filled with water and dissolved substances, that lies within the cytoplasm. Its functions include the storage of metabolic wastes as well as providing turgor pressure which gives the cells support.
- **Cytoplasm** - the protoplasm of the cell exclusive of the nucleus.
- **Chloroplasts** - the green spherical organelles containing the pigment chlorophyll that is involved in photosynthesis. As the microscope light heats up the water on the slide, you may observe the cytoplasm and the chloroplasts moving around the central vacuole in a process called ***cytoplasmic streaming***.
- **Nucleus** - the organelle within the cytoplasm that contains the genetic information in the form of DNA. Since dye has not been used to visualize cellular structures, the nucleus may be transparent and difficult to observe.

8. What three structures are observed in the Elodea cells that are unique to plants and not observed in animal cells ?

   _____

Procedure for Onion Epithelial Cells: (Optional)

1. Remove the thin, transparent epidermis (skin) from an onion leaf to prepare a wet mount slide. Alternatively, you may view a prepared slide of onion root tip. Do not discard commercially prepared slides.

2. Place on a clean slide and add a drop of methylene blue. Do not contaminate the dropper (do not touch the onion skin with the dropper). Cover with a clear coverslip.
3. Observe with the scanning objective lens using the coarse focus adjustment first, then the fine adjustment knob.
4. Observe using the low power objective lens. Make sure you see the rectangular shaped onion cells. Confirm with the instructor, if necessary. Sketch the onion cells in the circle below. On your drawing label the cell wall (lacking in animal cells), cell membrane, cytoplasm, central vacuole, and nucleus. Be sure to indicate the total magnification used in your drawing.

Sketch onion cells

Total Mag. ____

5. Estimate the size of one cell: _____ mm

Exercise 7: Stereo Microscope

Stereo microscopes (also called **dissecting microscopes; Figure 2**) also contain 2 sets of lenses (ocular and objective lenses). The ocular lenses on a stereo microscope, like a compound light microscope, magnify by a factor of 10x. The objective lenses, however, have relatively low magnification. There is a great deal of variation in stereo microscopes and the manner in which they achieve higher magnification. Stereo microscopes include an additional magnification system that makes the final image appear to be upright.

They are used for viewing and manipulating relatively large specimens which can be viewed in three dimensions. They have a binocular feature that creates a stereoscopic effect. They can be used to study entire small organisms since their depth of field is much greater than the compound light microscope. The light source can be directed down onto a specimen (reflected light) as well as up through the specimen (transmitted light), which permits the viewing of objects too thick to allow for the transmission of light. It is also possible to view multiple samples in a petri dish placed on the microscope's stage with light that is projected from below.

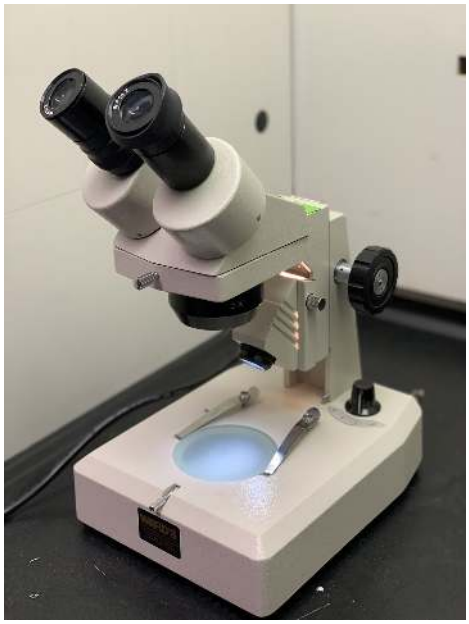

Figure 2. Stereo microscope (original photo)

- Stereo microscopes
- Variety of objects to be viewed (e.g. feather, shell, penny, preserved insects, crystals)

Procedure:

1. Your instructor will describe how to use the stereomicroscope.
2. Use the **stereomicroscope** to view the variety of materials provided for you.

Make a drawing of 2 of the items you viewed with the dissecting microscope.

Exercise 8: Electron Microscope

Electron microscopes, first developed in the 1940s, use a beam of electrons instead of light to magnify an object. They can magnify objects as small as 2 nanometers (0.00000004 inches) over 100,000 times.

Transmission electron microscopes are used to study internal cell structure and are analogous to compound light microscopes in that regard. Specimens are cut into thin sections, usually "stained" with heavy metal atoms (atoms with large atomic numbers) that attach to cell structures. An electron beam is then focused through the specimen. **Figure 3** is an example of the type of image an electron microscope can produce.

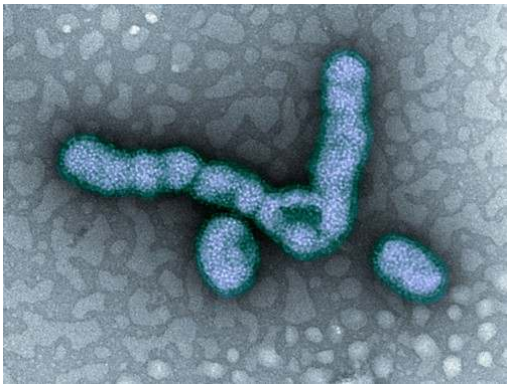

Figure 3. *"H1N1 Flu Virus"* ***by*** *NIAID* ***is licensed under*** *CC BY 2.0*

Scanning electron microscopes, analogous to stereo microscopes, allow a specimen's surfaces to be observed in detail. The object is chemically frozen and then coated with a thin film of metal. An electron beam excites surface electrons on the specimen and produces a three-dimensional image. **Figure 4** is an example image that is produced by an SEM.

Electron microscopes are expensive and require special training. Even though the preparation techniques used to prepare specimens for electron microscopy kill most living cells, some cells such as Tardigrades can withstand these harsh treatments.

Figure 4. *"Spider, false-coloured scanning electron micrograph"* ***by*** *ZEISS Microscopy* ***is licensed under*** *CC BY 2.0*

Questions For Review:

1. Why, specifically, did we look at the letter "e" slide, threads slide, and the ruler?
2. What cellular parts of your cheek cell could you observe?
3. What does it mean if a microscope is parfocal?
4. What is the relationship between the magnification and the diameter of the field of view? Explain.
5. What two parts of the microscope do you use to carry it?

Practical Challenge Questions:

1. Imagine you are looking at a slide on high magnification and you lose focus on it. What should you do?

2. You are using a microscope you haven't used before and you notice the microscope has a 10x ocular as well as a 73x objective lens. What will the total magnification be if this objective lens is positioned over the stage?

3. You are given several specimens to observe with a microscope but you are unsure what type of scope to use. What are some reasons that you may choose different microscopes?

4. Why is the resolving power of the transmission electron microscope so much greater than that of the compound light microscope?

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 3: Microscopy," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY https://oer.galileo.usg.edu/biology-textbooks/18

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1.3: Organic Molecules

Introduction

In its simplest definition, **organic compounds** include all molecules that contain carbon. By this definition, simple molecules such as carbon monoxide (CO) and carbon dioxide (CO2) would be defined as organic molecules, however, these simple molecules behave more like inorganic molecules than organic molecules. Therefore, other definitions of organic molecules state that **organic molecules are molecules containing both hydrogen and carbon**. For our studies, we define organic molecules using the latter definition.

The four main groups of biologically important organic compounds are **carbohydrates**, **lipids**, **proteins** and **nucleic acids**. These compounds are also known as biological macromolecules and all but the nucleic acids are the common food categories listed on Nutrition Facts panels. These biologically important macromolecules play essential roles in cell and organismal structure, energy and heredity. In addition to carbon and hydrogen, these biologically important organic compounds also contain the four other "building block" elements: **oxygen (O), nitrogen (N), phosphorus (P) and sulfur (S)**.

In this lab, we will use **chemical indicators** and chemical tests to detect the presence of biological macromolecules. Chemical indicators are substances that react in a characteristic fashion, often a color change, if a particular molecule is present.

Each test will include a **positive control** and a **negative control**. A ***positive* control** is a test substance that should reliably produce a positive result. It shows what a positive reaction looks like. It contains the compound for which we are testing and all the appropriate chemical indicator(s). A ***negative* control** is a test substance that should reliably produce a negative result. It shows what a negative reaction looks like. It usually contains *just distilled water* (**dH2O**) and the appropriate indicator(s). To be valid, a negative control is placed through ***all*** *the physical steps* of a positive control such as heating, changing of pH, etc., if required.

Positive and negative controls differ from the **control groups** we studied in the Scientific Method lab. Remember, a **control group** is a test group of subjects that does not receive the treatment under investigation and is used as the baseline for comparison to an **experimental group**.

1. **Flame test for Carbon (optional instructor demonstration)**

Exercise 1: *Wear protective goggles and gloves during this activity.*

Organic molecules contain carbon and hydrogen. Substances that contain carbon will burn and blacken. To test a substance for carbon, place the substance in a test tube and hold it over a flame for a few moments. If the substance blackens then it contains carbon and is an organic molecule.

Table 1 Results of Carbon Flame Test

Substance	Did it blacken?	Is it organic or inorganic?
Salt		
Sugar		
Gelatin		

2. **Carbohydrates**

Carbohydrates include sugars and starches and are composed of **monosaccharide** building blocks. Glucose and fructose are examples of monosaccharides and are often called simple sugars. Simple sugars can exist in linear or ring structures, but in most biological situations containing water they exist in the ring structure (Figure 1). Two simple sugars bound together form a **disaccharide**. An example of a disaccharide is sucrose, commonly known as table sugar. Sucrose is formed by a glycosidic covalent bond linking glucose and fructose (Figure 2). Lactose is also a disaccharide composed of galactose and glucose.

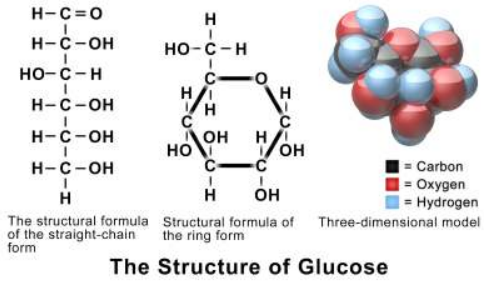

Figure 1. Glucose in ringed and linear forms. (Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436., CC BY 3.0 <https://creativecommons.org/licenses/by/3.0>, via Wikimedia Commons)

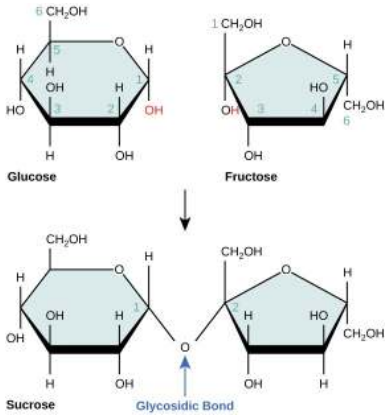

Figure_03_02_04.jpg">Sucrose ring structure. (CNX OpenStax, CC BY 4.0 <https://creativecommons.org/licenses/by/4.0>, via Wikimedia Commons)

Polysaccharides are long chains of many subunits of simple sugars covalently bound together. Polysaccharides are often referred to as complex carbohydrates due to their large structure. Starch and cellulose are polysaccharides found in plants. Plants store extra energy in the form of the polysaccharide starch. The complex carbohydrate, cellulose is an important structural material in many plants. Animals store some extra energy (for short-term storage) in the form of the polysaccharide glycogen.

Carbohydrates play important roles in organismal structure and as main sources of energy for cells. Simple sugars, such as glucose, enter directly into metabolic pathways (such as glycolysis) to provide ATP for cells. When larger polysaccharides, such as starch, are consumed by organisms the complex carbohydrates must be broken down by water and enzymes into simpler sugars before they can enter into metabolic pathways to yield ATP.

Exercise 2: Testing for Carbohydrates - Benedict's Test for Reducing Sugars

All Monosaccharides are reducing sugars. The disaccharides maltose (glucose + glucose) and lactose (glucose + galactose) have a free aldehyde group and are also reducing sugars. Reducing sugars are able to reduce (add electrons to) other molecules. Reducing sugars have a free carbonyl group (a carbon atom double-bonded to an oxygen atom) that can react to donate electrons. The disaccharide sucrose lacks free carbonyl groups due to the glycosidic bond that links glucose and fructose to create the disaccharide (Figure 2). Therefore, sucrose is not a reducing sugar. **Benedict's reagent** is a solution of copper sulfate, sodium carbonate and sodium citrate and **is the indicator used to test for the presence of reducing sugars**. In the absence of such sugars, Benedict's reagent is a bright royal blue color, and clear (not cloudy). However, when heated in the presence of a reducing sugar, it accepts electrons from the reducing sugar and changes color. It also becomes cloudy as it forms a **precipitate** (an insoluble solid that emerges from a liquid solution) of cuprous oxide. Any color change is considered a positive reaction. However, the degree of color change depends on the amount of reducing sugar present (Figure 3). A change from blue to yellow, or green, indicates a small amount of reducing sugar. A change from blue to red, or orange, indicates a large amount of reducing sugar. Note that heating the Benedict's reagent for too long can cause false positive results.

Figure 3. Results of Benedict's test for reducing sugars. Negative test remains blue (left tube) and positive results show color change (right three tubes). (original photo)

Benedict's Test for Reducing Sugars

Materials Required

Test tube rack Distilled water (dH2O) Test tube holder

7 test tubes Transfer pipettes Red wax pencil (or Sharpie)

Starch solution Benedict's reagent Boiling water bath (or heat block)

Unknown (#1- #4) Glucose solution Potato

Cow's milk Sucrose solution

*Each lab group should pick **only ONE** of the 4 unknown solutions to use for each of the following tests. Different lab groups will use different unknown solutions and then relay the results of their unknown solution to the other lab groups.

Procedure:

wear gloves and safety goggles when performing this activity

1. Use the red wax pencil (or Sharpie) to number seven clean test tubes #1 through #7.
2. Use a pipette to transfer **1 mL** of the test substances/solutions listed in Table 2 to the corresponding labeled test tubes.
3. Add **0.5 ml of Benedict's reagent** to each tube. Swirl to mix. Record the color of solution in each tube in Table 2 below before heating the tubes.
4. Place the tubes in a **gently boiling water bath (or heat block) for 2 minutes**. Using a test tube holder, carefully remove the test tubes from the water bath and place in the test tube rack to cool. Hold a white sheet of paper behind the tubes to more easily see the colors. Record any color changes, or changes in appearance, in Table 2.
5. Use color change information to determine the conclusion for each tube (whether or not each solution contains reducing sugars). A positive result indicates that the solution contains the macromolecule being tested for (reducing sugars). A negative result means that the solution does not contain reducing sugars.
6. **Discard contents of test tubes in the location indicated by your instructor**.
    1. Use test tube brushes to **wash tubes with soapy water.** Rinse thoroughly and shake out excess water. Return test tubes to the proper location.
    2. Some test tubes are disposable. If this is the case, recycle them in the proper receptacle.

Table 2. Results of Benedict's Test for Reducing Sugars

Test Tube	Substance Tested	Color before heating	Final color after Test	Conclusion: Positive (+) or Negative (-)
1	Distilled water			
2	Glucose solution			
3	Milk			
4	Starch solution			
5	Sucrose solution			
6	Potato			
7	Unknown # ____			

Exercise 3: Testing for Carbohydrates - Iodine Test for Starch

Polysaccharides are very long chains of monosaccharides and **do not react with Benedict's reagent**. Starch, cellulose and glycogen are examples of polysaccharides. Because these complex carbohydrates are not reducing sugars, and therefore do not chemically react with Benedict's reagent, a different indicator is required to test for the presence of these complex polysaccharides.

Starch is the storage polysaccharide of plants and is highly digestible when consumed by animals. **Iodine** (aka Lugol's Iodine) (I2KI), an amber-colored clear liquid, is the indicator used to detect the presence of starch. The starch molecules interact with iodine to produce a dark blue-black color (Figure 4). **Glycogen**, the storage polysaccharide in animals, reacts to a lesser extent with Lugol's to produce a red-brown or reddish-purple color.

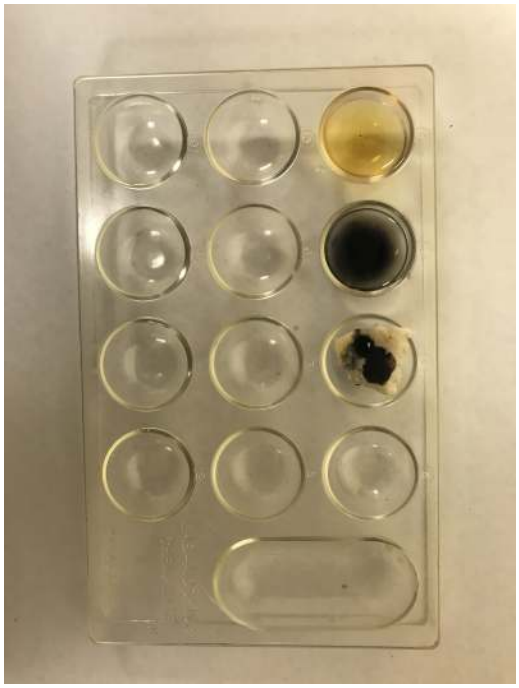

Figure 4. Iodine Test for Starch. Negative (left) and positive (right two wells) results of iodine tests for starch (original photo)

Iodine Test for Starch

Materials Required

dH2O Transfer pipettes sucrose solution Toothpicks

Starch solution Glucose solution

Iodine Unknown (#1 - #4) cracker

Potato paper 9 or 12-well spot plate (Figure 5)

Figure 5. Well plate (original photo)

Procedure:

1. Use the red wax pencil or Sharpie to label the wells of the well plate with #1-8 (if not already labeled).
2. Use a transfer pipette to transfer 1ml of the test substances listed in Table 3 to the appropriately numbered well. *For the solid substances, cut a small piece of the substance and place it into the appropriately numbered well.
3. Add **2 drops of Iodine** to each well (or onto the solid substance). Mix using a toothpick (for solutions). **Use a new toothpick for each well**.
4. Place the well plate over a white paper so any color changes are easily visible. Record the color of each reaction in Table 3.
5. **Discard the contents of the well plate as instructed. Rinse the plate thoroughly with soap and water.**

Table 3. Results of Iodine Test for Starch

Well	Substance Tested	Final color after Test	Conclusion: Positive or Negative
1	Distilled Water		
2	Starch Solution		
3	Glucose Solution		
4	Sucrose Solution		
5	Paper		
6	Potato		
7	Cracker		
8	Unknown # ____		

III. Proteins

Proteins are essential for organisms to survive and are a highly abundant macromolecule in the body. The monomer building blocks of proteins are **amino acids**. Amino acids are linked through covalent peptide bonds to form polypeptides, also known as proteins. **Proteins serve diverse and vital roles** in our bodies. Some proteins are important structural proteins in cells, such as tubulin. Other proteins play vital structural and protective roles in organisms, such as keratin. Actin and myosin are proteins that work together in muscle cells to provide movement. Most enzymes, such as DNA polymerase, are proteins and are essential to speed up biological reactions in cells. Ribulose-1,5- bisphosphate carboxylase (commonly known as Rubisco), catalyzes carbon fixation

during photosynthesis and is thought to be the most abundant enzyme on earth. Additionally, antibodies are proteins produced by the immune system to protect us from invading pathogens.

Exercise 4: Testing for Proteins

Biuret reagent, a light aquamarine-colored liquid, is used to detect the presence of proteins. Copper ions in the Biuret reagent react with peptide bonds causing a color change from its original color to purple or pink. Proteins with short peptide chains turn pink; those with longer chains turn purple (Figure 6). Other types of molecules can cause color changes, but *only the purple or pink colors indicate the presence of peptide bonds.* Note that a positive Biuret reaction only occurs at an elevated pH; therefore, Biuret reagent contains a strong base (NaOH) turning it a turquoise color. Some protocols include adding additional NaOH to test tubes at the time of protein testing.

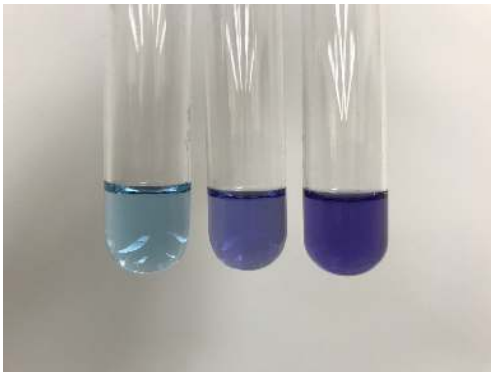

Figure 6. Biuret test for protein. Negative test remains light blue (left tube). Positive result shows change in color to violet (right two tubes). (original photo)

Biuret Test for Proteins

Materials Required

5 Test tubes Toothpicks Albumin

Unknown (#1 - #4) Biuret reagent Milk

Sucrose solution Transfer pipettes dH2O

Procedure:

wear gloves and safety goggles when performing this activity

1. Use the red wax pencil (or Sharpie) to number 5 clean test tubes # 1 through # 5.
2. Use a pipette to transfer **1 mL** of the test substances/solutions listed in Table 4 to the corresponding labeled test tubes.
3. Add **0.5 ml of Biruet reagent** to each test tube. Swirl to mix. Record the color of the liquid in each well in Table 4. Hold a white sheet of paper behind the test tubes to more easily see the colors of the solutions.
4. **Discard contents of the test tubes in location indicated by your instructor.**
    1. Use test tube brushes to **wash tubes with soapy water.** Rinse thoroughly and shake out excess water. Return test tubes to the proper location.
    2. Some test tubes are disposable. If this is the case, recycle them in the proper receptacle.

Table 4 Results of Biuret Test for Protein

Test Tube	Substance Tested	Final color after Test	Conclusion: Positive (+) or Negative (-)
1	Water		
2	Albumin (egg white)		
3	Sucrose Solution		
4	Milk		
5	Unknown # ___		

IV. Lipids

Lipids are a diverse group of nonpolar, hydrophobic, energy-dense organic molecules. Lipids such as triglycerides, phospholipids and sterols play many important biological roles. All membranes in a cell are composed of phospholipids. Many hormones important in sexual development are derivatives of sterol molecules. The most abundant type of lipid in the human diet and human body is **triglycerides**. Triglycerides consist of three fatty acids bound to one glycerol molecule. If the fatty acids contain only single bonds between the carbon atoms then the fatty acid (and the triglyceride) is "saturated" with hydrogens and referred to as a saturated fatty acid. If the fatty acids contain one or more double bonds between the carbon atoms then the fatty acid (and triglyceride) is referred to as an unsaturated fatty acid. Saturated triglycerides are solid at room temperature and are commonly called fats. Unsaturated triglycerides are liquid at room temperature and are commonly called oils.

Exercise 5: Ethanol Emulsion Test for Lipids

Lipids are nonpolar molecules and cannot dissolve in polar solvents such as water. However, lipids can dissolve in nonpolar solvents such as ethanol. The presence of lipids can be tested using an **ethanol emulsion test**. An emulsion is formed when two substances that do not dissolve into one another are mixed together. A common example of an emulsion is oil and vinegar salad dressing. When undisturbed, the oil and vinegar separate out into two distinct layers. When you shake it up, the oil and vinegar combine, and the oil forms tiny droplets floating in the vinegar.

Ethanol is an amphipathic molecule; it has both polar and nonpolar ends. Because of the nonpolar component of the molecule, ethanol can dissolve lipids; however, because of it's polar component, ethanol can also mix with water. The **ethanol emulsion test** works because of the amphipathic nature of ethanol. When lipids are present in a sample, they dissolve in the first step when mixed with ethanol, and the mixture remains clear. However, in the second step of the test when added to water, the lipids are forced out of solution and appear as tiny fat droplets, which reflect light and appear whitish (Figure 7). The ethanol emulsion test allows fats in solid materials (such as potato chips) to be extracted in ethanol and then form an emulsion when added to water.

Figure 7. Negative ethanol emulsion test (left) and positive ethanol emulsion test (right two tubes). (original photo)

Materials Required

Test tube rack Transfer pipettes dH2O

12 test tubes Ethanol Half and half

Red wax pencil (or Sharpie) Vegetable oil Unknown (#1 - #4)

Sucrose solution Mortar & pestle Parafilm

Procedure:

1. Use the red wax pencil (or Sharpie) to number 6 clean test tubes #1 through #6. Then, repeat, labeling a second set of test tubes #1 through #6.
2. Use a pipette to transfer **1 mL** of the test substances/solutions listed in Table 5 to the corresponding labeled test tubes. *For solid substances, crush the test substance into small pieces, using a mortar and pestle, and add it to the test tube to approximately the same height on the test tube as the liquid substances.
3. Use a clean pipette to transfer **2 mL of ethanol** into each tube. Use a gloved finger or parafilm to cap the tubes and shake well to mix.
4. Allow the contents to settle for about 30 seconds.
5. Use a clean pipette to remove the top half of the solution and transfer it to a clean labeled test tube.
6. Add **2mL of dH2O** to each tube, and observe the results. The appearance of a milky whitish layer indicates the presence of lipids in the sample. Record your observations in Table 5 below.
7. **Discard contents of test tubes in the location indicated by your instructor**.
    1. Use test tube brushes to **wash tubes with soapy water.** Rinse thoroughly and shake out excess water. Return test tubes to the proper location.
    2. Some test tubes are disposable. If this is the case, recycle them in the proper receptacle.

Table 5. Results of ethanol emulsion test for lipids

Test tube	**Substance Tested**	**Observations at end of test**	**Conclusion:** **Positive (+) or Negative (-)**
1	Distilled Water		
2	Vegetable Oil		
3	Sucrose Solution		
4	Half and half		
5	Potato chips		
6	Unknown #____		

Exercise 6: Grease Spot Test for Lipids (alternative lipid test)

The grease spot test is a simple test to observe the presence of lipids in a substance. In the grease spot test, a drop of the test substance is placed onto brown paper and allowed to dry. If the test substance is a solid substance, then the solid is crushed and rubbed onto the brown paper. The liquids must be given ample time to dry. Water placed onto brown paper will dry and the spot will disappear. Substances containing lipids dry but still appear wet, leaving a translucent spot that is easily visible when the brown paper is held up to the light (Figure 8). You might have observed this type of result if you've noticed grease spots on a paper bag when picking up greasy take-out foods.

Figure 8. Negative grease spot test (left) and positive grease spot test (right). (original photo)

Materials Required

Sucrose solution Transfer pipettes dH2O

Brown paper squares Half and half Pencil

Vegetable oil Unknown

Procedure:

1. Use a pencil to label 6 small squares of brown paper with the test substances listed in Table 6.
2. Place a drop of the test substance on the appropriately labeled brown paper and rub it into the paper. If the test substance is a solid material then thoroughly rub the solid onto the brown paper, making sure that there is thorough contact with the test substance and paper.
3. Place the brown papers in a location where they are exposed to air and will dry quickly (such as on top of a test tube rack).
4. When the distilled water sample has dried (approximately 5-10 minutes), observe the brown papers by holding them up to the light. A clear, translucent spot indicates lipids are present. If the spot on the brown paper has evaporated and disappeared completely then no lipids are present.
5. Record your observations in Table 6 below.
6. Dispose of the used brown paper squares in the trash.

Table 6. Results of grease spot test for lipids

Brown paper	Substance Tested	Observation after drying	Conclusion: Positive or Negative
1	Distilled Water		
2	Vegetable Oil		
3	Sucrose Solution		
4	Half and half		
5	Potato chips		
6	Unknown #____		

Exercise 7: Testing Unknown Substances

1. In Table 7 compile the data from all of the tests of your unknown.
2. Determine what compounds (reducing sugar, starch, lipid, protein) are present in your unknown.
3. Based on the macromolecules in the unknown, try to determine the identity of your unknown food. The unknowns are all foods/beverages that are commonly consumed at breakfast time.

Table 7. Analysis of Unknown Substances

Unknown Solution #	Benedict's Test (+ or -)	Iodine Test (+ or -)	Biuret Test (+ or -)	Ethanol Emulsion Test (+ or -)	Grease spot test (+ or -)	List Organic Molecule(s) Present
1						
2						
3						
4						

Questions for Review

1. What simple test can you perform to tell whether a substance is organic or not?

2. What is the purpose of a positive control? Give examples of positive controls used in this lab. Be specific.

3. What is the purpose of a negative control? Why did we use water as a negative control in many experiments?

4. Most monosaccharides and disaccharides are reducing sugars. Explain why the disaccharide, sucrose, is not able to function as a reducing sugar?

5. Complete the table below:

Macromolecule	Indicator/Test used for detection	Positive results appear as..
Organic molecule		
Reducing Sugars (most simple carbohydrates)		
Starch		
Ethanol Emulsion Test		
Greasy Spot test		
Proteins		

6. What major characteristic do ALL lipids have in common? Explain.

Practical Challenge Questions

1. You are given an unknown sample and get the following results:

Biuret	Light purple color
Iodine	Yellowish color
Benedicts	Orange color
Ethanol Emulsion	Clear liquid
Flame test	blackened

- Based on these results, what can you infer about which organic molecule(s) are in the unknown sample?

- Speculate on the identity of this test substance. (what do you think the test substance could be).

- Are these test results examples of quantitative or qualitative data? Explain.

2. Describe a “limitation” for each of the following tests.

- Benedict’s Test -

- Grease Spot Test -

3. True or False? When an Unknown Solution changes to a violet/black color during the iodine test, this constitutes a **positive control**. Explain your answer.

4. Diabetes is a chronic disorder that results in an increased level of glucose in the bloodstream. It is caused by inadequate insulin, a hormone produced by the pancreas that allows cells to use and store glucose. One symptom of diabetes is excess glucose in the urine (glycosuria). Which test performed in the lab today could be used to assay a person’s urine to indicate diabetes? Describe the results of that test that would indicate the person has diabetes.

References

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Natale, E. G., Laura Blinderman, &. Patrick. (2021, March 19). Book: Unfolding the Mystery of Life - Biology Lab Manual for Non-Science Majors (Genovesi, Blinderman & Natale). “Exercise 5: Biomolecules.” CC-BY Retrieved April 5, 2021, from https://bio.libretexts.org/@go/page/24114

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1.4: Diffusion and Osmosis

The **cell theory** states that all living things are composed of cells and that cells only arise from other cells. Some cells are fairly simple in structure, while others are extremely complex. For example, some organisms are unicellular—they exist as a single cell, while multicellular organisms are composed of many cells that form tissues and organs. In either case, all cells share some common properties: the presence of DNA, intracellular proteins that enable the cell to perform its functions, and a plasma membrane. Some cells, known as eukaryotic cells, also contain membrane-bound organelles that allow a more complex level of functioning.

Homeostasis is defined as the maintenance of a stable internal environment. In order to maintain homeostasis, cells continually transport substances in and out across their cell (plasma) membrane.

The cell membrane serves as a "gatekeeper" and is the cellular structure that regulates the transport of materials into and out of the cell. The phospholipid bilayer architecture of the cell membrane allows certain molecules to pass through while keeping others out, therefore the cell membrane is **selectively permeable (or semipermeable)**. Things that need to enter a cell for it to function properly include ions, nucleotides, sugars, oxygen, amino acids, water, vitamins, and some hormones. Cells also allow certain molecules like water, ions, and secreted proteins to leave. Additionally, cells must eliminate waste products like urea and carbon dioxide

In the following exercises, you will examine the semipermeable nature of the cell membrane. You will also explore the concept of **tonicity**, which refers to the solute concentration of a solution, and its inherent ability to influence the rate and direction of osmosis.

PART 1: DIFFUSION & OSMOSIS

Diffusion is the movement of molecules from an area in which they are high in concentration to an area in which they are low in concentration. Molecules move down a **concentration gradient** until they are equally distributed, or **equilibrium** is reached (Fig. 1). At equilibrium, there is no concentration gradient. Molecules still move once equilibrium is reached, but there is no **net** movement in any one direction.

Figure 1 - Diffusion of molecules from an area of high concentration to low concentration in coffee. Equilibrium is reached when the molecules are equally distributed. (Pixabay License; iirliinnaa from Pixabay)

Osmosis is a specific type of diffusion: the diffusion of **water** molecules across a semipermeable membrane. Like other molecules, water molecules diffuse down a concentration gradient, from an area of higher free water concentration to an area of lower free water concentration. This means that water will move across a semipermeable membrane, like the cell membrane, in the direction of the higher solute concentration. (In solution, high solute concentration = low free water concentration; conversely, low solute concentration = high free water concentration.) You will observe this concept in **Part 2: Osmosis & Tonicity**.

In living organisms, most substances are transported as **solutes**, dissolved in water, a **solvent**. For example, if we dissolve salt ($NaCl$) in a beaker of water, salt ($NaCl$) is the solute and water is the solvent. Examples of solutes in the human body include glucose, small proteins, and electrolytes like calcium and sodium ions. Waste products, such as CO_2 and urea are also transported as solutes. Solutes are carried by body fluids, such as blood plasma, and pass into and out of cells through **passive** and **active transport**. In either case, the cell membrane will either inhibit or facilitate the process of diffusion: some molecules can easily diffuse across a plasma membrane and some cannot. For example, small, nonpolar molecules (such as CO_2 and O_2) can cross a membrane by simple diffusion. Large molecules or polar molecules, however, cannot easily diffuse across a membrane. Cells must have specialized membrane-bound proteins that function to transport such substances across the membrane.

In **Part 1: Diffusion & Osmosis**, you will learn about diffusion and osmosis using dialysis membrane, a selectively permeable sheet of cellulose that permits the passage of water and small solutes, but does not allow larger molecules to diffuse across. This is because the membrane has microscopic pores that only allow small molecules through; anything larger than the size of the pores is prevented from crossing. Some of the solutes in this experiment, **sucrose** ($C_{12}H_{22}O_{11}$) and **starch** (Extra close brace or missing open brace are too large to pass through the pores of the dialysis tubing, but the solvent molecules (H_2O) and **glucose** ($C_{12}H_{22}O_{11}$), are small enough to pass easily.

Exercise 1: Molecular Weight and Diffusion Rate

Molecular weight is an indication of the mass and size of a molecule. The purpose of this experiment is to determine the relationship between molecular weight and the rate of diffusion through a semisolid gel. You will investigate two dyes, methylene blue and potassium permanganate.

Molecule	Molecular Weight	Color
Methylene blue	300 grams/mole	blue
Potassium permanganate	150 grams/mole	purple

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials:

- Petri dish of agar semi-solid gel (Mueller Hinton agar plates, 150 x 15 mm) - make sure the agar has been allowed to come to room temperature
- Methylene blue solution (0.2% in 25% EtOH)
- Potassium permanganate solution (0.1% KMnO4)
- Small straws
- Small plastic metric ruler

Procedure:

1. Obtain a petri dish of agar

2. Take the plastic straw and gently stick it down into one side of the agar. Lift up the straw, withdrawing a small plug of agar. Repeat on the other side of the dish.

3. Using a 1mL transfer pipet, place a single drop of each dye into the appropriate agar well. (Fig. 2).

Figure 2 - Diffusion Experimental setup (by BB)

4. After 20 minutes, place a small, clear metric ruler underneath the Petri dish to measure the distance (diameter) that the dye has moved. Enter the data in Table 1.

5. Repeat step 4 at 40, 60, and 80 minutes.

Table 1 - Diffusion results

	Molecular Weight (g/mole)	Diameter after 20 min. (mm)	Diameter after 40 min. (mm)	Diameter after 60 min. (mm)	Diameter after 80 min. (mm)
Methylene blue					
Potassium permanganate					

Questions:

- What is the relationship between molecular weight and the rate of diffusion? Explain.

  _____

- Go back and look at your initial hypothesis. Does your data support this hypothesis? Explain.

  _____

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 1.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Exercise 2: Diffusion Across a Membrane

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials:

- Dialysis tubing
- Plastic clips or string
- 5 x 400 mL beakers (or plastic cups)
- Electronic balance
- Weigh boats
- 15% Sucrose solution (MW sucrose = 342 g/mol)
- 30% Sucrose solution (MW sucrose = 342 g/mol)
- 30% Glucose solution (MW glucose = 180 g/mol)
- Graduated cylinders (10 mL and 100 mL)
- Wax pencil or sharpie
- 15% starch solution (MW = variable)

- Iodine solution (MW = 166 g/mol)
- Benedict's reagent
- Hot plate or heat block

Procedure:

1. Cut 5 pieces of dialysis membrane approximately 10 cm long. Soak the pieces in tap water until they are soft and pliable (3-5 minutes). *This step may be done for you; check with your instructor.
2. Obtain 5 beakers (plastic cups) and label them #1 - 5. Fill each beaker with 150 mL of a solution as follows:

- Beaker #1 – H2O
- Beaker #2 – H2O
- Beaker #3 – H2O
- Beaker #4 – 30% sucrose solution
- Beaker #5 - H2O and 1mL Iodine solution

3. Set beakers aside.
4. Remove one piece of dialysis membrane from the soaking water and open it, forming a tube. Close one end of the tube with a plastic clip, a piece of string, or simply tie it with a knot (Fig. 3)

Figure 3 - Dialysis tube "bag" (By BB)

5. Fill the bag with 10 mL of H2O. Remove excess air, and close the other end of the bag with a plastic clip, a piece of string, or tie with a knot. Set aside on a paper towel.
6. Repeat steps 4 and 5 for the 4 remaining dialysis tubes, filling them with 10 mL of a solution as follows:

• Bag #2 – 15% sucrose

• Bag #3 – 30% sucrose

• Bag #4 – H2O

• Bag #5 - 5 mL 30% glucose solution and 5 mL 15% starch solution

7. Rinse off the outside of the bags with water and carefully blot dry.
8. Weigh bags #1 - 4 to the nearest 0.5g. Record the weights in Table 1 below, in the column labeled "0 min."
9. Place each bag in the corresponding beaker (Bag #1 in Beaker #1, etc.). Make sure each bag is fully submerged in the solution.
10. Set a timer for 5 minutes.
11. At the end of 5 minutes, remove bags 1 - 4 from their beakers, blot excess fluid, and record the mass (in grams) in Table 1.
12. Return the bags to the appropriate beaker, and wait another 5 minutes.
13. Repeat steps 11 - 13 every 5 minutes and record the weights in Table 1.

Table 1. Osmosis - mass (g) over time for dialysis bags

Time (min.)		0	5	10	15	20
Beaker #1 (water)	**Bag #1 (water)**					
Beaker #2 (water)	**Bag #2 (15% sucrose)**					
Beaker #3 (water)	**Bag #3 (30% sucrose)**					
Beaker #4 (30% sucrose)	**Bag #4 (water)**					

14. Calculate the total weight change (weight change = final weight – initial weight) for each bag. Record the values in Table 2. Calculate the **rate** (g/min) of osmosis for each bag by dividing the weight change by the time change. Since all 4 bags were recorded for a total of 20 minutes, the time change for all 4 bags is 20 minutes. Record the rate of osmosis for all 4 bags in Table 2.

Table 2. Rate of osmosis

	Weight Change (g)	Time (min)	Rate (g/min)
Bag #1 (water)			
Bag #2 (15% sucrose)			
Bag #3 (30% sucrose)			
Bag #4 (water)			

15. Make observations about bag #5 and beaker #5 in Table 3.
16. Remove several mL of liquid from bag #5 and beaker #5 and add each to separate test tubes.
17. Add several drops of Benedict's solution to each of the two test tubes and heat to 100 degrees Celsius in a boiling water bath or heat block for 2 - 5 minutes. Record the test results in Table 3.

Table 3. Selective Permeability

	Appearance of liquid after 20 minutes	Appearance of liquid after heating
Bag #5 (5 mL 30% glucose solution and 5 mL 15% starch solution)		
Beaker #5 (H2O and 1mL Iodine solution)		

Questions

1. Did the weight of each bag (#1 - #4) change significantly over 20 minutes? Explain.
2. In which bag(s) was there a **net movement** of water?
3. Explain what is meant by “net movement”.
4. Which carbohydrate molecules (glucose, sucrose, starch) were not able to move across the membrane? Explain.
5. In terms of **solvent** (water) concentration, water moved from the area of _____ concentration to the area of _____ concentration across a selectively permeable membrane, which is defined as _____.
6. What can you conclude about the movement of Iodine, glucose, and starch across the dialysis membrane based on your results in Table 3? Support your answers for each with the observation from bag #5 and beaker #5.

- Iodine -
- Glucose -
- Starch -

7. We used the dialysis tubing to simulate a cell membrane. How is the dialysis tubing functionally the same as a cell membrane?
8. We used the dialysis tubing to simulate a cell membrane. How is the dialysis tubing functionally different from a cell membrane?

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Prepare a line graph using the data from Table 2.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

PART 2: OSMOSIS & TONICITY

Tonicity is the relative concentration of solute (particles), and therefore also a solvent (water), outside the cell compared with inside the cell.

• An **isotonic solution** has the same concentration of solute (and therefore of free water) as the cell. When cells are placed in an isotonic solution, there is no net movement of water.

• A **hypertonic solution** has a higher solute (therefore, lower free water) concentration than the cell. When cells are placed in a hypertonic solution, water moves out of the cell into the lower free water solution.

• A **hypotonic solution** has a lower solute (therefore, higher free water) concentration than the cell. When cells are placed in a hypotonic solution, water moves into the cell from the higher free water solution.

IMPORTANT NOTE: Notice that all of the above definitions have ‘solution’ as the noun. Sometimes the noun will refer to the cell instead of the solution. For example, a hypotonic cell will experience a net movement of water out of the cell. What this means is that if the ‘solution’ is hypotonic, the cell is hypertonic and vice versa.

Exercise 1: Observing Osmosis in Potato Strips

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials:

- Potatoes
- Cork borer
- Petri dish

- Wax pencil or Sharpie
- Metric ruler
- 10% NaCl solution
- 0.9% NaCl solution
- Forceps and scalpel

Procedure:

1. Obtain a potato and use a cork borer to prepare 3 cylinders of potato. Push the borer through the length of the potato. When the borer is filled, use the flat end of a wooden skewer to gently push out the potato cylinder into the petri dish. Use the scalpel to cut each potato cylinder into a length of 5 cm.
2. With a wax pencil or Sharpie, label 3 test tubes (#1, #2, #3).
3. Using the metric ruler, mark each tube at the 10 cm mark level from the bottom of the tube.
4. Set up three test tubes with 10 cm of solutions as follows:
    1. Tube #1 - distilled water
    2. Tube #2 - 10% sodium chloride (NaCl)
    3. Tube #3 - 0.9% NaCl
5. Place one potato cylinder into each test tube and allow them to soak for about 15 minutes in the solutions.
6. You can now move on to **Exercises 2 and 3** while your potatoes soak.
7. After the elapsed time, observe each strip for limpness (water loss, flaccid) or stiffness (water gain,turgor).

Questions

1. Which tube contained the limp (flaccid) potato strip? Explain.
2. Which tube contained the stiff (turgid) potato strip? Explain.
3. Which solution is isotonic to the inside of the potato cell?
4. What happened to the potato strip in the isotonic solution?

Osmoregulation in Living Cells

Some organisms, known as **osmoregulators**, have special adaptations to keep tight control over their internal osmotic conditions while still others, known as **osmoconformers**, are able to live in a variety of osmotic conditions. Most living cells, however, are often at the mercy of their surrounding osmotic environment. Many freshwater plants live in an isotonic or hypotonic environment, so they have no adaptations to protect them from a hypertonic environment. Likewise, mammalian red blood cells live in the isotonic plasma inside your circulatory system so they have no protection from either a hypertonic nor a hypotonic environment.

A plant cell is surrounded by a rigid cell wall, so when the cell is placed in a hypotonic environment, the net flow of water is from the surrounding medium into the cell, and it simply expands to the cell wall and becomes turgid. When the same plant cell is placed in a hypertonic environment, water leaves the central vacuole and the cytoplasm shrinks. This causes the cell membrane to pull away from the cell wall. In this situation, the plant cell will undergo plasmolysis and die. Animal cells have no cell wall so when they are in a hypotonic environment they will expand and fill with water until they burst in a condition known as lysis. **Figures 4 and 5** demonstrate these conditions.

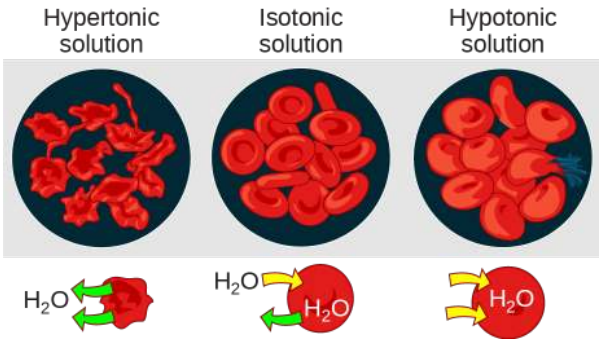

Figure 4 - Red blood cells in varied osmotic environments.(Diffusion and Osmosis. (2021, February 28). Retrieved April 1, 2021, from https://chem.libretexts.org/@go/page/34826 CCBY)

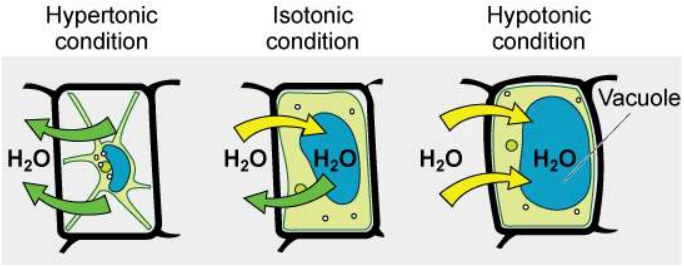

Figure 5 - Plant cell tonicity. The turgor pressure within a plant cell depends on the solution's tonicity in which it is bathed. (credit: modification of work by Mariana Ruiz Villareal CCBY)

Exercise 2: Observing Osmosis in Elodea Cells

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials

- Microscope
- Blank slides and coverslips
- DI water
- 10% salt solution
- Forceps
- Elodea leaf

Procedure

1. Place a drop of water onto a clean microscope slide.
2. Using the forceps, gently tear off a small leaf from the *Elodea* plant.
3. Prepare a wet mount by placing the *Elodea* leaf into the drop of water on your slide.
4. Place a coverslip onto the slide.
5. Use the **scanning** (4x) objective to bring the *Elodea* cells into focus. You may not be able to observe individual cells at this power.
6. Switch to the **low power** (10x) objective. The *Elodea* cell walls should be visible. They will look like dark green grid lines. Use the fine focus adjustment to focus the specimen.

7. Once you think you have located an *Elodea* cell, switch to the **high power** (40x) objective and refocus using the fine focus adjustment.
8. Next, add several drops of 10% salt (NaCl) solution to the edge of the coverslip to allow the salt to diffuse under the coverslip. Observe what happens to the cells (this may require you to search around along the edges of the leaf). Look for cells that have been visibly altered.
9. Record your observations in the following table. The cells in distilled water should look similar to the figure below.

Solution	Appearance of *Elodea* Cells
Distilled water (0% NaCl)	
10% NaCl	

Figure 3: *Elodea* cells at low power
"Anacharis 40x" by biologycorner, via Flickr

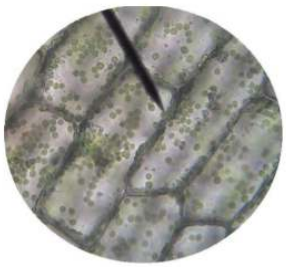

Figure 4: *Elodea* cells at high power
"Anacharis 400x" by biologycorner, via Flickr

Figure 3: *Elodea* cells at low power
"Anacharis 40x" by biologycorner, via Flickr

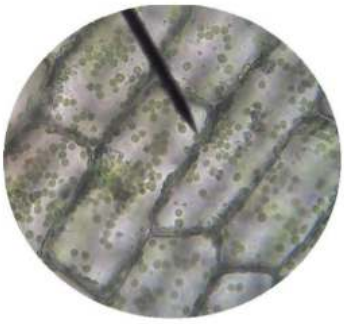

Figure 4: *Elodea* cells at high power
"Anacharis 400x" by biologycorner, via Flickr

Figure 6 Wet Mount of an Elodea **Leaf Cell (Burran, S., & DesRochers, D. (2021, March 19). Cells. Retrieved April 1, 2021, from https://chem.libretexts.org/@go/page/24084)**

Questions

1. Which solution is hypertonic to an Elodea cell? Use your observations to support your answer.
2. Would you expect pond water to be isotonic, hypotonic, or hypertonic to *Elodea* cells? Explain your answer.
3. Explain what happens to a plant cell that undergoes plasmolysis.

Exercise 3: Observing Osmosis in Red Blood Cells (Erythrocytes)

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials

- Microscope
- Wax pencil or Sharpie
- Blank slides and coverslips
- DI water
- 10% NaCl solution
- 0.9% NaCl solution
- Sheep red blood cells

Procedure

1. Obtain 3 test tubes and label them (#1, #2, #3) with a wax pencil or Sharpie.
2. Set up the three test tubes with 5 mL of solutions as follows:
   1. Tube #1 - Distilled water
   2. Tube #2 - 10% NaCl
   3. Tube #3 - 0.9% NaCl
3. Using a new transfer pipette, add 2 drops of sheep blood to each tube and swirl gently to mix the contents.
4. Hold each test tube up to a sheet of paper with printed text. Attempt to read the print through each tube and record your results in the following table.

Test Tube / Solution	Appearance of Solution	Can you read print?
#1 - Distilled water		
#2 - 10% NaCl		
#3 - 0.9% NaCl		

5. Label 3 microscope slides (#1, #2, #3) with a wax pencil or Sharpie.
6. Prepare wet mounts of each tube by placing a drop of the solution in each tube (#1 - 3) on the appropriate microscope slide (#1 -3). Add a coverslip to each slide.
7. View slide #1 through the microscope using the scanning (4x) objective first. Focus the image using the coarse adjustment. Then view the blood cells under low power and then high power. Only use the fine focus adjustment to focus the specimen.
8. Observe slide #2 and slide #3 in the same manner.
9. Record the appearance of the red blood cells in each solution in the following table.

Solution	Appearance of RBCs
#1 - Distilled water	
#2 - 10% NaCl	
#3 - 0.9% NaCl	

Questions

1. Which solution allowed you to read print through the solution? Explain.
2. Which solution is hypertonic to the RBCs? Use your observations to support your answer.
3. Which solution is hypotonic to the RBCs? Use your observations to support your answer.
4. Which solution is isotonic to the RBCs? Use your observations to support your answer.

Questions for Review

1. Define diffusion. What is the energy source for diffusion? Is diffusion considered an active or passive process? Explain.
2. Name a molecule that diffused through the artificial membrane (dialysis tubing) that we used in the laboratory. Can diffusion occur without a membrane? Give an example to support your answer.

3. What is osmosis? Is it an active or a passive process? Explain.
4. Fill in the blank in the following statements.
    1. A solution that has a lower solute concentration than another solution is said to be _____ when compared with the second solution.
    2. A solution that has the same solute concentration as another solution is said to be _____ when compared with the second solution.
    3. A solution that has a higher solute concentration than another solution is said to be _____ when compared with the second solution.
5. What does it mean when a membrane is selectively permeable?

Practical Challenge

6. Briefly explain what happens to a red blood cell when placed into the following solutions.
    1. Isotonic solution –
    2. Hypertonic solution –
    3. Hypotonic solution -
7. The concentration of glucose inside *Elodea* cells is 5 mM. What is the solution in moles (M)? What will happen to an *Elodea* cell if it is placed in a 1 M glucose solution? Explain.

8. Apply what you learned in the lab to explain why it is said that marine organisms, which live in saline environments, literally live in a desert environment.

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1.5: Mitosis and Meiosis I

In eukaryotic cells, the time and phases from the beginning of one cell division until the beginning of the next cell division is called the **cell cycle** (Figure 1)**.** The first phase of the cell cycle is **interphase.** Interphase is the time during which the cell performs its normal functions and prepares for cell division. Cells spend most of their time in this phase. During interphase, **chromosomes** are not visible because they are **decondensed** (present only as a tangled mass of thin threads of DNA with associated proteins, called chromatin). The nuclear membrane is present, and visible, as is the **nucleolus**.

Figure 1. The cell cycle.

Interphase includes two gap phases, G1 and G2, where the cell increases in size and synthesizes new organelles, enzymes, and other proteins that are needed for cell division. In between the two gap phases, the DNA replicates in preparation for cell division. This stage is called **S phase**. At the beginning of S phase, chromosomes are single and unreplicated. By the end of S phase, each chromosome has made an exact copy and consists of two **sister chromatids**. At this point in the cell cycle the sister chromatids are held together tightly at the **centromere**. While the two sister chromatids are physically joined together they are still considered one replicated chromosome (Figure 2). When the sister chromatids physically separate, later during the cell cycle, they are then considered to be individual chromosomes.

In animal cells, interphase is also when the **centrosome** (consisting of **two centrioles**) is replicated. Spindle fibers form from and radiate outward from the centrosomes to attach to and move chromosomes during cell division.

Figure 2. Chromosomes and sister chromatids.

Interphase is followed by **mitosis** (in the somatic cells) **or meiosis** (in reproductive cells), which is when replicated chromosomes and cytoplasm separate, during the process of karyokinesis and cytokinesis respectively.

Chromosomes that are the same length, have the same centromere location and the same gene sequences and positions are called homologous chromosomes. Human somatic cells contain pairs of homologous chromosomes. Each homologous pair consists of one maternal chromosome and one paternal chromosome. Cells that contain two copies of each chromosome are called **diploid** (2n, where n is the number of different chromosomes in a single set). Human sex cells (eggs and sperm) contain only one copy of each chromosome. Cells with only one copy of each chromosome are **haploid** (n). When the haploid sperm (n) and egg (n) combine during fertilization this forms a diploid zygote (2n).

MITOSIS

Mitosis is nuclear division that results in two cells containing the same number of chromosomes as the parent cell. Most human cells (skin, muscle, bone, etc.) divide by mitosis. This process is necessary for the normal growth and development of a multicellular eukaryotic organism from a zygote (fertilized egg), as well as growth and the repair and replacement of cells and tissues. At the end of mitosis, two daughter cells are formed that are identical to the original (parent) cell. Mitosis is also a form of asexual reproduction in unicellular eukaryotes.

Mitosis is a complex and highly regulated process. It occurs in the following 4 separate phases: prophase, metaphase, anaphase, and telophase. Telophase is quickly followed by cytokinesis.

Prophase: Cells prepare for division by coiling and condensing their chromatin into chromosomes. By late prophase, individual chromosomes can be seen, each consisting of two sister chromatids joined at a centromere. Spindle fibers begin to form from the centrosomes, which have begun to migrate to opposite “poles” of the cell. The nucleoli and the nuclear membrane degrade. (Figure 3)

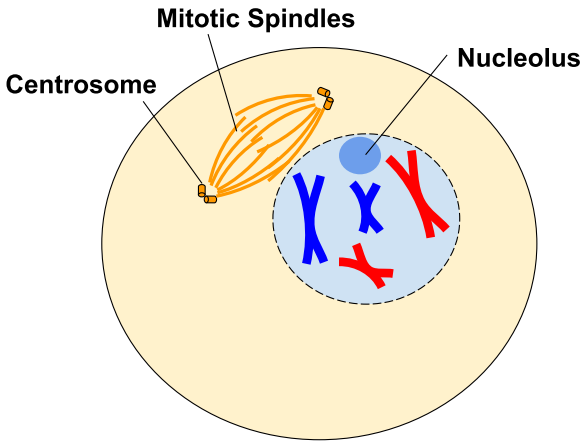


Figure 3. Cell in prophase

Metaphase: Spindle fibers (called kinetochore microtubules or kinetochore spindle fibers) that emanate from the centromeres attach to the kinetochore (a proteinaceous area) on the sister chromatids. The fibers pull and otherwise manipulate the chromosomes to align them on the plane that passes through the center of the cell (metaphase plate) (Figure 4). This "plate" is not an actual structure; it merely signifies the location of replicated chromosomes prior to their impending separation.

Other non-kinetochore spindle fibers or tubules (aka polar microtubules), emanating from the two centrosomes, elongate and eventually overlap with each other near the metaphase plate.

Figure 4. Spindle fibers attaching to kinetochores in metaphase.

Anaphase: The centromeres divide, with the help of separase enzymes, and separate the sister chromatids (Figure 5). This happens simultaneously in all the chromosomes. The kinetochore spindles shorten and pull each chromatid to which they are attached toward the pole (and centrosome) from which they originate. This equally distributes exactly half the chromosomal material to each side of the cell. In late anaphase, the non-kinetochore spindles begin to elongate, lengthening the cell.

Figure 5. Cell in anaphase.

Telophase: The non-kinetochore microtubules continue to elongate, further elongating the cell in preparation for cytokinesis (splitting of the cytoplasm). The chromosomes reach their respective poles. The kinetochores disappear. The two nuclear membranes (one in each half of the cell) begin to form around the chromosomes. Nucleoli reappear and the chromosomes in each soon-to-be new cell begin to decondense back into chromatin. Mitosis is complete at the end of this stage.

Cytokinesis (splitting of the cytoplasm):

In animal cells and all other eukaryotes without a cell wall, cytokinesis is achieved by means of a constricting "belt" of protein fibers that slide past each other near the equator of the cell. As this occurs, the diameter of the belt decreases, pinching the cell to form a **cleavage furrow** around the cell's circumference. As constriction proceeds, the furrow deepens until it eventually slices its way into the center of the cell. At this point, the cell is divided into two.

Plant cell walls are far too rigid to be split apart by contracting proteins. Instead, these cells assemble membrane proteins (in vesicles that bud off the Golgi apparatus) in their interior at right angles to the spindle apparatus. This expanding membrane partition, called a cell plate, continues to grow outward until it reaches the interior surface of the plasma membrane and fuses with it. This divides the cell in two.

The phases of Mitosis

Exercise 1: Modeling the Phases of Mitosis

Materials:

- Several sheets of blank paper (continuous printer paper is ideal)
- Chromosome modeling kits
  - Commercially available pop bead kits (e.g Carolina Biological Supply Company, Item #171100)
  - Homemade kits may consist of pipe cleaners or yarn or socks, etc. to represent chromosomes
- The following procedure will be described using a homemade kit consisting of pipe cleaners to represent chromosomes. The pipe cleaner chromosome kit contains:
  - 10 each – short red pipe cleaner sticks, short blue pipe cleaner sticks, long pipe cleaner red stick, long blue pipe cleaner sticks (Use as 2 homologous chromosome pairs)
  - 5 each – short red plastic lacing cord, short blue plastic lacing cord, long red plastic lacing cord, long blue plastic lacing cord (Use as 2 homologous chromatin pairs)
  - 20 white or grey beads (Use as centromeres)
  - Several red and blue beads (Use as genes for meiosis crossing-over)

Procedure:

Using models is a great way to represent natural structures and processes that are too small, or too large, or too complex to observe directly. By building chromosomes from the pipe cleaners and manipulating them to model cell division (mitosis and meiosis) you will enhance your understanding of the nature of chromosomes and the cellular structures needed to perform cell division. The pipe cleaner and plastic cord strands are intended to represent two pairs of homologous chromosomes. One pair of homologous chromosomes is longer than the other. Half of each pair is red and represents maternal DNA (genetic material contributed by a female's egg). The other half of each pair is blue and represents paternal DNA (genetic material contributed by a father's sperm). Thus, for each pair of homologous chromosomes, one should be red and one should be blue. The thin plastic lacing cord represents chromatin when chromosomes are in an uncoiled, decondensed state. The thicker pipe cleaner chromosomes represent the condensed chromosomes as they prepare for DNA replication and cell division. Alert your instructor if the chromosomes in your bag differ from those below.

This diploid cell with 2 homologous pairs of chromosomes will be modeled as it moves through the following phases of mitosis:

- Interphase (uncondensed DNA) **Before Synthesis** of DNA (G1)
- Interphase (uncondensed DNA) **After Synthesis** of DNA (G2)
- Prophase
- Metaphase
- Anaphase
- Telophase
- Cytokinesis

1. Use the lace cording chromosomes to model the **G1 phase of interphase** (before synthesis of the DNA). On the paper draw the cell membrane, nucleus, nucleolus, centrioles.
2. Use the lace cording chromosomes to model the **G2 phase of interphase** (after each chromosome was replicated during S phase). Use white beads to represent centromeres. Thread sister chromatids through a white bead to represent the duplicated chromosomes attached at the centromere. Centrioles would move toward opposite poles of the nucleus. Be sure to draw the cell membrane, nucleus, nucleolus, and centrioles on the paper.
3. Use the pipe cleaner chromosomes to model the **prophase** stage of mitosis. The chromosomes are condensed and distributed throughout the nucleus. On your paper be sure to note that the nuclear membrane begins to break down and spindle fibers begin to form and radiate toward the chromosomes. Spindle fibers attach to **kinetochore** proteins at the centromeres of the chromosomes.
4. Use the pipe cleaner chromosomes to model **metaphase**. Line up the individual chromosomes on the equator (middle) of the cell. Sister chromatids remain attached at the centromere during metaphase.
5. Model **anaphase** by removing the white beads (centromere) from the sister chromatids to separate and move them toward opposite poles of the cell. After separation at the centromere, the chromatids are now called chromosomes.

6. Anaphase ends and **telophase** begins when chromosomes reach opposite poles of the cell. Nuclear division happens in telophase. Nuclear envelopes and nucleoli reappear. Condensed chromosomes begin to decondense and uncoil. The formation of separate nuclear envelopes divide the nuclei and marks the end of telophase.
7. Model **cytokinesis** by drawing the formation of a cleavage furrow to divide the cytoplasm into two and form two separate cells.

How do the daughter cells you formed compare to the original parent cell?

_____

Observe the phases of Mitosis in Plant Cells

Exercise 2: Observing the Phases of Mitosis in the Onion Root Tip

Materials:

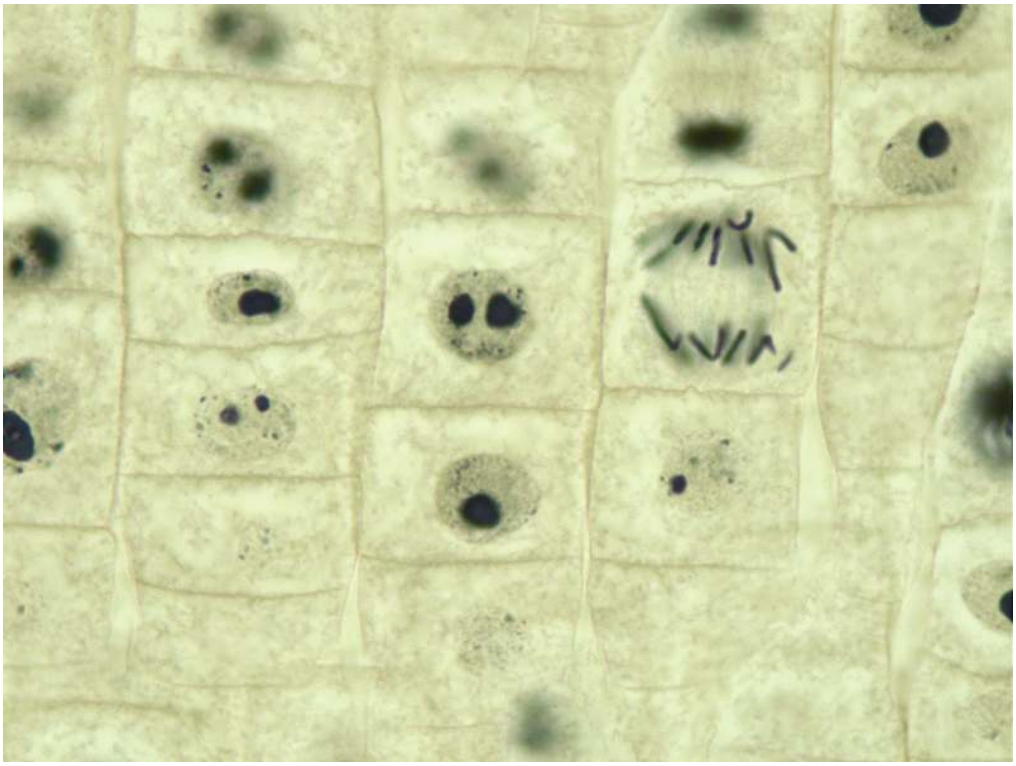
- Prepared slide of the onion root tip
- Compound light microscope

Procedure:

1. Examine a slide of a longitudinal section of an onion root tip. Adjust the slide to view the region just above the root cap, where there are likely to be dividing cells.
2. Focus on the dividing cells using the 4x scanning objective lens, then switch to the 10x objective and then the 40x objective.
3. Survey the slide to find a cell in each phase of mitosis. Draw a cell for each phase below.

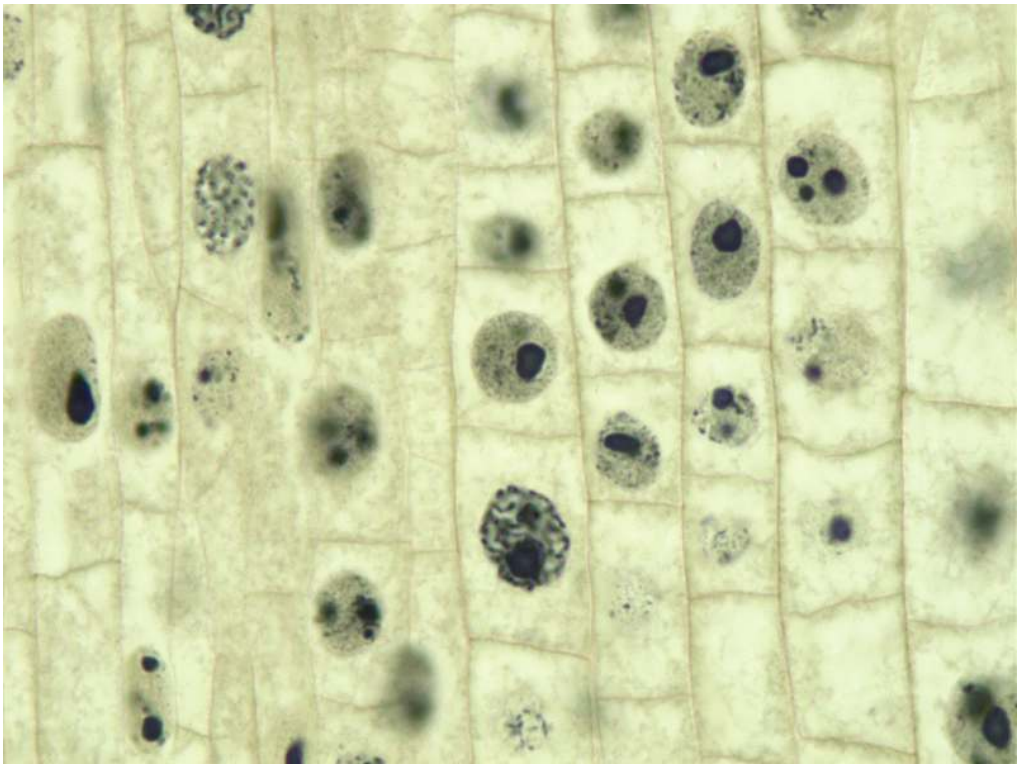
Interphase

DNA is uncondensed and in the form of chromatin. Individual chromosomes are not visible. The nuclear membrane is intact. The nucleolus is visible.



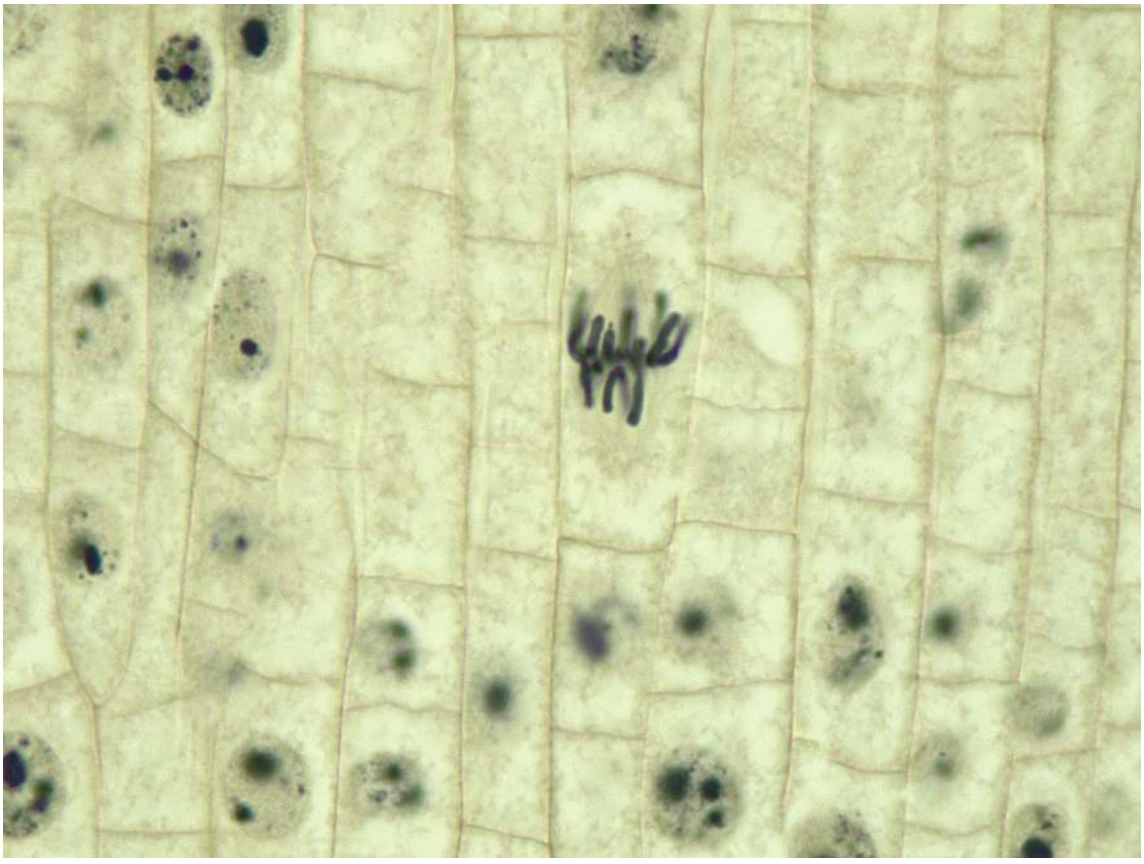
Prophase

Chromatin begins to condense into distinguishable chromosomes. These “puffy” structures are seen throughout the nucleus. Nucleoli begin to disappear. In late prophase (often called prometaphase) the nuclear membrane is no longer visible.



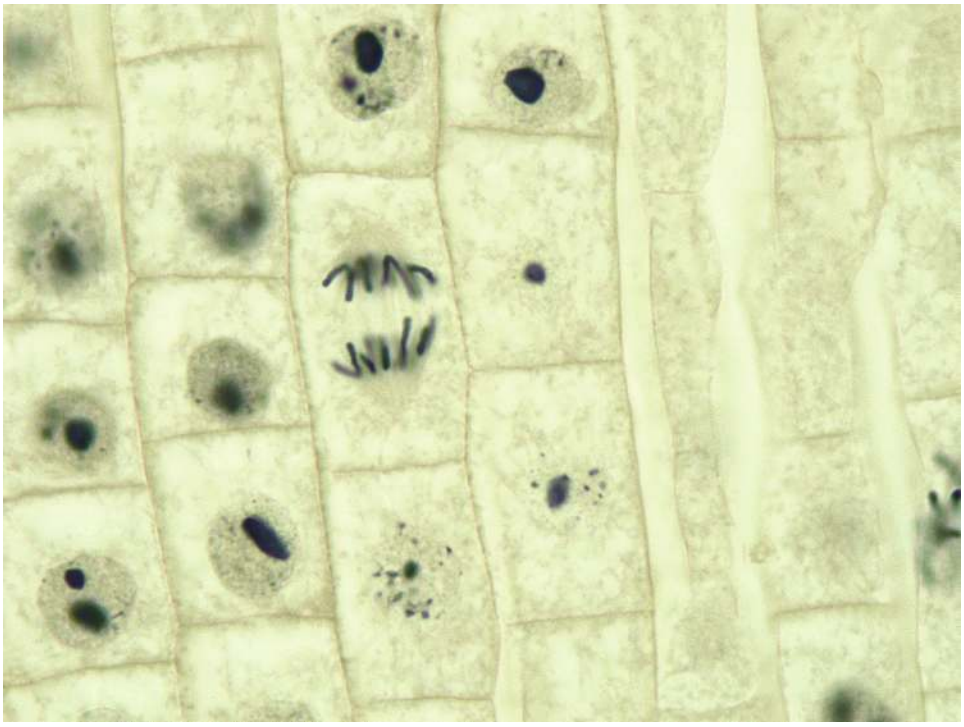
Metaphase

The chromosomes line up in the middle of the cell. Spindle fibers attach to kinetochores at the centromere and extend to the poles of the cell.



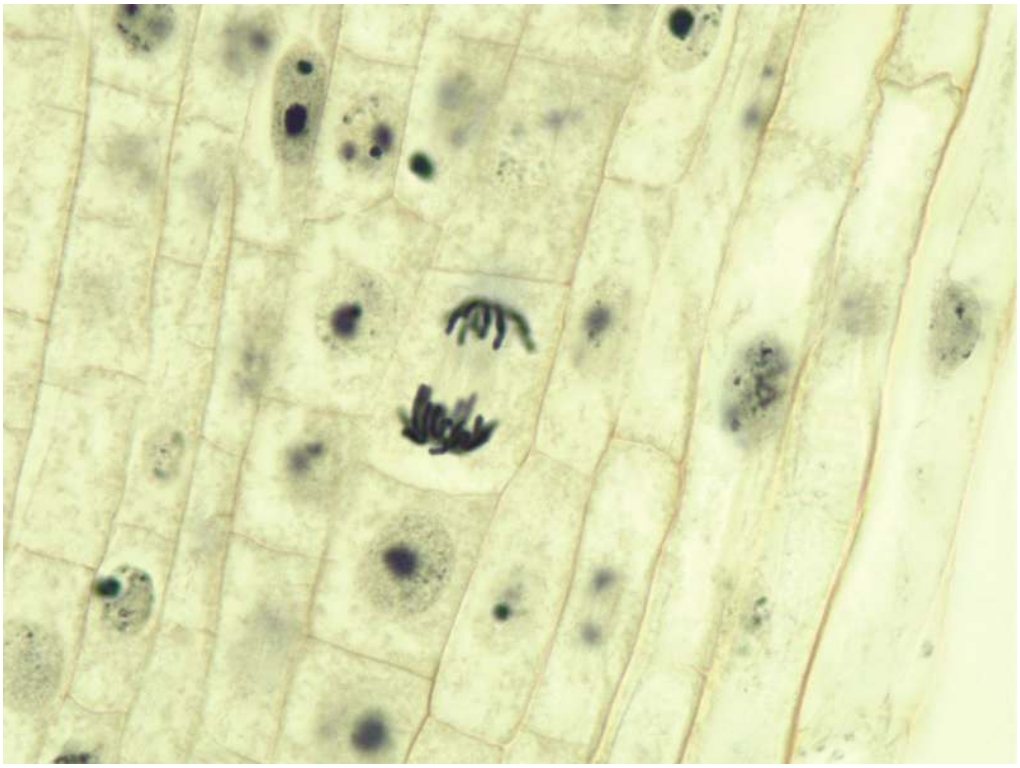
Anaphase

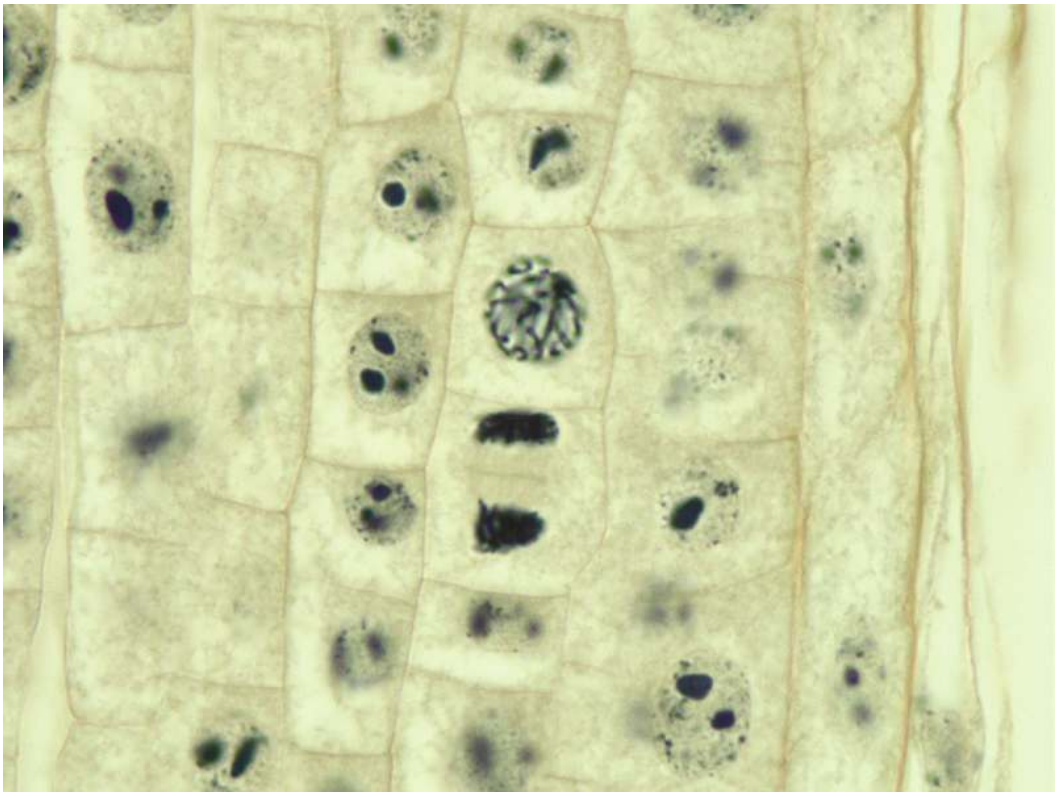
Centromeres split, separating each former chromatid into two individual chromosomes. The chromosomes move toward opposite poles.



Telophase and Cytokinesis

Chromosomes reach the poles. The nuclear envelopes begin to reform. The formation of a cell plate forms between the two cells to carry out cytokinesis.





Observe the phases of Mitosis in Animal Cells

Exercise 3: Observing the Phases of Mitosis in the Whitefish Blastula

Materials:

- Prepared slide of whitefish blastula

- Compound light microscope

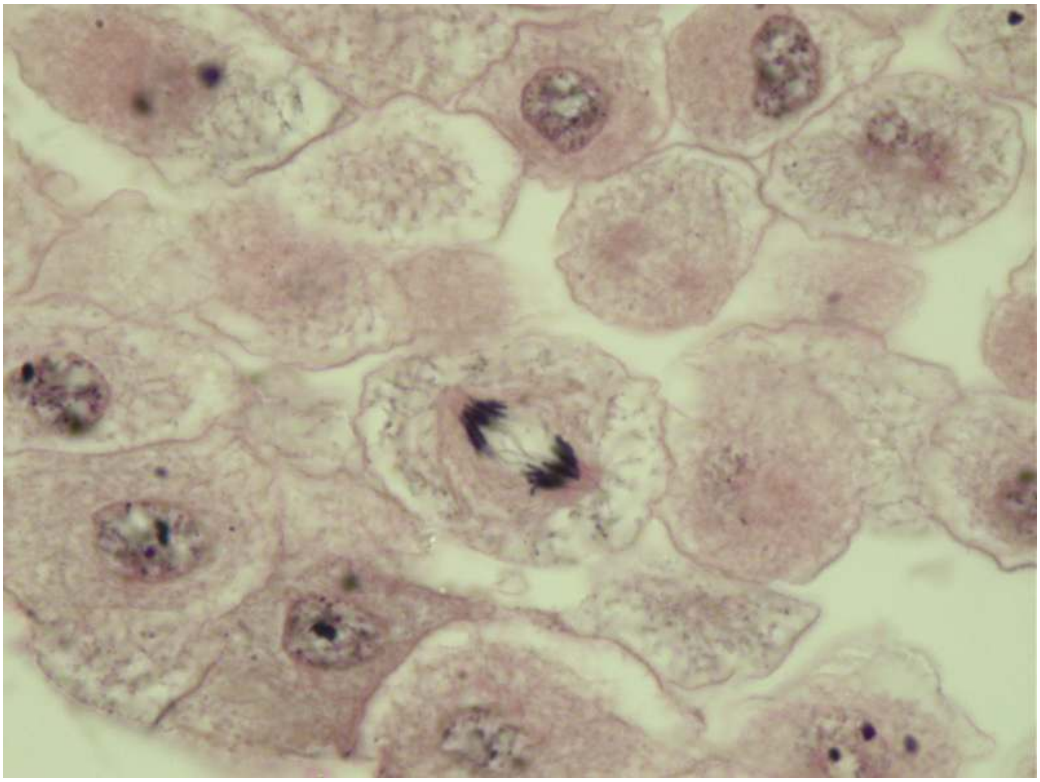
Procedure:

The blastula is an early embryonic stage where many of the cells are dividing at any one time.

1. Focus on the dividing cells using the 4x scanning objective lens, then switch to the 10x objective and then the 40x objective.
2. Survey the slide to find a cell in each phase of mitosis. Draw a cell for each phase below.

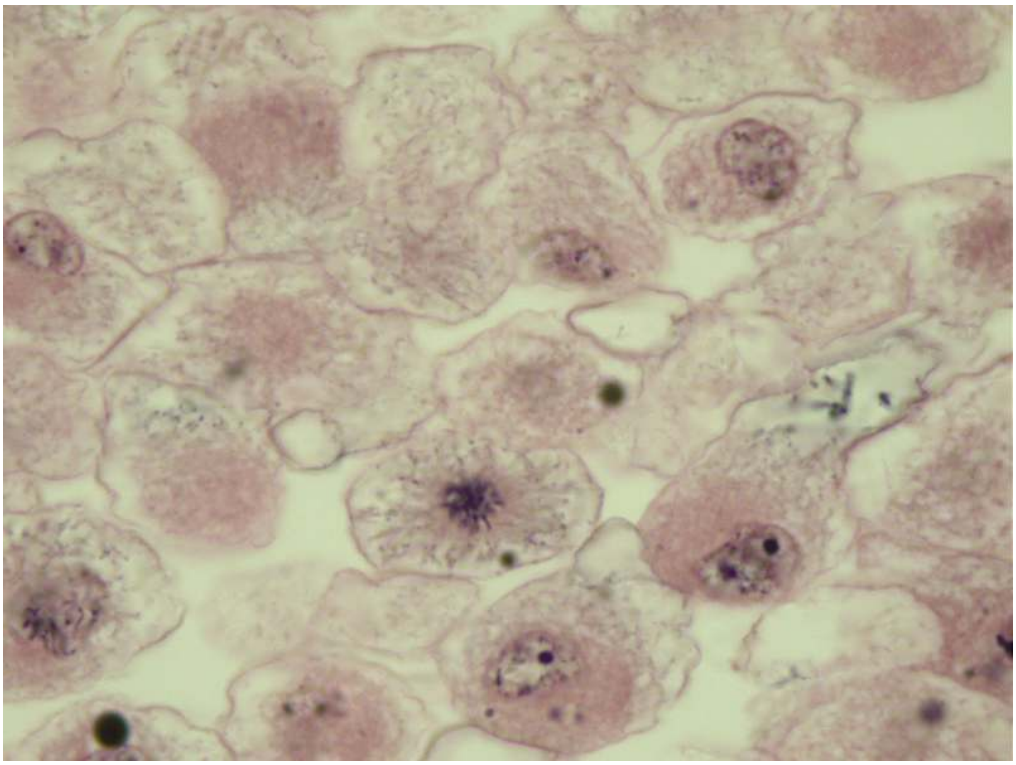
Interphase

The DNA is uncondensed and in the form of chromatin. Individual chromosomes are not visible. The nuclear membrane is intact. The nucleolus is visible.



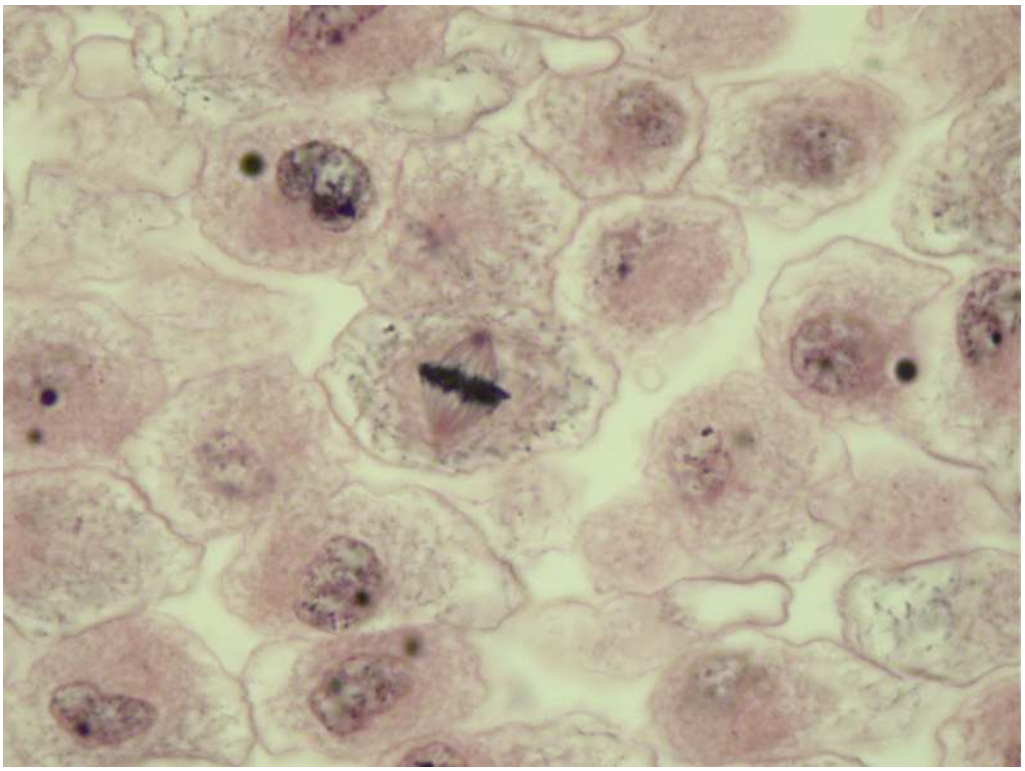
Prophase

Chromatin begins to condense and chromosomes are distinguishable. These “puffy” structures are seen throughout the nucleus. The nucleoli begin to disappear. In late prophase (often called prometaphase) the nuclear membrane is no longer visible.



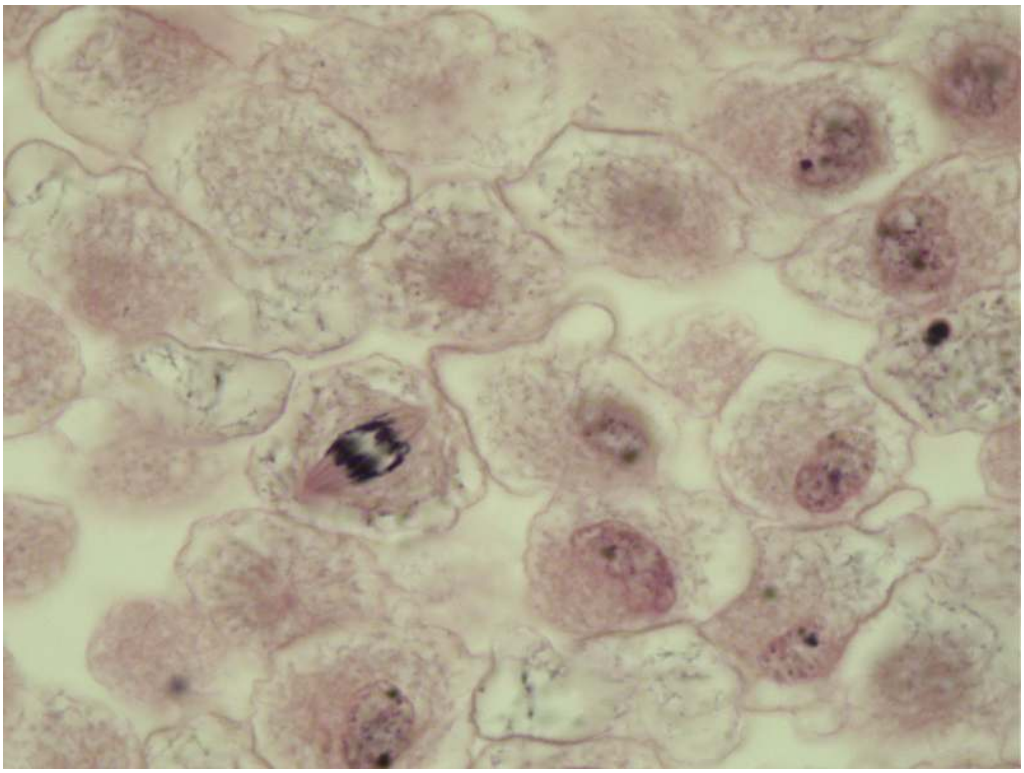
Metaphase

The chromosomes line up in the middle of the cell. Spindle fibers attach to kinetochores at the centromere and extend to the poles of the cell.



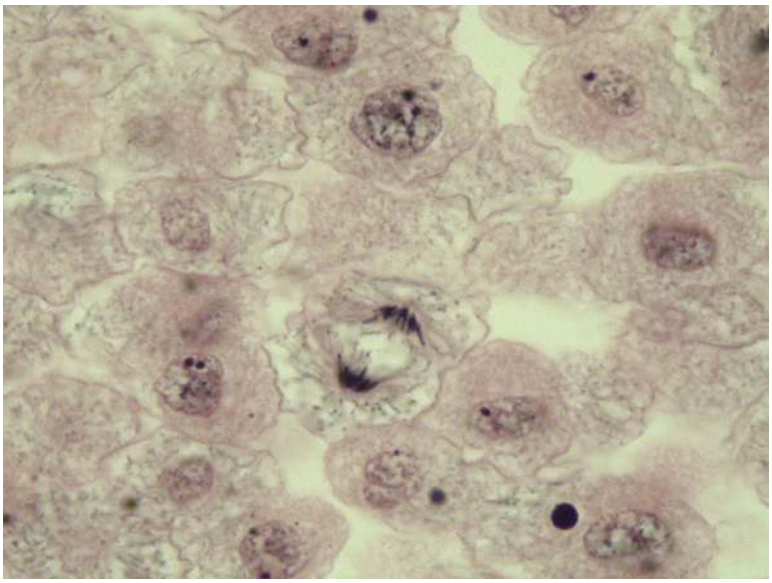
Anaphase

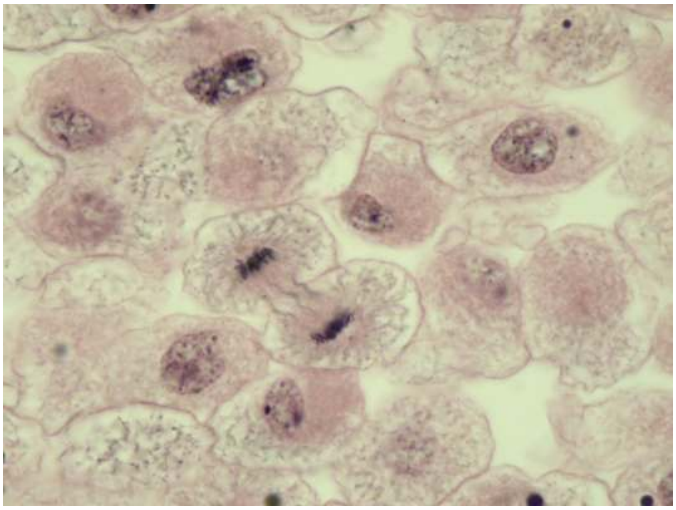
Centromeres split, separating each former chromatid into two individual chromosomes. The chromosomes move toward opposite poles.



Telophase and Cytokinesis

Chromosomes reach the poles. The nuclear envelopes begin to reform. A **cleavage furrow** forms between the two cells to carry out cytokinesis.





How Long Does a Cell Spend in Each Phase of the Cell Cycle?

Exercise 4: Determining Time Spent in Different Phases of the Cell Cycle (Optional)

Materials:

- Laptop
- Calculator

Procedure:

1. Obtain a laptop.
2. Open a web browser and go to the following site: **http://www.biology.arizona.edu**

1. This site will provide an interactive test of your ability to identify the stages of mitosis. It will also allow you to calculate the duration of the stages identified in the laboratory exercise you just completed, but the website will give standard results for the entire class.
2. Go to the **Cell Biology** section, and find the activity "**Online Onion Root Tips**".
3. Keep clicking on "Next" at the bottom of the page until you get to the screen: **Determining Time Spent in Different Phases of the Cell Cycle**

4. Click on “Next” at the bottom of the page.
5. Identify each stage shown to you by the program. When a picture of a cell pops up in a stage of mitosis, simply click on the phase in which the cell belongs. If you make a mistake, read the explanation for why you were mistaken before making a new selection.
6. When you are finished, use the formula given below and record your results in the table.
7. The duration of each stage of mitosis can be determined by using the following formula. Compute the length of time for each stage and place your calculations in the table below.

(Number of cells in a stage ÷ Total number of cells) x 1440 (min in a day) = minutes a cell spends

in each stage in one day

Number of cells in each stage	**Interphase** _____	**Prophase** _____	**Metaphase** _____	**Anaphase** _____	**Telophase** _____	**Total 36**
Percent of cells (# of cells / Total)						100%
Time (in minutes) spent in Stage – use calculation above						

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). “Lab 10: Mitosis & Meiosis,” (2019). *Biological Sciences Open Textbooks*. 18. CC-BY

https://oer.galileo.usg.edu/biology-textbooks/18

MEIOSIS

Introduction to Meiosis (aka “Reduction Division”)

Meiosis is a special type of cell division in which the daughter cells produced have half the number of chromosomes (n) as their parent cell. This division occurs in the reproductive organs (gonads -- testes of males or ovaries of females) of species that reproduce sexually, and results in the formation of gametes (eggs or sperm) that contain half the number of chromosomes as the original cell. Sexual reproduction involves the joining of gametes (fertilization) to form a zygote, which then has two copies of each chromosome (2n). Meiosis is a critical process, as it increases genetic diversity within a species.

Cells that divide by meiosis prepare for cellular division (during interphase) much like every other cell. Meiosis progresses through the same phases as mitosis (prophase, anaphase, metaphase, telophase, and cytokinesis). However, unlike mitosis, meiosis involves two rounds of cellular division (meiosis I and meiosis II). Meiosis involves only one round of DNA replication where each chromosome replicates to form sister chromatids. Therefore, when meiosis is completed, each daughter cell contains only half the number (n) of chromosomes as the original cell.

The stages of meiosis:

Meiosis I

Prophase l

Metaphase l

Anaphase l

Telophase l

Cytokinesis l

Meiosis II

Prophase ll

Metaphase ll

Anaphase ll

Telophase ll

Cytokinesis ll

Prophase I: During prophase of meiosis I, the chromosomes join in homologous pairs. Homologous chromosomes (aka homologs) are the same length, and carry genetic information (genes) for the same traits, but not necessarily the same versions (alleles) of the gene.

For example, human chromosome #19 contains a gene for eye color. In one person, one allele might code for blue eyes and the other allele codes for green eyes. Since every human inherits two copies of chromosome 19 (one from the mother's egg and one from the father's sperm) a person could have 2 blue alleles, 2 green alleles, or one of each.

Paired homologous chromosomes are called tetrads and are said to be in synapsis. During synapsis, equivalent pieces of homologous chromatids are exchanged between the chromosomes. This is called **crossing-over** and can occur several times along the length of the chromosomes. As occurs in the mitotic division, prophase of meiosis I also involves the degradation of the nuclear membrane and formation of spindle fibers.

Metaphase I: Metaphase of meiosis I occurs when the joined homologous chromosome pairs are moved to the center of the cell by spindle fibers (Figure 6). The fibers arrange the pairs so that homologs are on opposite sides of the metaphase plate (aka equatorial plane).

Figure 6. Homologous pairs line up at the equatorial plate in Metaphase l.

Anaphase I follows, as homologs are pulled apart, toward opposite poles of the cell (Figure 7). [*Note: this is significantly different from the separation of sister chromatids that occurs during mitosis].

Figure 7. In Anaphase l mitotic spindles pull homologs to opposite poles of the cell.

Telophase I marks the end of meiosis I, as new nuclei form and cytokinesis separates the cytoplasm forming two daughter cells.

At the end of meiosis I, the two daughter cells have half the number of chromosomes as did their parent cell. Thus, the cells have been reduced from diploid (2n) to haploid (n) (Figure 8). [n refers to the number of chromosomes in a set that are characteristic for a species. Humans have one set (n) of 23 unique chromosomes (n = 23). A diploid human cell has 2 sets (2n) of 23 unique chromosomes (2n = 46).

Figure 8. Meiosis l results in two haploid cells.

Meiosis II follows meiosis I, which proceeds very much like mitosis.

During **Prophase II**, chromosomes containing two sister chromatids are lined up on the equator of each daughter cell by the spindle fibers. This is completed by the end of **Metaphase II** (Figure 9).

Figure 9. Metaphase ll of Meiosis

The centromeres separate and sister chromatids are pulled to each pole of the cell during **Anaphase ll** (Figure 10)**.** Cytokinesis II occurs after Telophase II to complete cell division and ultimately the production of four (4) daughter cells (Figure 11). The cells produced (egg or sperm, in humans) are haploid (n rather than 2n) and will either unite (via fertilization) or die. They do not divide further on their own as meiosis is not a cycle.

Figure 10. Anaphase ll of Meiosis

Figure 11. Meiosis results in four haploid cells.

The Phases of Meiosis

Exercise 1: Modeling the Phases of Meiosis

Materials:

- Several sheets of blank paper (continuous printer paper is ideal)
- Chromosome modeling kits
  - Commercially available pop bead kits (e.g Carolina Biological Supply Company, Item #171100)
  - Homemade kits may consist of pipe cleaners or yarn or socks, etc. to represent chromosomes
- The following procedure will be described using a homemade kit consisting of pipe cleaners to represent chromosomes. The pipe cleaner chromosome kit contains:
  - 10 each – short red pipe cleaner sticks, short blue pipe cleaner sticks, long pipe cleaner red stick, long blue pipe cleaner sticks (Use as 2 homologous chromosome pairs)
  - 5 each – short red plastic lacing cord, short blue plastic lacing cord, long red plastic lacing cord, long blue plastic lacing cord (Use as 2 homologous chromatin pairs)
  - 20 white or grey beads (Use as centromeres)
  - Several red and blue beads (Use as genes for meiosis crossing-over)

Procedure:

A diploid cell with 2 homologous pairs of chromosomes (as in the previous modeling exercise) will be modeled as it moves through the meiosis. First, you will model meiosis l. Then, you will model meiosis ll as described below.

Model Meiosis l (1 diploid cell → 2 haploid cells)

- **tetrads form, crossing over occurs, homologues separate**
  - **Interphase Before Synthesis of DNA (G1)**
  - **Interphase After Synthesis of DNA (G2)**
  - **Prophase l**
  - **Metaphase l**
  - **Anaphase l**
  - **Telophase l**
  - **Cytokinesis l**

Model Meiosis ll (2 haploid cells → 4 haploid cells)

- **sister chromatids separate**
- Prophase ll
- Metaphase ll
- Anaphase ll
- Telophase ll
- Cytokinesis ll

Modeling Meiosis l

1. Use the lace cording chromosomes to model the **G1 phase of interphase** (before synthesis of the DNA). On the paper draw the cell membrane, nucleus, nucleolus, centrioles.
2. Use the lace cording chromosomes to model the **G2 phase of interphase** (after each chromosome was replicated during S phase). Use white beads to represent centromeres. Thread sister chromatids through a white bead to represent the duplicated chromosomes attached at the centromere. Centrioles would move toward opposite poles of the nucleus. Be sure to draw the cell membrane, nucleus, nucleolus and centrioles on the paper.
3. Use the pipe cleaner chromosomes to model **prophase l** of meiosis. Prophase l of meiosis is notably different than prophase of mitosis. Arrange condensed chromosomes so that homologous chromosomes are paired. Put homologous chromosomes next to each other on your lab bench to simulate this process of **synapsis** (homologs pairing). Homologous pairs of chromosomes paired in this way are called **tetrads**.

When tetrads form, the inner non-sister chromatids of the tetrad pair can exchange DNA by a process known as **crossing over**. Pieces of equivalent segments of non-sister chromatids can be exchanged from one chromatid to the other. Crossing over can occur several times along the length of the chromosomes. Use red and blue beads to represent exchanged segments of chromatids on the inner non-sister chromatids of the tetrad pairs. Place a blue bead on an inner red (maternal) chromatid to represent DNA exchanged from the paternal chromatid. Place a red bead on an inner blue (paternal) chromatid to represent DNA from the maternal chromatid. Make a minimum of 1 crossover for each pair of homologous chromosomes.

4. Use the pipe cleaner chromosomes to model **metaphase l**. Metaphase l of meiosis is also notably different than metaphase of mitosis. Spindle fibers move homologous pairs to line up along the equatorial plane of the cell. Line up the homologous pairs of chromosomes so that homologues are paired together but the maternal and paternal chromosomes are on opposite sides of the equatorial plate along the middle of the cell. Every pair of chromosomes is arranged independent of another. The side of the equatorial plate where each chromosome is arranged is completely random and independent of the side of the equatorial plate on which other chromosomes are located. Therefore, there are several different arrangements that can occur (Figure 12).

Figure 12. Independent Assortment in a cell with 2 homologous pairs.

5. In **anaphase l** of meiosis, homologous chromosomes separate toward opposite poles of the cell. For each homologous pair, move the duplicated maternal chromosome and duplicated paternal chromosome to opposite poles of the cell.
6. Anaphase ends and **telophase l** begins when chromosomes reach opposite poles of the cell. Arrange the chromosomes in groups at opposite ends of the cell. Nuclear division happens in telophase. The formation of separate nuclear envelopes divide the nuclei and mark the end of telophase.
7. Model **cytokinesis l** by drawing the formation of a cleavage furrow to divide the cytoplasm into two and form two separate cells. These two cells will enter meiosis ll. Note that each daughter cell has half the number of chromosomes as the parental cell. Thus, the cells have been reduced from **diploid (2n)** to **haploid (n)**. **[n** refers to the **number of *pairs* of chromosomes that are characteristic for a species**. Humans have a “n” of 23, so a diploid human cell has 2(23), or 46 chromosomes].

Meiosis ll

8. There is no DNA replication before the second cell division stage of meiosis. The stages of meiosis ll proceed very much like mitosis. The two cells created in meiosis l will enter into **prophase ll**. The chromosomes in each cell contain two sister chromatids, which are condensed and distributed throughout the nucleus. On your paper be sure to note that the nuclear membrane begins to break down and spindle fibers begin to form and radiate toward the chromosomes. Spindle fibers attach to **kinetochore** proteins at the centromeres of the chromosomes.
9. Use the pipe cleaner chromosomes to model **metaphase ll**. Line up the individual chromosomes on the equator (middle) of the cell. Sister chromatids remain attached at the centromere during metaphase ll.
10. Model **anaphase ll** by removing the white beads (centromere) from the sister chromatids to separate and move them toward opposite poles of the cell. After separation at the centromere, the chromatids are now called chromosomes.
11. Anaphase ll ends and **telophase ll** begins when chromosomes reach opposite poles of the cell. Nuclear division happens in telophase. Nuclear envelopes and nucleoli reappear. Condensed chromosomes begin to decondense and uncoil. The formation of separate nuclear envelopes divide the nuclei and marks the end of telophase.
12. Model **cytokinesis ll** by drawing the formation of a cleavage furrow to divide the cytoplasm of each cell into two. A total of four cells exist at the end of cytokinesis ll.

How many chromosomes are in the original parental cell? _____

How many chromosomes are in each daughter cell? _____

Are the chromosomes in daughter cells identical to the chromosomes in the original parental cell? _____

Questions for Review

1. What is the meaning of diploid? What abbreviation do we use to represent diploid? Name 2 diploid cells in humans.

2. What is the meaning of haploid? What abbreviation do we use to represent haploid? Name 2 haploid cells in humans.

3. In what stage of the cell cycle does S phase occur? Explain why the DNA must be duplicated during the S phase of the cell cycle, prior to mitosis taking place.

4. What specific feature of cytokinesis in animal cells can you use to distinguish this process from cytokinesis in plant cells?

Practical Challenge Questions

1. In the circle below, sketch a 2n=6 diploid cell in metaphase of mitosis. Be sure to label the centromere, centrioles, and spindle fibers.

2. In the circle below, sketch a 2n=6 haploid cell in metaphase l of meiosis.
3. A monogenic gene gives rise to a trait from a single set of alleles. A polygenic gene gives rise to a trait from several sets of alleles. Give an example of a monogenic and polygenic trait.

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). “Lab 10: Mitosis & Meiosis,” (2019). *Biological Sciences Open Textbooks*. 18. CC-BY

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1.6: Mitosis and Meiosis II

Introduction to Mitosis

In eukaryotic cells, the time and phases from the beginning of one cell division until the beginning of the next cell division is called the **cell cycle** (Figure 1)**.** The first phase of the cell cycle is **interphase.** Interphase is the time during which the cell performs its normal functions and prepares for cell division. Cells spend most of their time in this phase. During interphase, **chromosomes** are not visible because they are **decondensed** (present only as a tangled mass of thin threads of DNA with associated proteins, called chromatin). The nuclear membrane is present, and visible, as is the **nucleolus**.

Figure 1. The cell cycle.

Interphase includes two gap phases, G1 and G2, where the cell increases in size and synthesizes new organelles, enzymes, and other proteins that are needed for cell division. In between the two gap phases, the DNA replicates in preparation for cell division. This stage is called the **S phase**. At the beginning of S phase, chromosomes are single and unreplicated. By the end of S phase, each chromosome has made an exact copy of itself and consists of two **sister chromatids**. At this point in the cell cycle the sister chromatids are held together tightly at the **centromere**. While the two sister chromatids are physically joined together they are still considered one replicated chromosome (Figure 2). When the sister chromatids physically separate, later during the cell cycle, they are then considered to be individual chromosomes.

In animal cells, interphase is also when the **centrosome** (consisting of **two centrioles**) is replicated. Spindle fibers form from and radiate outward from the centrosomes to attach to and move chromosomes during cell division.

Figure 2. Chromosomes and sister chromatids.

Interphase is followed by **mitosis** (in the somatic cells) **or meiosis** (in reproductive cells), which is when replicated chromosomes and cytoplasm separate, during the process of karyokinesis and cytokinesis respectively.

Chromosomes that are the same length, have the same centromere location and the same gene sequences and positions are called homologous chromosomes. Human somatic cells contain pairs of homologous chromosomes. Each homologous pair consists of one maternal chromosome and one paternal chromosome. Cells that contain two copies of each chromosome are called **diploid** (2n, where n is the number of different chromosomes in a single set). Human sex cells (eggs and sperm) contain only one copy of each chromosome. Cells with only one copy of each chromosome are **haploid** (n). When the haploid sperm (n) and egg (n) combine during fertilization this forms a diploid zygote (2n).

MITOSIS

Mitosis is nuclear division that results in two cells containing the same number of chromosomes as the parent cell. Most human cells (skin, muscle, bone, etc.) divide by mitosis. This process is necessary for the normal growth and development of a multicellular eukaryotic organism from a zygote (fertilized egg), as well as growth and the repair and replacement of cells and tissues. At the end of mitosis, two daughter cells are formed that are identical to the original (parent) cell. Mitosis is also a form of asexual reproduction in unicellular eukaryotes.

Mitosis is a complex and highly regulated process. It occurs in the following 4 separate phases: prophase, metaphase, anaphase, and telophase. Telophase is quickly followed by cytokinesis.

Prophase: Cells prepare for division by coiling and condensing their chromatin into chromosomes. By late prophase, individual chromosomes can be seen, each consisting of two sister chromatids joined at a centromere. Spindle fibers begin to form from the centrosomes, which have begun to migrate to opposite “poles” of the cell. The nucleoli and the nuclear membrane degrade. (Figure 3)

Figure 3. Cell in prophase

Metaphase: Spindle fibers (called kinetochore microtubules or kinetochore spindle fibers) that emanate from the centromeres attach to the kinetochore (a proteinaceous area) on the sister chromatids. The fibers pull and otherwise manipulate the chromosomes to align them on the plane that passes through the center of the cell (metaphase plate) (Figure 4). This “plate” is not an actual structure; it merely signifies the location of replicated chromosomes prior to their impending separation.

Other non-kinetochore spindle fibers or tubules (aka polar microtubules), emanating from the two centrosomes, elongate and eventually overlap with each other near the metaphase plate.

Figure 4. Spindle fibers attaching to kinetochores in metaphase.

Anaphase: The centromeres divide, with the help of separase enzymes, and separate the sister chromatids (Figure 5). This happens simultaneously in all the chromosomes. The kinetochore spindles shorten and pull each chromatid to which they are attached toward the pole (and centrosome) from which they originate. This equally distributes exactly half the chromosomal material to each side of the cell. In late anaphase, the non-kinetochore spindles begin to elongate, lengthening the cell.

Figure 5. Cell in anaphase.

Telophase: The non-kinetochore microtubules continue to elongate, further elongating the cell in preparation for cytokinesis (splitting of the cytoplasm). The chromosomes reach their respective poles. The kinetochores disappear. The two nuclear membranes (one in each half of the cell) begin to form around the chromosomes. Nucleoli reappear and the chromosomes in each soon-to-be new cell begin to decondense back into chromatin. Mitosis is complete at the end of this stage.

Cytokinesis (splitting of the cytoplasm):

In animal cells and all other eukaryotes without a cell wall, cytokinesis is achieved by means of a constricting “belt” of protein fibers that slide past each other near the equator of the cell. As this occurs, the diameter of the belt decreases, pinching the cell to form a **cleavage furrow** around the cell’s circumference. As constriction proceeds, the furrow deepens until it eventually slices its way into the center of the cell. At this point, the cell is divided into two.

Plant cell walls are far too rigid to be split apart by contracting proteins. Instead, these cells assemble membrane proteins (in vesicles that bud off the Golgi apparatus) in their interior at right angles to the spindle apparatus. This expanding membrane partition, called a cell plate, continues to grow outward until it reaches the interior surface of the plasma membrane and fuses with it. This divides the cell in two.

The phases of Mitosis

Exercise 1: Modeling the Phases of Mitosis with Pop Beads

Materials:

- Chalk (can be used directly on lab bench to draw cellular structures and then washed off)
- Chromosome modeling kits
  - Commercially available pop bead kits (e.g Carolina Biological Supply Company, Item #171100)
- 40 pop beads of one color (red)
- 40 pop beads of another color (yellow)
- 8 magnetic centromeres
- 4 laminated pictures of centrosomes (each consisting of a pair of centrioles)
- Paper towels / Kimwipes

Procedure:

Using models is a great way to represent natural structures and processes that are too small, or too large, or too complex to observe directly. By building chromosomes from the pop beads and manipulating them to model cell division (mitosis and meiosis) you will enhance your understanding of the nature of chromosomes and the cellular structures needed to perform cell division. In this simulation, your cell will be a **diploid (2n)** cell with 4 chromosomes. It will contain 2 homologous pairs of chromosomes. In order to differentiate between the pairs of homologs, one pair of homologous chromosomes will be longer than the other. One chromosome of each pair is red and represents maternal DNA (genetic material contributed by a female’s egg). The other chromosome of each pair is yellow and represents paternal DNA (genetic material contributed by a father’s sperm). Thus, for each pair of homologous chromosomes, one should be red and one should be yellow. The strands of pop beads represent the DNA in the form of chromatin during the G1, S, and G2 phases. They then represent chromosomes as they enter the phases of mitosis.

This diploid cell with 2 homologous pairs of chromosomes will be modeled as it moves through the following phases of mitosis:

- Interphase (uncondensed DNA) **Before Synthesis** of DNA (G1)

- Interphase (uncondensed DNA) **After Synthesis** of DNA (G2)
- Prophase
- Metaphase
- Anaphase
- Telophase
- Cytokinesis

1. You will build 2 pairs of homologous chromosomes (2n = 4). The first homologous pair of single chromosomes should be constructed using 12 red beads for one member of the long pair and 12 yellow beads for the other member of the pair. You may place the magnetic centromere at any position along the chromosome, but it must be at the same position on both members of the homologous pair.
2. The second homologous pair of single chromosomes should be constructed using 6 red beads for one member of the short pair and 6 yellow beads for the other member of the pair. You may place the magnetic centromere at any position along the chromosome, but it must be at the same position on both members of the homologous pair.
3. Use the pop beads to model the **G1 phase of interphase** (before synthesis of the DNA). On your lab bench, use the chalk to draw the cell membrane, nucleus, and nucleolus. Place a laminated centrosome in the cytoplasm of your cell. Place the 4 assembled chromosomes in the nucleus of your cell drawing. Remember that these represent a mass of chromatin.
4. DNA replication takes place during the S phase of interphase. Model the **S phase of interphase** by assembling a second strand that is identical to each of the 4 single chromosomes. The identical strands are considered sister chromatids, which are held together by their magnetic centromeres. (Note: In a living cell, the centromere is a single unit until it separates in anaphase. Therefore, consider the pair of magnets to be a single centromere.)
5. Model the **G2 phase of interphase** by letting your 4 assembled replicated chromosomes rest in the nucleus of your cell. Duplicate the centrosomes by placing another laminated centrosome in the cytoplasm of your cell drawing. Begin to move them towards opposite poles of the cell drawing.
6. To model the **prophase** stage of mitosis, leave the chromosomes where they are in your cell drawing. In this stage, the chromatin coils and condenses into chromosomes. Using the paper towel (or Kimwipe) start erasing some of the nuclear membrane on your cell drawing. This will simulate the breakdown of the nuclear membrane. Also erase the nucleolus, as the nucleolus disappears in this stage. Continue to move your centrosomes to opposite sides of the cell. Use the chalk to draw spindle fibers beginning to form and radiating outward toward the chromosomes.
7. At **prometaphase** the centrosomes are at opposite poles of the cell. During this phase the chromosomes continue to condense, the nuclear membrane completely breaks down, and the spindle fibers start to attach to the **kinetochores** of the centromeres. These are called **kinetochore microtubules**. Other spindle fibers radiate outward from the centrosomes, but these do not attach to the kinetochores. These spindle fibers are called nonkinetochore microtubules.
8. To model **metaphase**, move the centromeres of your chromosomes to lie on an imaginary plane midway between the centrosomes that are now positioned at opposite poles of the cell. Line up the individual chromosomes in the middle (equator) of the cell. Sister chromatids remain attached at the centromere during metaphase.
9. To model **anaphase**, pull the magnetic centromeres apart and slide them towards opposite poles of the cell. Keep them attached to your drawn kinetochore microtubules, but use the paper towel to erase them, making them shorter. With the chalk, lengthen the nonkinetochore microtubules and have them overlap in the middle of your cell. After separation at the centromere, each sister chromatid is now referred to as an individual chromosome. Anaphase ends when the chromosomes reach the opposite ends of the cell.
10. Model **telophase** by piling up your chromosomes at each pole. Erase your spindle fibers, as they disappear during this stage. Even though we can't simulate it, the chromosomes will start to decondense and uncoil back into chromatin. Redraw a nuclear envelope around each pile of chromosomes. Also add nucleoli to your drawing. The formation of separate nuclear envelopes divide the nuclei and marks the end of telophase.
11. The division of the cytoplasm, or cytokinesis, results in the formation of two separate cells. To model **cytokinesis** in animal cells, fungi, and slime molds, leave the piles of chromosomes at their separate poles. Draw indentations of the cell membrane

inwards, towards the cytoplasm, on the sides of the cell where there are no centrosomes. This represents a **cleavage furrow**,which eventually "pinches" the cell's cytoplasm into two separate cells. The proteins actin and myosin contribute to the formation of the cleavage furrow. In plant cells, membrane-bound vesicles migrate to the center of the cell (the equatorial plane) and fuse together to form a **cell plate**. The cell plate eventually divides the cytoplasm into two separate cells. Materials needed to build the cell wall are released from the vesicles at the cell plate, forming a new cell wall.

- How do the daughter cells you formed compare to the original parent cell?

_____

Observe the phases of Mitosis in Plant Cells

Exercise 2: Observing the Phases of Mitosis in the Onion Root Tip

Materials:

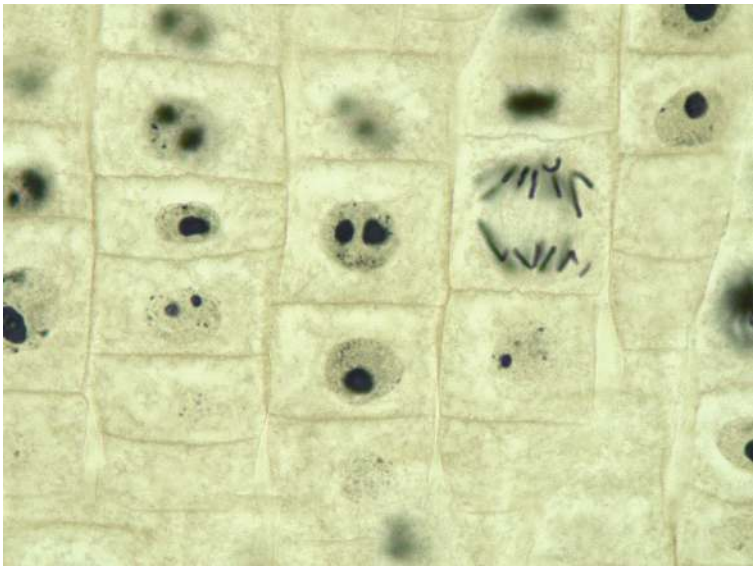
- Prepared slide of the onion root tip
- Compound light microscope

Procedure:

1. Examine a slide of a longitudinal section of an onion root tip. Adjust the slide to view the region just above the root cap, where there are likely to be dividing cells.
2. Focus on the dividing cells using the 4x scanning objective lens, then switch to the 10x objective and then the 40x objective.
3. Survey the slide to find a cell in each phase of mitosis. Draw a cell for each phase in the boxes provided.

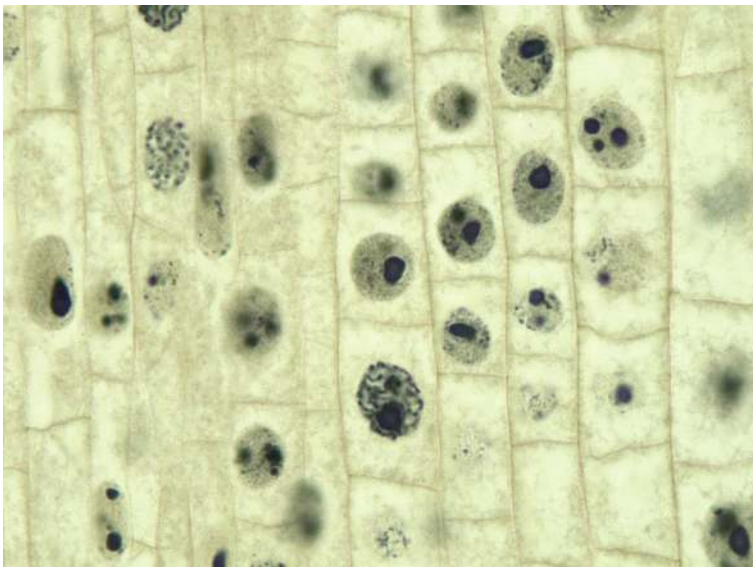
Interphase

DNA is uncondensed and in the form of chromatin. Individual chromosomes are not visible. The nuclear membrane is intact. The nucleolus is visible.



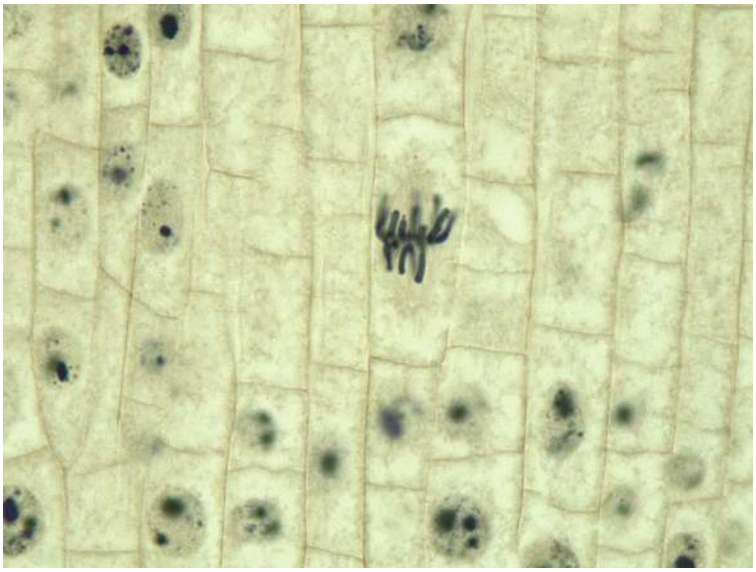
Prophase

Chromatin begins to condense into distinguishable chromosomes. These “puffy” structures are seen throughout the nucleus. Nucleoli begin to disappear. In late prophase (often called prometaphase) the nuclear membrane is no longer visible.



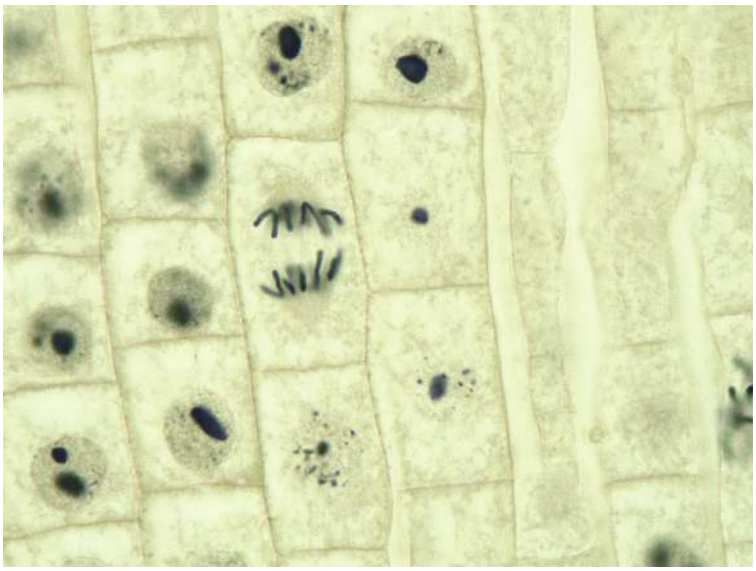
Metaphase

The chromosomes line up in the middle of the cell. Spindle fibers attach to kinetochores at the centromere and extend to the poles of the cell.



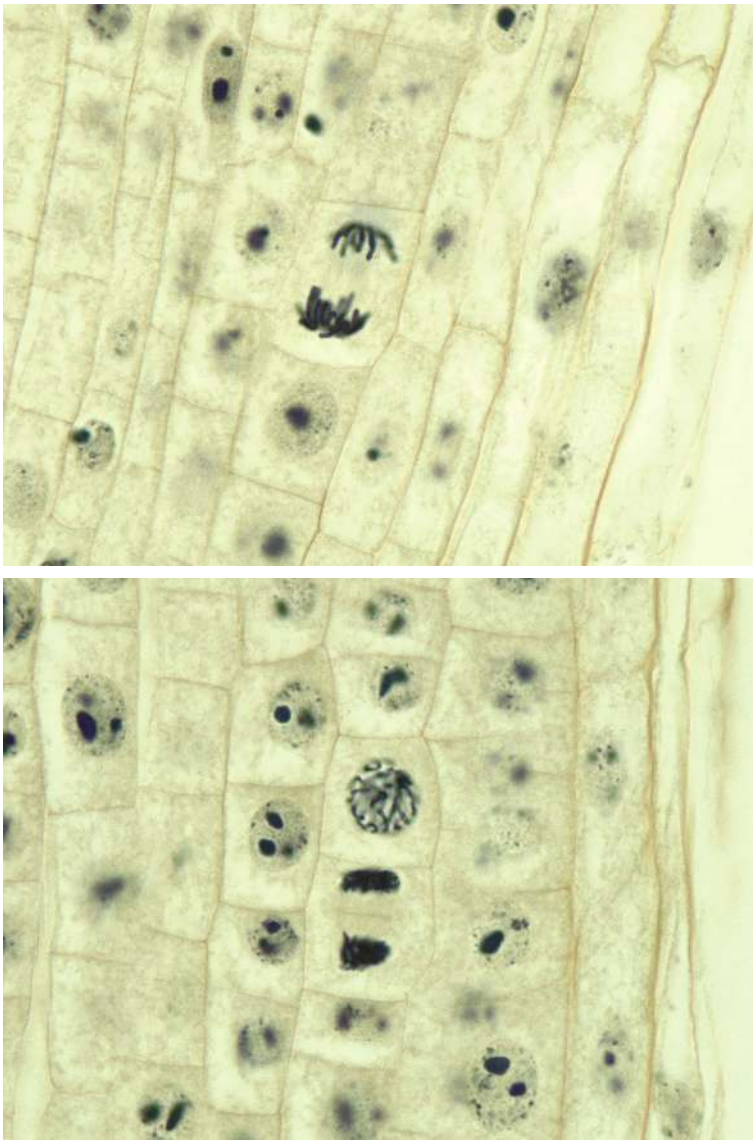
Anaphase

Centromeres split, separating each former chromatid into two individual chromosomes. The chromosomes move toward opposite poles.



Telophase and Cytokinesis

Chromosomes reach the poles. The nuclear envelopes begin to reform. The formation of a cell plate forms between the two cells to carry out cytokinesis.



Observe the phases of Mitosis in Animal Cells

Exercise 3: Observing the Phases of Mitosis in the Whitefish Blastula

Materials:

- Prepared slide of whitefish blastula
- Compound light microscope

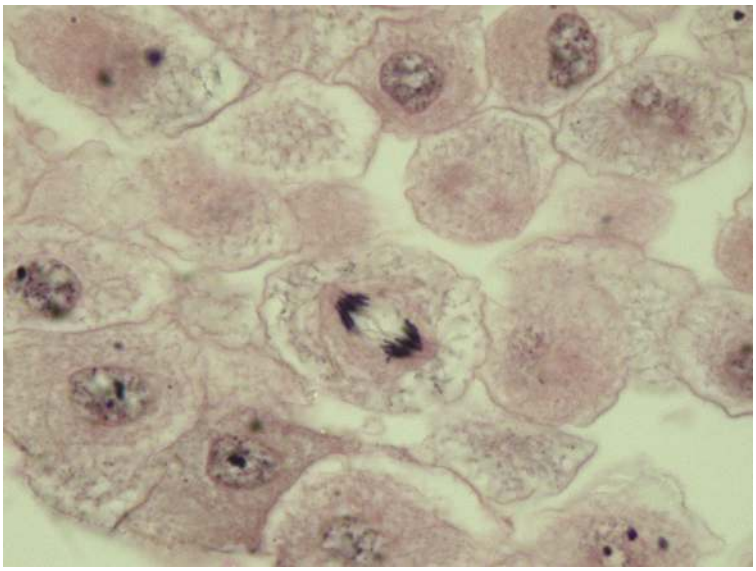
Procedure:

The blastula is an early embryonic stage where many of the cells are dividing at any one time.

1. Focus on the dividing cells using the 4x scanning objective lens, then switch to the 10x objective and then the 40x objective.
2. Survey the slide to find a cell in each phase of mitosis. Draw a cell for each phase in the boxes provided.

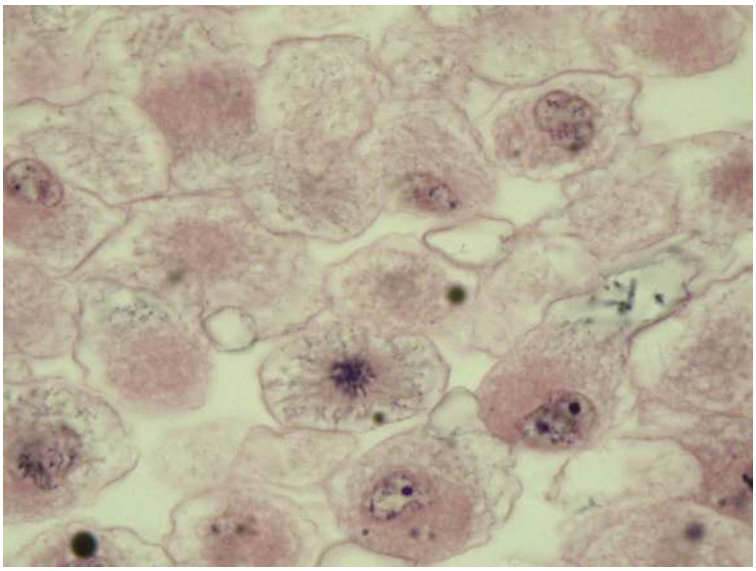
Interphase

The DNA is uncondensed and in the form of chromatin. Individual chromosomes are not visible. The nuclear membrane is intact. The nucleolus is visible.



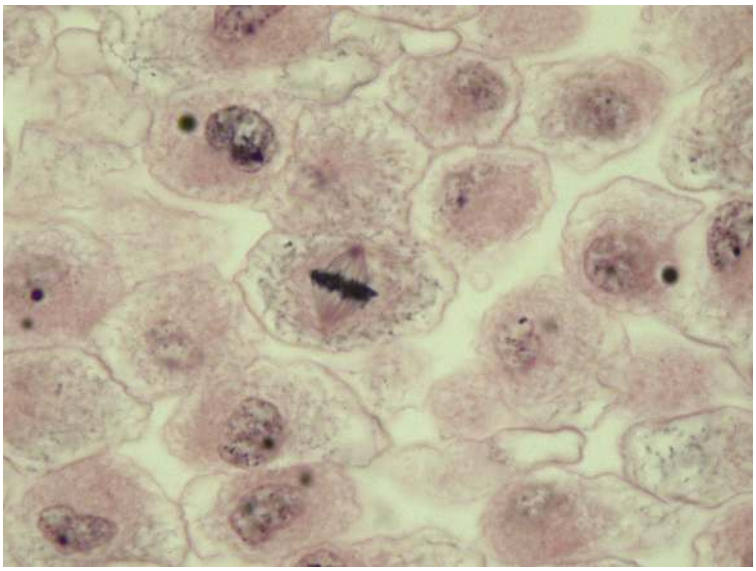
Prophase

Chromatin begins to condense and chromosomes are distinguishable. These “puffy” structures are seen throughout the nucleus. The nucleoli begin to disappear. In late prophase (often called prometaphase) the nuclear membrane is no longer visible.



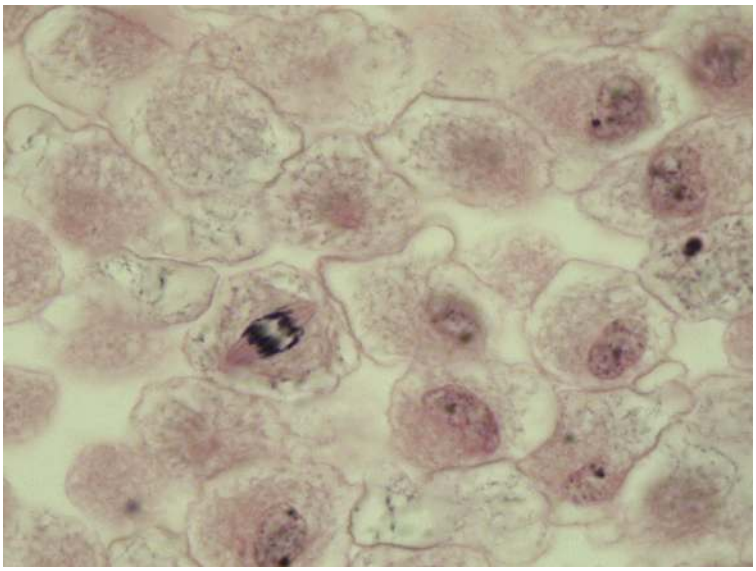
Metaphase

The chromosomes line up in the middle of the cell. Spindle fibers attach to kinetochores at the centromere and extend to the poles of the cell.



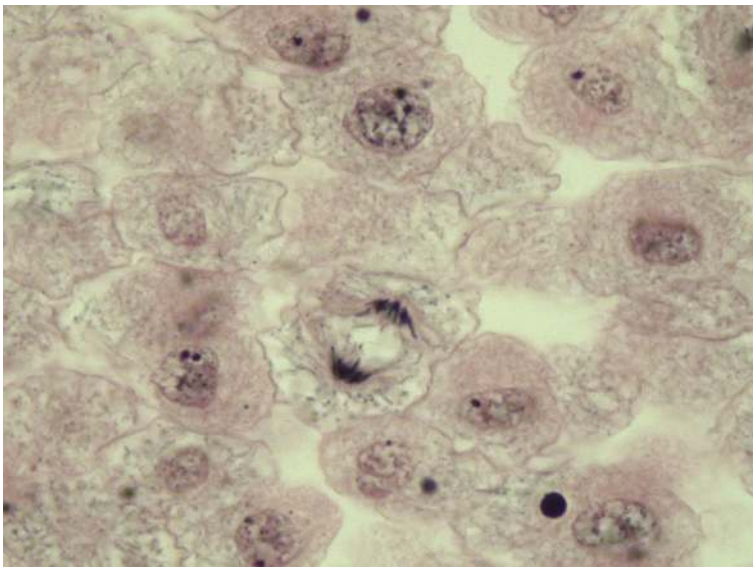
Anaphase

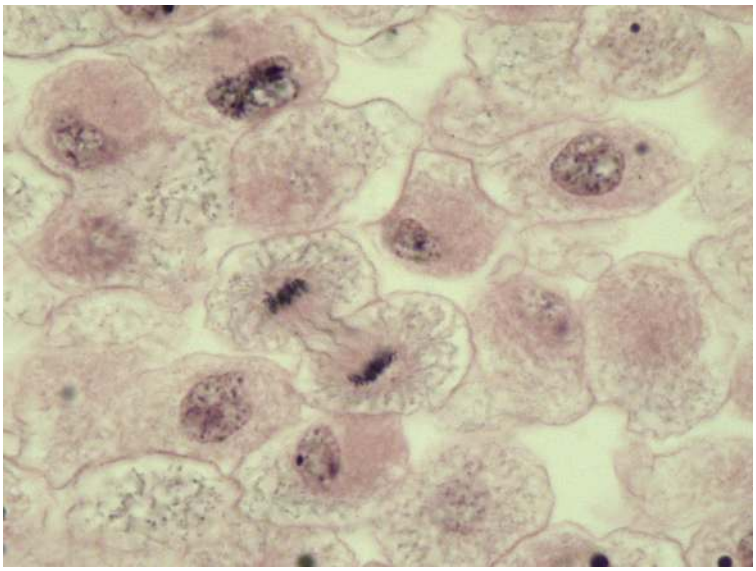
Centromeres split, separating each former chromatid into two individual chromosomes. The chromosomes move toward opposite poles.



Telophase and Cytokinesis

Chromosomes reach the poles. The nuclear envelopes begin to reform. A **cleavage furrow** forms between the two cells to carry out cytokinesis.





- Why would the method of cytokinesis in animal cells not work in plant cells? Explain.

_____

How Long Does a Cell Spend in Each Phase of the Cell Cycle?

Exercise 4: Determining Time Spent in Different Phases of the Cell Cycle (Optional)

Materials:

- Laptop
- Calculator

Procedure:

1. Obtain a laptop.
2. Open a web browser and go to the following site: **http://www.biology.arizona.edu**
3. This site will provide an interactive test of your ability to identify the stages of mitosis. It will also allow you to calculate the duration of the stages identified in the laboratory exercise you just completed, but the website will give standard results for the entire class.
4. Go to the **Cell Biology** section, and find the activity "**Online Onion Root Tips**".

5. Keep clicking on “Next” at the bottom of the page until you get to the screen: **Determining Time Spent in Different Phases of the Cell Cycle**
6. Click on “Next” at the bottom of the page.
7. Identify each stage shown to you by the program. When a picture of a cell pops up in a stage of mitosis, simply click on the phase in which the cell belongs. If you make a mistake, read the explanation for why you were mistaken before making a new selection.
8. When you are finished, use the formula given below and record your results in the table.
9. The duration of each stage of mitosis can be determined by using the following formula. Compute the length of time for each stage and place your calculations in the table below.

(Number of cells in a stage ÷ Total number of cells) x 1440 (min in a day) = minutes a cell spends

in each stage in one day

Number of cells in each stage	**Interphase** _____	**Prophase** _____	**Metaphase** _____	**Anaphase** _____	**Telophase** _____	**Total 36**
Percent of cells (# of cells / Total)						100%
Time (in minutes) spent in Stage – use calculation above						

Practical Challenge Questions

1. In the circle below, sketch a 2n=6 diploid cell in metaphase of mitosis. Be sure to label the centromere, centrioles, and spindle fibers.

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). “Lab 10: Mitosis & Meiosis,” (2019). *Biological Sciences Open Textbooks*. 18. CC-BY

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Meiosis

Introduction to Meiosis(aka “Reduction Division”)

Meiosis is a special type of cell division in which the daughter cells produced have half the number of chromosomes (n) as their parent cell. In animals, this division occurs in the reproductive organs (gonads -- testes of males or ovaries of females) of species that reproduce sexually, and results in the formation of gametes (eggs or sperm) that contain half the number of chromosomes as the original cell. Sexual reproduction involves the joining of gametes (fertilization) to form a zygote, which then has two copies of each chromosome (2n). Meiosis is a critical process, as it increases genetic diversity within a species.

Cells that divide by meiosis prepare for cellular division (during interphase) much like every other cell. Meiosis progresses through the same phases as mitosis (prophase, anaphase, metaphase, telophase, and cytokinesis). However, unlike mitosis, meiosis involves two rounds of cellular division (meiosis I and meiosis II). Meiosis involves only one round of DNA replication where each

chromosome replicates to form sister chromatids. Therefore, when meiosis is completed, each daughter cell contains only half the number (n) of chromosomes as the original cell.

The stages of meiosis:

- Meiosis I
- Prophase l
- Metaphase l
- Anaphase l
- Telophase l
- Cytokinesis l
- Meiosis II
- Prophase ll
- Metaphase ll
- Anaphase ll
- Telophase ll
- Cytokinesis ll

Prophase I: During prophase of meiosis I, the chromosomes join in homologous pairs. Homologous chromosomes (aka homologs) are the same length, have their centromere in the same position, and carry genetic information (genes) for the same traits, but not necessarily the same versions (alleles) of the gene.

For example, human chromosome #19 contains a gene for eye color. In one person, one allele might code for blue eyes and the other allele codes for green eyes. Since every human inherits two copies of chromosome 19 (one from the mother's egg and one from the father's sperm) a person could have 2 blue alleles, 2 green alleles, or one of each.

Paired homologous chromosomes are called tetrads and are said to be in synapsis. During synapsis, equivalent pieces of homologous chromatids are exchanged between the chromosomes. This is called **crossing-over** and can occur several times along the length of the chromosomes. As occurs in the mitotic division, prophase of meiosis I also involves the degradation of the nuclear membrane, disassembly of the nucleolus, and formation of spindle fibers.

Metaphase I: Metaphase of meiosis I occurs when the joined homologous chromosome pairs are moved to the center of the cell by spindle fibers (Figure 6). The fibers arrange the pairs so that homologs are on opposite sides of the metaphase plate (aka equatorial plane).

Figure 6. Homologous pairs line up at the equatorial plate in Metaphase l.

Anaphase I follows, as homologs are pulled apart, toward opposite poles of the cell (Figure 7). [*Note: this is significantly different from the separation of sister chromatids that occurs during mitosis].

Figure 7. In Anaphase l mitotic spindles pull homologs to opposite poles of the cell.

Telophase I marks the end of meiosis I, as new nuclei form and cytokinesis separates the cytoplasm forming two daughter cells.

At the end of meiosis I, the two daughter cells have half the number of chromosomes as did their parent cell. Thus, the cells have been reduced from diploid (2n) to haploid (n) (Figure 8). [n refers to the number of chromosomes in a set that are characteristic for a species. Humans have one set (n) of 23 unique chromosomes (n = 23). A diploid human cell has 2 sets (2n) of 23 unique chromosomes (2n = 46).

Figure 8. Meiosis l results in two haploid cells.

***Meiosis II follows meiosis I, which proceeds very much like mitosis.**

During **Prophase II**, chromosomes containing two sister chromatids are lined up on the equator of each daughter cell by the spindle fibers. This is completed by the end of **Metaphase II** (Figure 9).

Figure 9. Metaphase ll of Meiosis

The centromeres separate and sister chromatids are pulled to each pole of the cell during **Anaphase ll** (Figure 10)**.** Cytokinesis II occurs after Telophase II to complete cell division and ultimately the production of four (4) daughter cells (Figure 11). The cells

produced (egg or sperm, in humans) are haploid (n rather than 2n) and will either unite (via fertilization) or die. They do not divide further on their own as meiosis is not a cycle.

Figure 10. Anaphase ll of Meiosis

Figure 11. Meiosis results in four haploid cells.

The Phases of Meiosis

Exercise 1: Modeling the Phases of Meiosis

Materials:

- Chalk (can be used directly on lab bench to draw cellular structures and then washed off)
- Chromosome modeling kits
  - Commercially available pop bead kits (e.g Carolina Biological Supply Company, Item #171100)
- 40 pop beads of one color (red)
- 40 pop beads of another color (yellow)
- 8 magnetic centromeres
- 8 laminated pictures of centrosomes (each consisting of a pair of centrioles)
- Paper towels / Kimwipes
- Fine point sharpie
- Small round stickers

Procedure:

A diploid cell with 2 homologous pairs of chromosomes (as in the previous modeling exercise) will be modeled as it moves through the meiosis. First, you will model meiosis l. Then, you will model meiosis ll as described below.

Model Meiosis l (1 diploid cell → 2 haploid cells)

- **tetrads form, crossing over occurs, homologues separate**
  - **Interphase Before Synthesis of DNA (G1)**
  - **Interphase After Synthesis of DNA (G2)**
  - **Prophase l**
  - **Metaphase l**
  - **Anaphase l**
  - **Telophase l**
  - **Cytokinesis l**

Model Meiosis ll (2 haploid cells → 4 haploid cells)

- **sister chromatids separate**

- Prophase ll
- Metaphase ll
- Anaphase ll
- Telophase ll
- Cytokinesis ll

Modeling Meiosis l

1. You will build 2 pairs of homologous chromosomes (2n = 4). The first homologous pair of single chromosomes should be constructed using 12 red beads for one member of the long pair and 12 yellow beads for the other member of the long pair. You may place the magnetic centromere at any position along the chromosome, but it must be at the same position on both members of the homologous pair.
2. The second pair of single chromosomes will represent the X and Y sex chromosomes in a male. The X chromosome should be constructed using 8 red beads. To make manipulation of the chromosomes easier, place the magnetic centromere in the middle

of the X chromosome so that you have 4 red beads on one side of the centromere and 4 red beads on the other side. The Y chromosome should be constructed using 2 yellow beads with a magnetic centromere between them.

3. Alternative forms of genes are called alleles. We will use the letter “B” or “b” to represent the gene for eye color. Labeled stickers will be used to represent alleles. With the Sharpie, label one small sticker with a “B” and another small sticker with a “b”. “B” will represent the dominant allele for brown eyes and “b” will represent the recessive allele for blue eyes. You may place the “B” sticker on any of the red beads of the long red chromosome. The “b” sticker must be placed at the same bead position of the “B” sticker, but on the long yellow chromosome.
4. To model the **G1 phase of interphase** (before synthesis of the DNA). On your lab bench, use the chalk to draw the cell membrane, nucleus, and nucleolus. Place a laminated centrosome in the cytoplasm of your cell. Place the 4 assembled chromosomes in the nucleus of your cell drawing. Remember that these represent a mass of chromatin.
5. DNA replication takes place during the S phase of interphase. Model the **S phase of interphase** by assembling a second strand that is identical to each of the 4 single chromosomes. The identical strands are considered sister chromatids, which are held together by their magnetic centromeres. (Note: In a living cell, the centromere is a single unit until it separates in anaphase. Therefore, consider the pair of magnets to be a single centromere.)
6. Model the **G2 phase of interphase** by letting your 4 assembled replicated chromosomes rest in the nucleus of your cell. Duplicate the centrosomes by placing another laminated centrosome in the cytoplasm of your cell drawing. Begin to move them towards opposite poles of the cell drawing.
7. To model the **prophase I** stage of meiosis, leave the chromosomes where they are in your cell drawing. In this stage, the chromatin coils and condenses into chromosomes. Using the paper towel (or Kimwipe) start erasing some of the nuclear membrane on your cell drawing. This will simulate the breakdown of the nuclear membrane. Also erase the nucleolus, as the nucleolus disappears in this stage. Continue to move your centrosomes to opposite sides of the cell. Use the chalk to draw spindle fibers beginning to form and radiating outward toward the chromosomes.
8. **Prophase l** of meiosis has some notable differences compared to prophase of mitosis. To further model **prophase l** of meiosis, arrange the condensed chromosomes so that homologous chromosomes are paired. Put homologous chromosomes next to each other in your cell drawing to simulate this process of **synapsis** (homologs pairing). Homologous pairs of chromosomes paired in this way are called **tetrads**. When tetrads form, the inner non-sister chromatids of the tetrad pair can exchange DNA by a process known as **crossing over**. Pieces of equivalent segments of non-sister chromatids can be exchanged from one non-sister chromatid to the other. Crossing over can occur several times along the length of the chromosomes.

- How many tetrad complexes do you have in your cell, which is 2n = 4?

_____

9. Exchange segments of the inner non-sister chromatids of the red and yellow beads that contain the two different alleles, “B” and “b”. Remember that the red beads represent DNA from the maternal chromatid and the yellow beads represent DNA from the paternal chromatid. You now have one crossover event for this tetrad.
10. **Metaphase l** of meiosis is also notably different than metaphase of mitosis. To model **metaphase l** of meiosis, move your tetrads to the equator, midway between the two centrosomes, which should now be positioned at the two opposite poles of the cell. Draw spindle fibers radiating out from the centrosomes to the homologous pairs (tetrads) which are lined up along the equatorial plane of the cell. Homologs are paired together but the maternal and paternal chromosomes are on opposite sides of the equatorial plate along the middle of the cell. Every pair of chromosomes is arranged independent of another. The side of the equatorial plate where each chromosome is arranged is completely random and independent of the side of the equatorial plate on which other chromosomes are located. Therefore, there are several different arrangements that can occur (Figure 12).

Figure 12. Independent Assortment in a cell with 2 homologous pairs.

11. In **anaphase l** of meiosis, homologous chromosomes separate toward opposite poles of the cell. **Centromeres do not split as they do in mitosis.** For each homologous pair, move the duplicated maternal chromosome and duplicated paternal chromosome to opposite poles of the cell. Remember that in our model, the two magnets (one of each of the two sister chromatids) represent one centromere. Erase the spindle fibers and redraw them shorter and shorter as each homologous pair moves away from one another towards opposite poles of the cell.

- How does the structure of chromosomes in anaphase I of meiosis differ from that in anaphase of mitosis?

_____

12. Anaphase ends and **telophase l** begins when chromosomes reach opposite poles of the cell. Arrange the chromosomes in groups at opposite ends of the cell. You should have one long chromosome of 12 beads and either the X chromosome or the Y chromosome at each pole. Nuclear division happens in telophase. The formation of separate nuclear envelopes divide the nuclei and mark the end of telophase.
13. Model **cytokinesis l** by drawing the formation of a cleavage furrow to divide the cytoplasm into two and form two separate cells. Redraw the nuclear membrane around the chromosomes and draw a nucleolus inside of each nucleus. These two cells will now enter meiosis ll. Note that each daughter cell has half the number of chromosomes as the parental cell. Thus, the cells have been reduced from **diploid (2n)** to **haploid (n)**. **[n** refers to the **number of *pairs* of chromosomes that are characteristic for a species**. Humans have a "n" of 23, so a diploid human cell has 2(23), or 46 chromosomes].

Meiosis ll

1. There is no DNA replication before the second cell division stage of meiosis. The stages of meiosis ll proceed very much like mitosis. The two cells created in meiosis l will enter into **prophase ll**. The chromosomes in each cell contain two sister chromatids, which are condensed and distributed throughout the nucleus. Add another laminated centrosome. There should be two centrosomes in each new cell. In your drawing be sure to note that the nuclear envelope begins to break down, the nucleolus disappears, the centrosomes move towards opposite poles, and spindle fibers begin to form and radiate toward the chromosomes. Spindle fibers attach to **kinetochore** proteins at the centromeres of the chromosomes.
2. To model **metaphase ll**, line up the individual chromosomes on the equator (middle) of each cell. Sister chromatids remain attached at the centromere during metaphase ll.
3. Model **anaphase ll** by pulling the two magnetic centromeres of each duplicated chromosome apart. The sister chromatids should be separated and moved toward opposite poles of each cell. After separation at the centromere, the chromatids are now called chromosomes. In anaphase II single chromosomes move towards opposite poles.
4. Anaphase ll ends and **telophase ll** begins when the chromosomes reach opposite poles of the cells. Nuclear division happens in telophase. The spindle fibers disassemble. Nuclear envelopes and nucleoli reappear. Condensed chromosomes begin to decondense and uncoil. The formation of separate nuclear envelopes divide the nuclei and marks the end of telophase.
5. Model **cytokinesis ll** by drawing the formation of a cleavage furrow to divide the cytoplasm of each cell into two separate cells.

- What is the total number of nuclei and cells now present? _____
- How many cells were present when meiosis began? _____
- How many chromosomes were present in the original parental cell? _____
- How many chromosomes are in each new daughter cell? _____
- Are the chromosomes in the new daughter cells identical to the chromosomes in the original parental cell? Explain your results in terms of independent assortment and crossing over. _____

Questions for Review

1. What is the meaning of diploid? What abbreviation do we use to represent diploid? Name 2 diploid cells in humans.

2. What is the meaning of haploid? What abbreviation do we use to represent haploid? Name 2 haploid cells in humans.
3. In what stage of the cell cycle does S phase occur? Explain why the DNA must be duplicated during the S phase of the cell cycle, prior to mitosis taking place.

4. What specific feature of cytokinesis in animal cells can you use to distinguish this process from cytokinesis in plant cells?

Practical Challenge Questions

1. In the circle below, sketch a 2n=6 haploid cell in metaphase l of meiosis.
2. A monogenic gene gives rise to a trait from a single set of alleles. A polygenic gene gives rise to a trait from several sets of alleles. Give an example of a monogenic and polygenic trait.

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 10: Mitosis & Meiosis," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY

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1.7: Enzyme Function

In every living cell, many chemical reactions are performed. In chemical reactions, the **reactants** are molecules that undergo a change, which results in the **products**. The arrow indicates the direction of the reaction as the reactants proceed to be transformed into the product(s). As you can see in the figure below, the number of reactants and products can vary, but the number of atoms is the same on both sides of the arrow. During synthesis reactions (Example 1), the reactants are joined to form a product. During decomposition reactions (Example 2), the reactant is broken down into products. During exchange reactions (Example 3) both decomposition and synthesis take place. The decomposition of two reactants is followed by an exchange and synthesis of two new compounds from them.

Example 1 (Synthesis Reaction): $\mathrm{A + B \longrightarrow AB}$

$$\mathrm{2\,H_2 + O_2 \longrightarrow 2\,H_2O} \tag{1.7.1}$$

Example 2 (Decomposition Reaction): $\mathrm{AB \longrightarrow A + B}$

$$\mathrm{2\,H_2O \longrightarrow 2\,H_2 + O_2} \tag{1.7.2}$$

Example 3 (Exchange Reaction): $\mathrm{A + BC \longrightarrow AC + B}$

$$\mathrm{2\,Na + 2\,H_2O \longrightarrow 2\,NaOH + H_2} \tag{1.7.3}$$

Enzymes are very efficient catalysts for biochemical reactions. These catalysts speed up reactions by providing an alternative reaction pathway that requires a lower activation energy needed for a reaction to get started. The lower the activation energy for a reaction, the faster the rate at which it will proceed. Enzymes only lower the activation energy, but do not change the difference in energy levels between the reactants and the products. They work by binding to the reactants and converting them to a different compound (the product). Enzymes are specific to a type of reactant, and therefore can catalyze only one type of reaction. This **enzyme specificity** is the result of the particular shape of the enzyme that only permits binding to one type of reactant, much like a key fits into a lock (Figure 2). The reactants in an enzymatic chemical reaction are called **substrates**. Notice how the shape of the enzyme fits its substrate. The location where the enzyme binds a substrate is called the **active site** because the reaction occurs here. At the end of the reaction, the product is released, and the enzyme can then bind to more substrate. Thus, an enzyme can perform the reaction over and over again, as long as the substrate is present.

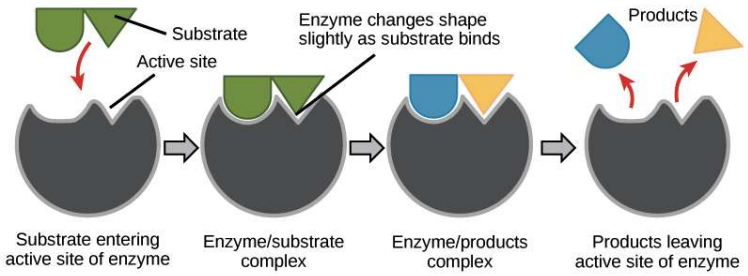

Figure 2: Enzymes bind a specific substrate and convert it into the products. The products are released, and the enzyme is unchanged. (CC By 4.0; OpenStax)

Most enzymes are complex proteins that function most efficiently within a specific range of temperature and pH. Extremes in temperature or pH will **denature** the enzyme by permanently altering its chemical structure. Even a small change in the protein's structure will change the enzyme's shape enough to prevent the substrate from binding, and thus keep the reaction from occurring. In this laboratory, you will demonstrate how altering the temperature, enzyme concentration, and pH will affect an enzyme's ability to function. Specifically, you will be studying the actions of the enzyme **catalase**.

Exercise 1: Catalase Activity

Materials:

- Water (distilled or deionized)
- Disposable pipets

- Catalase (400 units/mL solution)
- Hydrogen peroxide (3% solution)
- Sucrose solution (5% solution)
- Small test tubes (10 ml test tubes) for Exercises 1, 2, and 4
- Large test tubes (20 ml test tubes) for Exercise 3
- Ruler
- Wax pencil / Sharpie
- Ice
- Boiling water bath
- Refrigerator
- Incubator (37o Celsius)
- 0.1 M HCl
- 0.1 M NaOH
- Buffer pH 7.0
- Safety glasses

Catalase is an enzyme that speeds up the breakdown of hydrogen peroxide to water and oxygen:

Hydrogen peroxide → water + oxygen gas

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2 \tag{1.7.4}$$

- What is the **reactant** in this reaction? _____
- What is the **substrate** for catalase? _____
- What are the products for this reaction? _____
- Bubbling occurs as the reaction proceeds. Explain why.

  _____

Procedure:

1. With a wax pencil (or Sharpie), label three small test tubes (#1, #2, #3).
2. Using the ruler, mark each tube at the 1 cm (first mark) and 5 cm (second mark) levels from the bottom of the tube.
3. Fill tube #1 to the first mark with catalase. Then fill it to the second mark with hydrogen peroxide.
4. Swirl the contents to mix and wait at least 20 seconds for bubbles to develop.
5. Using the ruler, measure the height of the bubble column (in millimeters), and record your results in Table 1 below.
6. Fill tube #2 to the first mark with water. Then fill it to the second mark with hydrogen peroxide.
7. Swirl the contents to mix and wait at least 20 seconds for bubbles to develop.
8. Using the ruler, measure the height of the bubble column (in millimeters), and record your results in Table 1 below.
9. Fill tube #3 to the first mark with catalase. Then fill to the second mark with sucrose solution.
10. Swirl the contents to mix and wait at least 20 seconds for bubbles to develop.
11. Using the ruler, measure the height of the bubble column (in millimeters), and record your results in Table 1 below.

Table 1: Catalase Activity

Tube	Contents	Bubble Column Height (mm)
1	catalase, hydrogen peroxide	
2	water, hydrogen peroxide	
3	catalase, sucrose solution	

- Which tube showed the most bubbling? Explain why.

  _____

_____

- Which tube was the negative control? _____
- Which tube was the positive control? _____
- Describe what you observe in test tube #3. What was the purpose of this tube?

_____

_____

Exercise 2: Effect of Temperature on Enzyme Activity

In general, cold temperatures slow chemical reactions, and warm temperatures speed up chemical reactions. Every enzyme, however, has an **optimal temperature** at which it works best. Some enzymes prefer cooler temperatures, and others prefer warm temperatures. However, extreme increases in temperature (i.e. boiling) will denature enzymes, making them inactive.

In the following exercise, you will test enzyme function at four different temperatures (on ice, in a refrigerator, in a warm incubator, and in a boiling water bath). Before setting up this experiment, formulate a hypothesis regarding the effect on enzyme activity. For example, do you think the enzyme will perform better at a warmer temperature? A cooler temperature? On ice? At boiling temperatures?

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. With a wax pencil (or Sharpie), label four small test tubes (#1, #2, #3, #4) and mark each at the 1 cm and 5 cm levels.
2. Fill each tube to the first mark with catalase.
3. Place tube #1 on ice, tube #2 at room temperature, tube #3 in a 37 degree Celsius incubator or warm water bath, and tube #4 in a boiling water bath or heat block. Wait 15 minutes.
4. While you are waiting, use a thermometer to measure the temperature of the environment for all four tubes. Record the values in Table 2 below.
5. After the 15-minute incubation, fill each tube to the second mark with hydrogen peroxide.
6. Swirl the contents to mix and wait 20 seconds.
7. Measure the height of the bubble column (in mm) for each tube and record your results in Table 2 below.

Table 2: Effect of Temperature on Enzyme Activity

Tube	Temperature (oC)	Bubble Column Height (mm)
1 - on ice		
2 - room temperature		
3 - warm water bath		
4 - boiling		

- Which tube showed the most enzyme activity? Explain.

  _____

- What is the **optimal temperature** for catalase activity? Explain

  _____

- Was your hypothesis supported? _____
- What is your conclusion concerning the effect of temperature on enzyme activity?

  _____

- What is the **optimal temperature** for enzymes in the human body?

  _____

- What effect could a fever have on enzymatic activity in the human body?

  _____

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 2.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Exercise 3: Effect of Concentration on Enzyme Activity

In general, the amount of product produced in a given amount of time should increase if you increase the enzyme concentration.

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. With a wax pencil (or Sharpie), label three large test tubes (#1, #2, #3). Make sure to use the larger tubes provided by your instructor.
2. Mark tube #1 at the 1 cm and 5 cm levels.
3. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
4. Swirl the contents to mix and wait 10 seconds.
5. Measure the height of the bubble column (in mm) and record your results in Table 3 below.
6. Mark tube #2 at the 2 cm and 6 cm levels.
7. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
8. Swirl the contents to mix and wait 10 seconds.
9. Measure the height of the bubble column (in mm) and record your results in Table 3 below.
10. Mark tube #3 at the 3 cm and 7 cm levels.

11. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
12. Swirl the contents to mix and wait 10 seconds.
13. Measure the height of the bubble column (in mm) and record your results in Table 3.

Table 3: Effect of Concentration on Enzyme Activity

Tube	Amount of Enzyme	Bubble Column Height (in mm)
1	1 cm	
2	2 cm	
3	3 cm	

- The amount of bubbling corresponds to the degree of enzyme activity. Which tube showed the most activity. Explain.

_____

_____

- If we waited for an unlimited amount of time, would the results be the same in all tubes? Explain.

_____

_____

- In this experiment, was the amount of substrate the same in all three tubes?

_____

- Generate a hypothesis about what you might observe if the amount of substrate concentration were increased while the enzyme concentration remained the same. Explain. _____

_____

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 3.
2. What is the dependent variable? Which axis is used to graph this data?

_____

3. What is your independent variable? Which axis is used to graph this data?

_____

Exercise 4: Effect of pH on Enzyme Activity

Each enzyme has an optimal pH, or level of acidity or alkalinity at which it functions best. A higher or lower pH affects hydrogen bonding and can alter the structure of the enzyme, leading to reduced activity.

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

_____

2. Record a **Hypothesis** for the question stated above.

_____

3. Predict the results of the experiment based on your hypothesis (if/then).

_____

4. Perform the experiment below and collect your data.

Procedure:

1. With a wax pencil (or Sharpie), label three small test tubes (#1, #2, #3) and mark at the 1 cm, 2 cm, and 6 cm levels. Fill each tube to the 1 cm level with catalase.
2. Fill tube #1 to the second mark with 0.1 M HCl.
3. Fill tube #2 to the second mark with buffer (pH 7.0).
4. Fill tube #3 to the second mark with 0.1 M NaOH.
5. Carefully swirl to mix and wait 20 seconds.
6. Fill all three tubes to the third mark with hydrogen peroxide.
7. Carefully swirl to mix and wait 20 seconds.
8. Measure the height of the bubble column (in mm) for each tube and record your results in Table 4 below.

Table 4: Effect of pH on Enzyme Function

Tube	Acid / Buffer / Base	Bubble Column Height (in mm)
1	0.1 M HCl	
2	Buffer pH 7.0	
3	0.1 M NaOH	

- Which tube showed the most activity? _____
- What is the **optimal pH** for catalase? _____

Questions for Review

1. What is the function of an enzyme? What specific **monomers** (i.e. building blocks) make up enzymes?
2. What does it mean when an enzyme becomes **denatured**?

Describe at least **2** ways in which an enzyme can become denatured.

1.
2.

3. What types of bonds or interactions are broken when an enzyme becomes denatured (name at least 3)?

4. Explain the difference between **substrate** and **active site**.

5. If an enzyme functions well at a pH of 4, would you expect it to also work well at a pH of 7? Explain.

Practical Challenge

1. Eggs can contain bacteria such as *Salmonella*. Considering what you've learned in this laboratory exercise, explain how cooking eggs makes them safe to eat.
2. What can happen to an enzyme when the pH of the environment becomes too acidic or too alkaline? **Use your observations from the lab to support your answer**.
3. If you don't want a cut potato to get brown, what could you do to prevent it assuming that the 'browning' occurs because of an enzymatic reaction? **Explain** your answer.
4. What would happen to enzyme activity if the pH of your blood decreased (became more acidic) or increased (became more basic)? Explain.

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 5: Enzyme Function," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY https://oer.galileo.usg.edu/biology-textbooks/18

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1.8: Respiration and Fermentation

Introduction

All organisms must break down organic molecules to release chemical energy to synthesize adenosine triphosphate (ATP). The energy stored in ATP can be released to perform cellular work. Organisms break down organic molecules, such as glucose, through the common processes of **cellular respiration** and **fermentation** (Figure 1). Cellular respiration is generally described as an aerobic process, requiring oxygen, which yields the most possible ATP generated from one molecule of glucose. But, technically, cellular respiration can occur in an anaerobic environment in some microorganisms. Anaerobic cellular respiration yields variable amounts of ATP, but much less than is generated in aerobic cellular respiration. In this laboratory, our discussion of cellular respiration will focus on **aerobic cellular respiration**.

Both aerobic cellular respiration and fermentation involve many chemical reactions that release high energy electrons from organic molecules and transfer the electrons to other molecules, often referred to as electron carriers (or coenzymes). These chemical reactions involving the transfer of electrons are called reduction-oxidation reactions, or **redox reactions**. In a redox reaction, one of the molecules gains electrons and becomes reduced (rig, **r**eduction **i**s **g**ain of electrons) and one of the molecules loses electrons and becomes oxidized (oil, **o**xidation **i**s **l**oss of electrons). In cellular respiration, electrons are often transferred to the electron carrier nicotinamide adenine dinucleotide (NAD+). When this redox reaction occurs, the organic molecule that loses the electrons has been oxidized. When NAD+ gains the electrons it forms NADH. NADH is the reduced form of NAD.

$$\mathrm{NAD^+ + 2\,e^- + H^+ \xrightarrow{Redox\ Reaction} NADH} \tag{1.8.1}$$

Aerobic cellular respiration involves a series of three processes of enzymatic chemical reactions: **glycolysis**, the **citric acid cycle** (also known as the Kreb's cycle), and the **electron transport chain**. Aerobic cellular respiration begins in the cytoplasm with glycolysis and ends in the mitochondria with the citric acid cycle and the electron transport chain. Aerobic cellular respiration results in fully oxidizing glucose, and can yield a maximum of 32 ATP per glucose molecule. At the culmination of the electron transport chain, the electrons are passed to oxygen, a highly electronegative element, to form water. Therefore, at the end of this process, the high energy electrons that were previously a part of glucose are now at a lower energy state, as they are held very closely by the electronegative oxygen.

Fermentation is an anaerobic process of breaking down organic molecules. It occurs in the absence of oxygen. Fermentation breaks down organic molecules, such as glucose, into smaller organic molecule end products. Fermentation begins with the process of glycolysis to produce pyruvic acid and 2 net ATP. Enzymes then carry out chemical reactions to convert pyruvic acid into various fermentation end products. Two common types of fermentation are named for their end products, **alcoholic fermentation** and **lactic acid fermentation**. Fermentation produces organic end products that still contain high-energy electrons. Fermentation does not fully oxidize glucose, and yields only 2 net molecules of ATP, along with organic end products.

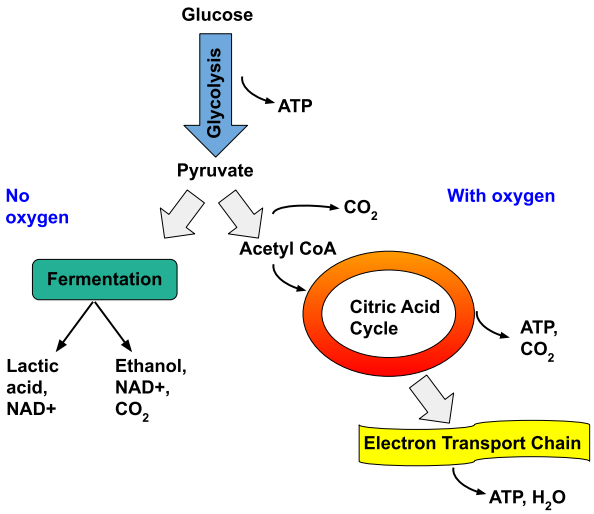


Figure 1. Cellular respiration and fermentation phases (original figure)

PART 1: CELLULAR RESPIRATION

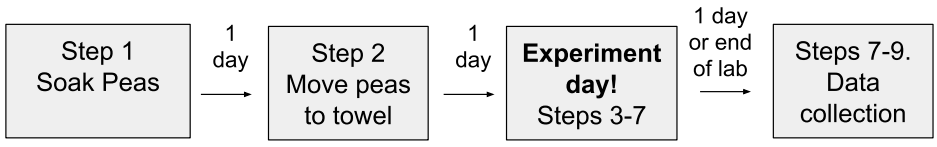
Exercise 1 : Investigating Cellular Respiration in Plants

This part of the lab investigates cellular respiration in pea seeds. Seeds of plants are stuffed full of sugars like starch. Cellular respiration involves breaking down sugars to generate ATP. Therefore, this process allows plants to harvest energy necessary to produce roots, shoots, and leaves. The process of cellular respiration also results in the release of carbon dioxide gas. Carbon dioxide will react with water to form carbonic acid. The formation of carbonic acid will affect the pH of an aqueous solution. Since carbon dioxide is colorless, odorless, and very hard to detect, we are going to use a pH indicator to detect the presence of carbonic acid and thus carbon dioxide. pH indicators, like red cabbage juice, bromothymol blue, and phenol red are chemicals that change color when pH is altered. In this experiment, we will observe cellular respiration in germinating pea seeds by detecting the production of carbon dioxide and monitoring the changes in the pH of the solution.

Materials:

- Pea seeds (20 germinating/ lab group and 20 dormant / lab group)
- Large sealable bag
- Test tube rack (that can accommodate wide mouth test tubes)
- Wide mouth test tubes with rubber stoppers (3/ lab group)
- Distilled water or spring water (non-chlorinated water)
- Paper towels
- Nonabsorbent cotton plugs
- Glass beads (20 / group)
- Glass rods
- Gloves and safety goggles
- Sharpie or red wax pencil
- Indicator reagent [Choose 1: red cabbage juice, or bromothymol blue (0.04% solution), or phenol red (0.04% solution)] (need 15 ml of indicator solution / lab group)

Overall Timeline:



Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. Two days before beginning the experiment, pour half of the peas into a glass container and cover with several inches of non-chlorinated water to compensate for the expansion of the seeds as they swell. Allow the seeds to soak overnight.
2. The next day, pour the water off of the seeds. Place the seeds onto a wet paper towel, place in a plastic sealable bag, seal the bag, and store the seeds overnight in the dark.
3. On the day of the experiment carefully remove the rehydrated (germinating) seeds from the paper towel.
4. Label 3 wide mouth tubes #1 - #3.
5. Wear gloves when handling the indicator solution. Place 5 ml of the indicator solution into each test tube.
6. Using the glass rod, push a plug of nonabsrobent cotton into each test tube until it sits right above the indicator solution.
7. Add the following to tubes #1 - #3.
   1. **Tube 1:** add 20 glass beads
   2. **Tube 2:** add 20 germinating peas
   3. **Tube 3:** add 20 dry dormant peas
8. Tightly cap the tubes with rubber stoppers. (If the rubber stoppers have a hole, cover the stoppers with cling wrap, and then place each stopper into a tube.)
9. Observe the color of the indicator reagent at the beginning of the experiment and record your results in Table 1.
10. Observe the color of the indicator reagent after the 2-hr incubation and record your results in Table 1.
11. Observe the color of the indicator reagent after the 24-hr incubation and record your results in Table 1.
12. Once the experiment has been completed, carefully pour the indicator reagent into the appropriate location as indicated by your instructor, being sure to collect the glass beads by pouring through a wire mesh filter.
13. Rinse and wash the test tubes thoroughly.

Table 1. Cellular Respiration of Germinating Peas

Indicator Used:				
Tube contents	**Initial color of indicator**	**Color after 2 hrs**	**Color after 24 hrs**	**Acidic or basic?**
1. Control				
2. Germinating peas				
3. Dormant Peas				

Questions for Review

1. What is the color of the indicator at at

- Neutral pH?
- Basic pH?
- Acidic pH?

2. What was the purpose of **Tube #1**?

3. What **specifically** was produced as a result of cellular respiration that changed the color of the indicator?
4. How is carbon dioxide an indicator that cellular respiration is taking place in these peas?
5. Germination is the process by which a dormant seed begins to sprout and grow into a seedling. What are some possible metabolic processes that are required for seed germination?
6. During respiration, a seed metabolizes sugars. What is the source of the sugar metabolized by the seed?
7. What variables do you think may affect the respiration rate of the seeds?
8. The equation for cellular respiration is:

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O + 32\,ATP \tag{1.8.2}$$

The energy released from the complete oxidation of glucose under standard conditions is 686 kcal/mol. The energy released from the hydrolysis of ATP to ADP and inorganic phosphate under standard conditions is 7.3 kcal/mol. Using the equation for cellular respiration above, **calculate** the efficiency of respiration (i.e. the percentage of chemical energy in glucose that is transferred to ATP). *For help with answering this question, refer to Concept 9.4 (Campbell Textbook).

9. How might the process of photosynthesis affect pH? Form a hypothesis.

PART 2: AEROBIC RESPIRATION IN YEAST

Optional Activity or Demonstration

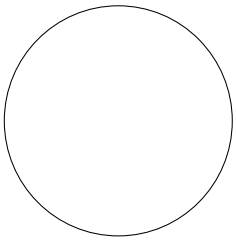
This part of the lab investigates aerobic cellular respiration by *Saccharomyces cerevisiae*, also referred to as “baker’s yeast” and “brewer’s yeast.” Yeast is a unicellular fungus that can convert glucose into carbon dioxide and ATP when oxygen is present. Methylene blue dye can be used as an indicator for aerobic respiration in yeast. Aerobic respiration releases hydrogen ions and electrons that are picked up by the methylene blue dye, gradually turning the dye colorless. This redox reaction can be observed when viewing a wet mount of yeast and methylene blue under the compound light microscope. The mitochondria of yeast cells undergoing aerobic respiration will appear as a clear area surrounded by a ring of light blue cytoplasm. If cellular respiration is not taking place, the mitochondria will absorb the blue dye and will not turn colorless.

Materials:

- Yeast (not quick rise)
- Distilled water
- Transfer pipette
- Methylene blue dye (in dropper bottle)
- Compound light microscope
- Microscope slide and cover slip
- Electronic balance, spatula, and weigh paper

Procedure:

1. Prepare yeast suspension: Add 7 grams yeast to 50 ml warm tap water. Stir to mix. Save the yeast suspension for Part 3.
2. Place a drop of yeast suspension on a clean microscope slide with a transfer pipette.
3. Add one drop of methylene blue dye and place a cover slip on the microscope slide over the yeast suspension.
4. Observe the yeast using the scanning objective lens. Use the coarse adjustment knob to focus on the yeast cells. Switch to the low power objective lens and then to the high power objective lens.
5. In the circle below, draw several yeast cells undergoing aerobic respiration and several yeast cells not undergoing aerobic respiration. Label the cytoplasm and nucleus if visible.



PART 3: ALCOHOLIC FERMENTATION IN YEAST

This part of the lab investigates alcoholic fermentation by *Saccharomyces cerevisiae*, also referred to as “baker’s yeast” and “brewer’s yeast.” Yeast converts pyruvate from glycolysis into acetaldehyde, releasing carbon dioxide gas. Acetaldehyde is then enzymatically converted by the enzyme alcohol dehydrogenase into ethanol (Figure 2). In this lab, we will measure the accumulation of carbon dioxide released in the first enzymatic reaction as an indicator of the progression of fermentation.

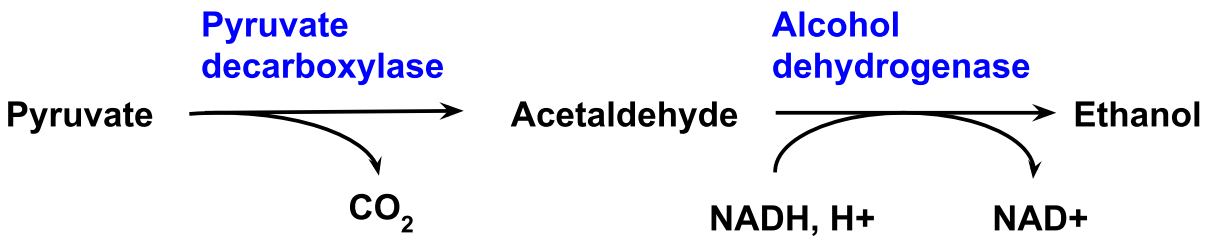


Figure 2. Alcoholic Fermentation (original figure)

Exercise 1: Investigating Different Concentrations of Yeast

Materials:

- 4 identical saccharometers (glass fermentation hydrometer with either a 10-cm or a 15-cm vertical tube, Figure 3) / lab group
- Yeast (not quick rise)
- Wax pencil or Sharpie
- Distilled water
- 10% glucose solution
- Transfer pipettes
- Test tube rack
- 4 large (20 ml) test tubes or small Erlenmeyer flasks for larger volumes
- Large plastic tray
- Masking tape or lab tape
- Large weigh boat (4/group)
- Metric ruler
- Electronic balance
- Spatula
- Weigh paper
- Red food coloring (optional)

Figure 3. Saccharometer

Table 2. Contents of Saccharometers when testing fermentation with various yeast concentrations.

Saccharometer	DI Water	Glucose Solution	Yeast Suspension
1	*8 ml	*6 ml	0 ml
2	*12 ml	0 ml	*2 ml
3	*6 ml	*6 ml	*2 ml
4	*2 ml	*6 ml	*6 ml

***Double these amounts if using saccharometers that have a 15-cm vertical tube. See table below**

Saccharometer	DI Water	Glucose Solution	Yeast Suspension
1	16 ml	12 ml	0 ml
2	24 ml	0 ml	4 ml
3	12 ml	12 ml	4 ml
4	4 ml	12 ml	12 ml

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. Prepare yeast suspension: Add 7 grams yeast to 50 ml warm tap water. Stir to mix. Alternatively, you can use the yeast suspension from Part 2. Optional: Add a few drops of red food coloring to the yeast to increase contrast, allowing easier measuring of the height of yeast in saccharometers.
2. Label 4 test tubes and 4 saccharometers # 1- 4. Use a transfer pipette to add the appropriate amount of glucose and distilled water listed in Table 2 to the corresponding labeled test tubes.
3. Use a transfer pipette to add the appropriate amount of yeast solution listed in Table 1 to the corresponding labeled test tubes. It is important to work carefully and quickly after adding the yeast solution to the glucose and water.

4. Carefully pour the contents of the test tubes into the correspondingly labeled saccharometer, ensuring that the solutions are well mixed.
5. Carefully tilt the saccharometers to allow any air bubbles that are trapped in the arms of the vertical tube to escape.
6. Begin the timer for the experiment and measure the size of any bubbles (in mm) that are trapped in the vertical arms of the saccharometers. Record this measurement as the 0 time point.
7. Position the saccharometers on the large plastic tray, positioning them around a plastic weigh boat to catch any fermentation overflow that may occur.
8. Carefully tape the saccharometers to the large plastic tray to prevent them from falling and breaking.
9. Every 2 minutes measure and record the total amount of bubbles that accumulate in the top of the vertical arm of the saccharometer. Record the mm of carbon dioxide (bubble) measurements in Table 3.
10. Continue recording the total amount of carbon dioxide released every 2 minutes for 20 minutes.
11. After completing the experiment carefully carry the saccharometers to a sink for washing. Carry only one saccharometer at a time. Spill the yeast mixture into the sink and wash the saccharometer carefully and thoroughly. Return the saccharometer to the plastic tray, laying it down on its side when not in use.

Table 3. Carbon dioxide evolved during fermentation by different concentrations of yeast. Carbon dioxide levels measured in millimeters (mm).

Time (min.)	Sacch. 1	Sacch. 2	Sacch. 3	Sacch. 4
0 (initial)				
2				
4				
6				
8				
10				
12				
14				
16				
18				
20				

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 3.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Exercise 2: Investigating the fermentation of different carbohydrates

Materials:

- 4 identical saccharometers (glass fermentation hydrometer with either a 10-cm or a 15-cm vertical tube) / lab group
- Yeast (not quick rise)
- Wax pencil or Sharpie
- Distilled water
- 10% glucose solution
- 1% starch solution
- 10% sucrose solution
- Metric ruler
- Transfer pipettes
- Test tube rack
- 4 large (20 ml) test tubes or small Erlenmeyer flasks for larger volumes
- Large plastic tray
- Masking or lab tape
- Large weigh boat
- Electronic balance
- Spatula
- Weigh paper
- Red food coloring (optional)

Table 4. Contents of Saccharometers when testing fermentation of different carbohydrates.

Saccharometer	DI Water	Yeast Suspension	Carbohydrate
1	*2 ml	*6 ml	*6 ml glucose
2	*2 ml	*6 ml	*6 ml sucrose
3	*2 ml	*6 ml	*6 ml starch

***Double these amounts if using saccharometers that have a 15-cm vertical tube. See table below**

Saccharometer	DI Water	Yeast Suspension	Carbohydrate
1	4 ml	12 ml	12 ml glucose
2	4 ml	12 ml	12 ml sucrose
3	4 ml	12 ml	12 ml starch

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. Prepare yeast suspension: Add 7 grams yeast to 50 ml warm tap water. Stir to mix. Optional: Add a few drops of red food coloring to the yeast to increase contrast, allowing easier measuring of the height of yeast in saccharometers.
2. Label 3 test tubes and 3 saccharometers # 1- 3. Use a transfer pipette to add the appropriate amounts of carbohydrates and distilled water listed in Table 4 to the corresponding labeled test tubes.
3. Use a transfer pipette to add 6 ml yeast solution to each of the test tubes. It is important to work carefully and quickly after adding the yeast solution to the carbohydrate.
4. Carefully pour the contents of the test tubes into the correspondingly labeled saccharometer, ensuring that the solutions are well mixed.
5. Carefully tilt the saccharometers to allow any air bubbles that are trapped in the arms of the vertical tube to escape.
6. Begin the timer for the experiment and measure the size of any bubbles (in mm) that are trapped in the vertical arms of the saccharometers. Record this measurement as the 0 time point.
7. Position the saccharometers on the large plastic tray, positioning them around a plastic weigh boat to catch any fermentation overflow that may occur.
8. Carefully tape the saccharometers to the large plastic tray to prevent them from falling and breaking.
9. Every 2 minutes measure and record the total amount of bubbles that accumulate in the top of the vertical arm of the saccharometer. Record the mm of carbon dioxide (bubble) measurements in Table 5.
10. Continue recording the total amount of carbon dioxide released every 2 minutes for 20 minutes.
11. After completing the experiment carefully carry the saccharometers to a sink for washing. Carry only one saccharometer at a time. Spill the yeast mixture into the sink and wash the saccharometer carefully and thoroughly. Return the saccharometer to the plastic tray, laying it down on its side when not in use.

Table 5. Carbon dioxide evolved during the fermentation of various carbohydrates. Carbon dioxide levels measured in millimeters (mm).

Time (min.)	Sacch. 1	Sacch. 2	Sacch. 3
0 (initial)			
2			
4			
6			
8			
10			
12			
14			
16			
18			
20			

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 5.

2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Questions for Review

1. Fermentation involves **redox reactions**. Explain what happens to electrons during a redox reaction and how this changes a molecule's potential energy.
2. Why did we add the *Saccharomyces cerevisiae* (baker's yeast) to the fermentation tubes? Specifically, what did the yeast provide to the fermentation mixture?

3. What is the purpose of *Saccharomyces cerevisiae* ("baker's yeast) in the bread-making process?
4. We measured the formation of what end product to determine the fermentation rate? Name the end product that we measured.

5. List two specific factors (as they relate to the experiment performed in our lab) that affect the rate of fermentation.

Practical Challenge Questions:

1. What other variables could be investigated that might affect the rate of alcoholic fermentation by yeast?

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1.9: Photosynthesis

Introduction

Have you ever wondered why leaves change colors in the fall but remain green during other seasons of the year? Leaves contain multiple types of pigments that are involved in many different functions in the plant, including photosynthesis, protection from UV radiation, and even attracting pollinators. Pigment molecules are stored inside of **plastids**, a class of cellular organelles that includes **chloroplasts**, the organelles responsible for photosynthesis. Found inside chloroplasts, **chlorophyll** is the most common pigment in a leaf. It comes in two varieties (**chlorophyll *a*** and **chlorophyll *b***). During the spring and summer months, when day length is longest and the sunlight is most direct, plants produce a large amount of chlorophyll, which is used to capture sunlight energy that drives photosynthesis.

All of the wavelengths of energy emitted by the sun are collectively called the **electromagnetic spectrum**. Waves at the low end of the spectrum (those with the longest wavelengths), like radio waves and microwaves, emit less energy, while waves at the high end (those with the shortest wavelengths), like x-rays and gamma rays, emit higher energy. The very small section of the electromagnetic spectrum that humans can see is called the **visible light spectrum**. Plants can use these same wavelengths of light, from red at the low end to violet at the high end of the visible light spectrum, to power photosynthesis(**Fig. 1**).

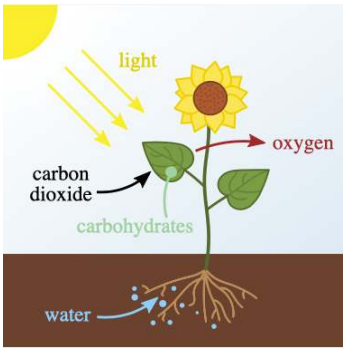

Figure 1. Photosynthesis Credit: [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)

Photosynthesis is the process by which plants use energy from the sun and water from the soil to convert atmospheric carbon dioxide into sugar (glucose) that can be used for energy production (**Fig. 2**).

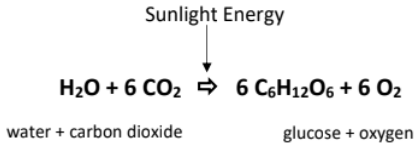

Figure 2. Chemical Equation for Photosynthesis.

Different pigments absorb and reflect different wavelengths of light and therefore appear different colors to us. During the spring and summer months, when plants are most productive, they produce a huge amount of chlorophyll molecules. Chlorophyll *a* and chlorophyll *b* absorb mostly at the red-to-orange and blue-to-violet ends of the visible light spectrum; they reflect the rest of the wavelengths. The reflected wavelengths, mostly in the yellow-to-green range, are the wavelengths that are detected by the human eye. This is why plants appear green to us (**Fig. 3**).

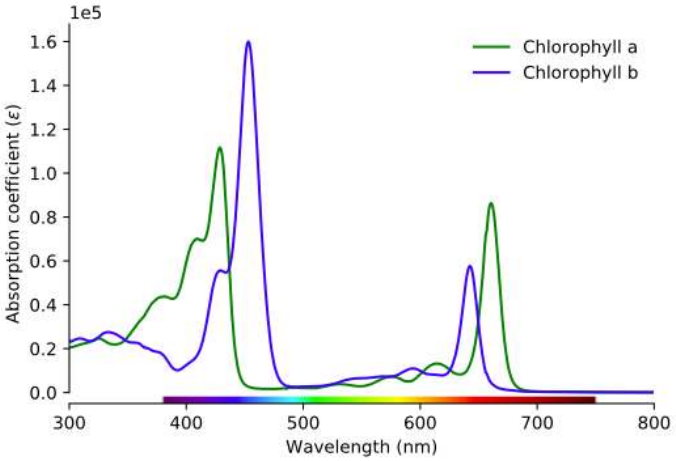

Figure 3. Absorption spectrum of chlorophyll *a* and chlorophyll *b* Credit: "File:Chlorophyll Absorption Spectrum.svg" by Serge Helfrich is licensed under CC BY-SA 4.0

Another class of pigments found in plants is the **carotenoids.** Carotenoids include **carotenes**, which appear yellow-orange, and **xanthophylls**, which appear mostly yellow. These pigments are found in the plant all year round but are largely masked by the abundant chlorophyll molecules that are present during the spring and summer. Carotenoids play a minor role in photosynthesis, but they play a larger role in protecting the plant tissues from damage caused by UV radiation from the sun.

As summer gives way to fall, day length gets shorter, temperatures decrease, and water may become less available. Plants stop producing new chlorophyll molecules, and the remaining chlorophyll molecules are broken down and reabsorbed to be used again in the following spring. Carotenoids break down more slowly, which is why we can see their yellow and orange colors showing through as chlorophyll is lost from the leaves.

Exercise 1: Separation of Plant Pigments Using Chromatography

In this lab, you will first extract pigments from spinach leaves and then separate pigments from one another using a technique called **chromatography.** Chromatography is used to separate chemicals based on their varying solubilities in selected solvents. Recall from our discussions of the Chemistry of Life (textbook chapter), molecules and compounds are classified as **polar** or **nonpolar**, and that "like dissolves like." In other words, polar compounds dissolve in polar solvents, but not in non-polar solvents, and vice versa. An example of this concept is seen when oil, a nonpolar substance, comes in contact with water, a polar substance. The two substances do not mix; they are repelled by one another. Chromatography works by separating chemicals according to their varying degrees of polarity. In chromatography experiments, there are two "phases," the stationary phase, which does not move, and the mobile phase, which travels across the stationary phase.

In this experiment, you will use **paper chromatography**. Paper, which is made of cellulose, is very polar and acts as the stationary phase. The solvent you will use is a mixture of petroleum ether and absolute acetone, both of which are nonpolar; the solvent acts as the mobile phase. Extracted plant pigment is applied to the paper, and the paper is placed in the solvent. As the solvent moves up the paper through capillary action, it carries the pigments along with it. Pigments that are more polar are attracted to the polar cellulose molecules in the stationary phase (the paper), so they move more slowly as the solvent travels up the paper. Nonpolar pigments are more strongly attracted to the nonpolar solvent and tend to stay in solution longer, thus moving farther up the paper. The pigments are carried at different rates because they are not equally soluble. Since different pigment molecules have different molecular structures and varying degrees of polarity, this technique works well to separate pigments from one another, giving us a clear look at each pigment individually.

The distance that each pigment travels will be unique to that pigment based on the solvent selected. These migration distances can be used to calculate the R_f (retention factor) value, which is simply calculated by dividing the distance traveled by the pigment over the distance traveled by the solvent.

$$R_f = \frac{\text{distance traveled by pigment (cm)}}{\text{distance traveled by solvent (cm)}} \tag{1.9.1}$$

Materials

- Spinach leaves
- Electronic balance / weigh boats
- A small amount of clean quartz sand
- Mortar and pestle
- Acetone (keep this in the hood at all times)
- Test tube
- Chromatography paper (11 cm square)
- Metric ruler
- Chromatography jars - glass quart jars (which will hold 11 cm square paper) with lids or can cover with aluminum foil
- Pencil
- Glass capillary tubes
- Small metric rulers
- Chromatography solvent (Use 9 parts petroleum ether and 1 part acetone)
- Waste container for discarded acetone/chlorophyll extract
- Handheld UV light (Turn lights off in lab and shine handheld UV light on students' chlorophyll extracts to cause them to fluoresce red)

Procedures

Pigment Extraction

1. Obtain spinach leaves. Tear leaves into small pieces, discarding the large midvein. Weigh out approximately 4.0 grams of leaf tissue.
2. Place leaf tissue plus a pinch of clean quartz sand into a clean mortar and pestle; grind to a fine pulp.
3. Add 6mL of acetone to the pulp and continue to grind.
4. When leaf tissue is thoroughly ground into a paste-like consistency, allow the solution to rest for 1 minute. Carefully pour off the liquid portion of the mixture into a clean test tube, leaving the pulpy remains in the mortar. *Note: Dispose of acetone/chlorophyll waste in the waste container in the fume hood. Wash the mortar and pestle well with warm soapy water, rinse, and set aside to dry.
5. You may wish to examine your acetone/chlorophyll extract under UV light. Turn off the lights in the lab and shine the fluorescent black light onto the extract. Do not look into the light directly. The chlorophyll will fluoresce red.
6. Can you explain why the extract containing the chlorophyll pigment turns from a green color to a fluorescent red color when exposed to UV light?

Pigment Separation

NOTE: The organic solvents used in this step are extremely volatile and flammable. The chromatography jars must be kept in the fume hood at all times.

Paper chromatography requires that the atmosphere within the chromatography jar be completely saturated with solvent. Be sure that the lid or aluminum foil covering the jar stays in place before and during chromatogram development.

1. **Prepare chromatography sheet:** Use the pencil to draw a line 1.5 - 2 cm from the bottom of the paper. This line will serve as a guide when applying the spinach extract. It will help ensure that the extract is applied evenly, in a straight line, and at a level **above the solvent** in the chromatography jar.
2. **Apply pigment:** Using a glass capillary tube, apply the pigment extract to the paper in a linear series of small dots (follow the line you created as a guide for application). **NOTE: Leave a 1 cm margin at each edge that is free of pigment extract. DO NOT TOUCH THE PAPER; oils from your skin can interfere with the process.**
3. After you have completed the pigment line across the chromatography paper, allow the extract to dry for about 5 min. Go back and re-apply more pigment over the first line, allowing each application to dry before reapplying more extract. Repeat the

procedure until ***all*** the pigment extract is used, or until you have applied enough extract (consult with your instructor). Allow the sample to dry.

4. Once your sample has dried, take your chromatography paper to the fume hood. Add approximately 20 mL of chromatography solution to the jar (this may have already been done for you). This should result in the solvent being about 1 cm deep from the bottom of the jar. Roll the paper into a cylinder so that the line faces outward and is toward the bottom of the jar. You can secure this cylinder shape by using a small stapler to staple the sides together at the top and the bottom of the cylinder. Place the rolled paper into the jar so that it is in the chromatography solution. **The chromatography solution should not be higher than the line on your chromatography paper.**
5. Cover the jar with the lid or aluminum foil and observe as the chromatography solvent (the mobile phase) travels up the paper (the stationary phase).
6. When all of the pigments are clearly separated and **before** the solvent front has reached the top edge, remove the chromatogram and allow it to dry in the hood. Make sure to use a pencil to draw a line to indicate where the solvent front ended.
7. You should be able to see at least four distinct bands. (There may be as many as six bands.)
8. Make a sketch of your chromatogram using the template that follows. Using colored pencils, note the color of the bands. Use Table 1 to help you identify each pigment. On your sketch, label each band with the name of the pigment. Mark the distance (in cm) from the initial pigment band to each colored band as well as the total distance from the initial pigment band to the solvent front.

Sketch of Resulting Chromatogram

Table 1: Different classes of pigments found in leaves and their respective colors.

Pigment	Band Color
Beta-carotene	**Orange**
Xanthophylls	**Yellow**
Chlorophyll *a*	**Blue-green**
Chlorophyll *b*	**Yellow-green**

9. Use the data from your sketch to complete the data table below. Calculate the R_f value and include that in your data table.

$$R_f = \frac{\text{distance traveled by pigment (cm)}}{\text{distance traveled by solvent (cm)}}$$

A	B	C	D	E
Name of Pigment	Description of color	Distance solvent front traveled from initial pigment band	Distance pigment traveled from initial pigment band	Rf value of pigment (column D / column C)

Review Questions:

1. Which plant pigments are most polar?
2. Which are least polar?
3. How do these differences in polarity affect the movement of the pigments up the chromatography paper? Explain why this is observed.
4. What does the abbreviation *Rf* stand for? How is it calculated? What does it tell us?

5. If Solution A moves 4 cm and Solution B moves 4.5 cm on a piece of chromatography paper, when the solvent moves 10 cm, which is the most polar solution? Explain your answer.

Practical Challenge Questions:

1. Use the chromatogram below to calculate R_f values

$$R_f = \frac{\text{distance traveled by pigment (cm)}}{\text{distance traveled by solvent (cm)}}$$

2. Based on these calculations, what do you think both high and low Rf values can tell you about the substances?

Exercise 2: Analysis of the Absorption Spectrum of Leaves

While we know most leaves are green, we have now seen that other pigments are also found in the leaves. The green color that we observe is a consequence of reflected light in the wavelengths of about 550-500nm in size (**Fig. 3**). In the autumn months of the year, many plants stop producing chlorophyll, revealing the colors of the other pigments beneath. Every chemical has a specific set of wavelengths it can absorb and thus be identified. By analyzing the absorption spectrum of leaf pigments, we can infer what wavelengths of light will be useful in photosynthesis.

Materials

- PASCO Wireless Spectrometer
- iPad or laptop to connect the spectrometers to
- 4 cuvettes
- Leaf samples
- 3 test tubes
- 100 mL 95% ethanol
- 20 mL graduated cylinder
- Scissors
- Mortar and pestle set up from the previous section

Procedure

1. Connect the spectrometer to a Laptop via a USB cable or wirelessly to an iPad.
2. Open the PASCO spectrometer application.
3. Select the ANALYZE Solution setting from the menu at the top.
4. Choose CALIBRATE DARK from the menu at the bottom. Cover the sample well with your finger to block light until a check mark appears indicating that calibration is complete.
5. Fill a cuvette ¾ full with ethanol. Only handle the cuvettes from the ribbed sides since fingerprint smudges on the clear sides will affect light passing through. Place the cuvette in the well so that the clear side is facing the light source.
6. Choose CALIBRATE REFERENCE from the menu until the checkmark appears. You do not need to cover the cuvette with your finger for this step.

SAMPLE PREPARATION:

1. Obtain 3 different samples of plant leaves.
2. Label 3 test tubes: Sample 1, Sample 2, Sample 3
3. Tear the first sample of plant leaves into small pieces, discarding the large midvein. Weigh out approximately 4.0 grams of leaf tissue.
4. Place the leaf tissue plus a pinch of clean quartz sand into a clean mortar and pestle; grind to a fine pulp.
5. Add 6mL ethanol to the pulp and continue to grind. Make sure to use ethanol and **NOT** acetone. The cuvettes are plastic and acetone will cloud the plastic and not allow light to pass through freely.
6. When the leaf tissue is thoroughly ground into a paste-like consistency, allow the solution to rest for 1 minute. Carefully pour off the liquid portion of the mixture into a clean test tube, labeled sample 1, leaving the pulpy remains in the mortar.

7. Repeat the extraction for the remaining two leaf samples. Be sure to add the appropriate leaf extraction mixture to the correctly labeled test tubes.

DATA COLLECTION

1. Place the first leaf sample extraction mixture into a cuvette in the well and press START RECORDING. The graph that appears is the absorbance spectrum of the extract. If any of the peaks are flattened, add more ethanol to the cuvette to further dilute the sample.
2. When the peaks have stabilized, select STOP RECORDING and name your run for the sample analyzed.
3. Use the ADD COORDINATE tool to find the peak of each sample and SNAPSHOT each of the sample runs. Record the values in a data table.
4. Table 2 shows the absorption spectrum of some common plant pigments. Use this information to predict what pigments were in your samples.

Table 2: Absorption of Plant Pigments

Pigment	Peak Absorption Wavelengths (nm)	Sample 1	Sample 2	Sample 3
Chlorophyll A	430 and 662			
Chlorophyll B	453-642			
Carotenoids	460-550			
Anthocyanins	520*			
Xanthophyll	494			
Betalains	535 or 480*			

*peaks may vary and are pH-dependent.

Questions

1. How do you think the knowledge obtained from your chromatogram and the spectrograms relate to our understanding of plant pigments?
2. Why do leaves change color in the fall?

Exercise 3: Measuring the Rate of Photosynthesis

(remixed from https://batch.libretexts.org/print/Letter/url=https://bio.libretexts.org/Bookshelves/Ancillary_Materials/Laboratory_Experiments/General_Biology_Labs/BCCC_MASTER_Biology_PLC/Biology_102/02%3A_Photosynthesis/2.1%3A_Photosynthesis_Protocol.pdf CCBY 2.1.1)

Materials:

- Five spinach leaves (Dark green, fresh spinach leaves produce better, faster results.)
- Two cups (Short clear plastic cups which hold ~300 mL work best.)
- Index cards
- Forceps
- Plastic petri dish
- 500 mL of water with 0.2% sodium bicarbonate (NaHCO3) (or baking soda; you may prefer to use 0.3%-0.5% sodium bicarbonate which will result in a faster rate of photosynthesis and more rapid floating for the leaf disks)
- 500 mL of water without sodium bicarbonate (NaHCO3)
- Two drops of dilute detergent (To prepare the dilute detergent, add approximately 5 mL of dishwashing liquid soap to 250 mL of water. Aliquot into microfuge tubes and label with a “DD”)

- Hole punch (or a straw)
- Two 10 ml syringes without needles (syringes can be rinsed, dried and reused)
- Lamp with 23 W spiral compact fluorescent bulb (If you have a clamp lamp, obviously this could be attached to a ring stand or other vertical pole; if you don't have enough vertical poles, you could rig up lamp support using a cardboard box.)
- Stopwatch

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. Label one cup **'sodium bicarbonate'** and fill it about one-quarter full (~75 mL) with the sodium bicarbonate solution. Label the second cup **'water'** and fill it about one-quarter full (~75mL) with water. Add one drop of dilute detergent (microfuge tube labeled “DD”) to each cup.
2. Next, you will prepare your leaf disks, taking care not to damage them. First, prepare two index cards by folding each of them in half and then unfolding them and laying them flat. Use the hole punch to prepare the leaf disks. Punch out a piece of leaf tissue, avoiding large veins. (If you are using a hole punch, keep it clamped shut as you tap the punch on the table to release the leaf disk onto the piece of paper.) Repeat until you have 10 leaf disks on each index card.
3. Remove the plunger from a syringe, and use one of the folded papers to pour 10 leaf disks into the syringe. Tap them down to the tip of the syringe.
4. Replace the plunger, and push it down to about the 1 mL mark, being careful not to squash the leaf pieces.
5. Suck up ~5 mL of the sodium bicarbonate/detergent solution into the syringe. Hold the syringe upright and push out as much of the air as possible.
6. Put your thumb over the tip of the syringe, and pull back slowly on the plunger to about the 10 mL mark. Additionally, while pulling back on the plunger and keeping your thumb over the tip of the syringe, shake the solution in the syringe. This creates a vacuum and pulls air out of the leaf discs. Tilt and swirl the syringe to make sure all disks are submerged in the solution, then hold it for 10 seconds, and then gently let go of the plunger without removing your thumb from the tip of the syringe. The plunger will “snap” back into position and the solution will enter the leaf disks. If the leaf disks drop to the bottom of the solution in the syringe, you are done. If not, do this again.
7. Remove the plunger and empty the disks into the cup containing sodium bicarbonate solution. Then swirl the cup to dislodge any disks that are stuck to the side of the cup. Make sure that all of the disks are settled on the bottom of the cup.
8. Repeat steps 3 - 7 but use the water/detergent solution instead of sodium bicarbonate.
9. Place both cups under a bright light. Place half a petri dish full of water on top of each cup to act as a temperature buffer.

Safety Precaution

Be careful to keep all liquids away from the light source and electrical cord.

10. At the end of each minute, record the number of floating disks (any disk that is no longer touching the bottom) in the following table.

Table 2: Number of Floating Leaf Disks

Minute	In Water/Detergent	In Bicarbonate/Detergent
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 2, using different symbols for the leaf disks in water vs. the leaf disks in bicarbonate solution.
2. What is the dependent variable? Which axis is used to graph this data?

   _____
3. What is your independent variable? Which axis is used to graph this data?

   _____

Questions

1. Complete the following sentence.

Photosynthesis is a set of _____ in which _____energy is converted to _____energy.

2. Why do some trees appear green in the summer but change colors in the fall?
3. What **optimal wavelengths** (or peaks) did you observe for both chlorophyll *a* and *b*?
4. Do your leaf disks float? Use the information in this diagram of a cross-section of a leaf to explain why a leaf disk would float.

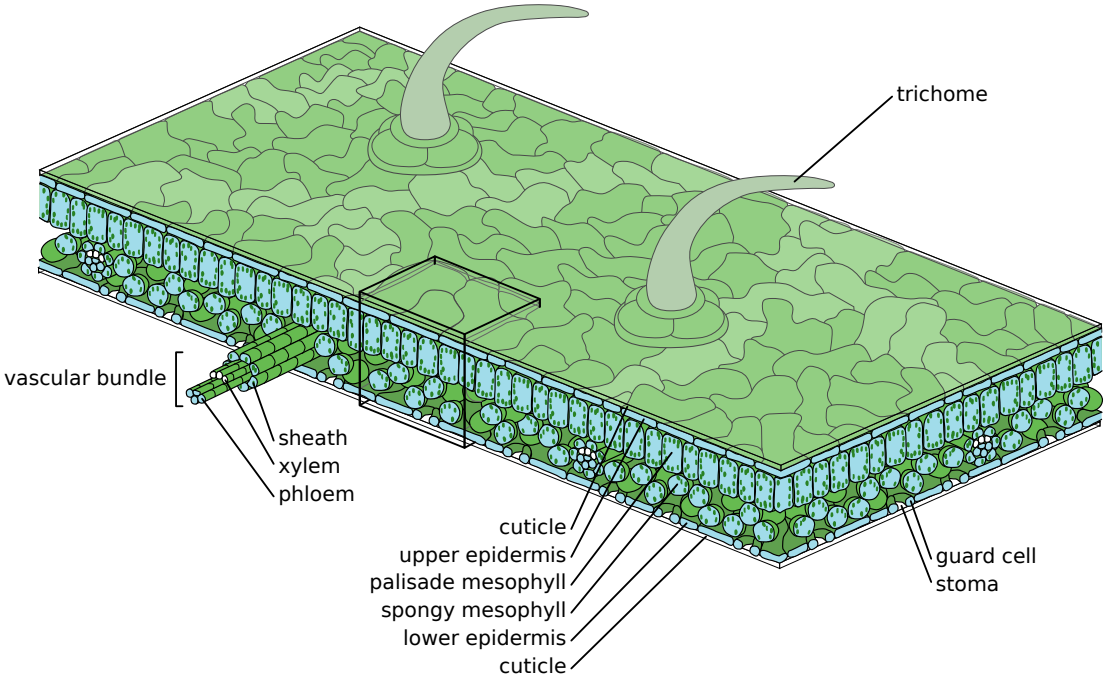

Figure 1.9.1: Copy and Paste Caption here. (CC BY-SA 3.0 Unported; Zephyris via Wikipedia)

5. Where does photosynthesis occur in a leaf? State which organelles carry out photosynthesis and which type or types of leaf cells have this organelle.
6. Explain why it is useful to the plant to have air spaces around the spongy mesophyll cells in the leaves. (Hint: Recall the chemical equation for photosynthesis.)
7. What was the purpose of the sodium bicarbonate in this experiment?
8. Leaf disks normally float. What caused the leaf disks to sink?
9. To measure the rate of photosynthesis, you replaced the air in the spongy mesophyll in your leaf disks with a liquid. This caused the leaf disks to sink. Then you put these leaf disks in water with dissolved CO_2 and measured the amount of time it took for the leaf disks to float. Which product of photosynthesis accumulated in the spongy mesophyll and caused the leaf disks to float?
10. Suppose that a leaf disk that has had the air sucked out is placed in a bicarbonate solution under a dim light that results in a low rate of photosynthesis that just equals the rate of cellular respiration. Would you expect this leaf disk to float? Explain why or why not.

Exercise 4: Observing and Quantifying Stomata (Optional)

Leaves are the primary food factories in a typical plant. Powered by the sun's energy, leaves take in water and carbon dioxide and produce oxygen and glucose through **photosynthesis**. Leaves have specialized cells on their surfaces called **guard cells** which surround openings called **stomata** (singular = stoma). Stomata are tiny openings in the epidermis of terrestrial (land) plants. Gases, primarily O_2 and CO_2, can pass through these openings to allow for photosynthesis to occur. Likewise, H_2O will evaporate out of the stomata, leading to potential dehydration.

Stomata typically remain open during the day (since sunlight stimulates them to open) and close at night, when photosynthesis doesn't occur, allowing for water conservation. Stomata can also close during especially hot days, during droughts, or as a response to plant growth regulators. Desert plants have a special form of photosynthesis which allows them to open their stomata at night, to perform gas exchange, and close them during the day to conserve water.

Materials:

- Potted plant with leaves
- Bottles of clear nail polish
- Clear packing tape
- Microscopes
- Microscope slides
- **Forceps**
- Scissors
- Calculator

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Develop a **hypothesis** about the number of open stomata found on the upper side of a leaf as compared to the lower side of the leaf. _____
3. Develop a **hypothesis** about the number of open stomata found on the upper side of a leaf as compared to the lower side of the leaf. Write your hypothesis in the space below. _____
4. Predict the results of the experiment based on your hypothesis (if/then).

   _____

5. Perform the experiment below and collect your data.

Procedure:

1. Obtain 2 cut sections of leaves from the plant provided in the lab.
2. Using a small amount of clear nail polish, coat the underside (lower surface) of one of the leaves with a THIN layer. Set the leaf on a paper towel (painted surface up) and allow it to dry completely (~ 30 min.)
3. Coat the upper surface of the remaining leaf in the same way.
4. As the nail polish dries, it will conform to the surface of the leaf.
5. Place a piece of the clear cellophane tape over the entire surface of the leaf covered with the nail polish. Use your fingertip to gently adhere the tape to the entire surface of the leaf, rubbing back and forth until you feel you have pressed the tape into the surface of the leaf.
6. Use a corner of the tape to gently peel back the tape. While peeling back the tape you will start to separate the dried nail polish from the surface of the leaf. This is the leaf impression you will examine under the microscope.
7. Tape each of your peeled impressions to a clean microscope slide. Use scissors to trim away any excess tape.
8. Examine the leaf cell imprints under a total magnification of 40x. Scan the slide until you find a good area where you can see plenty of the stomata. *Note: You may need to adjust the iris diaphragm to reduce the amount of light and add contrast to the slide to more clearly see the stomata.
9. Increase the total magnification to 100x for counting.
10. Each stoma is surrounded by two sausage-shaped cells that are smaller than the surrounding epidermal cells. These cells are called guard cells and, unlike other cells in the epidermis, contain chloroplasts.
11. Identify the epidermal guard cells and the stomata. Fix the field of your microscope in place, and count the number of visible stomata. Stomata on the edge of the field of view are to be counted! Record this number in Table 3 under "**View 1**".
12. Move the field of view to a new position on the leaf and repeat the procedure. Record this number in Table 3 under "**View 2**".
13. Do a third count in a new position and record this number in Table 3 under "**View 3**". (Do three different counts for the upper surface leaf imprint and the lower surface leaf imprint.)

Table 3: Number of Stomata Observed by Surface

Surface of Leaf	# of Stomata: View 1	# of Stomata: View 2	# of Stomata: View 3	Average # of Stomata
Upper				
Lower				

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 3.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Questions:

1. What are stomata?
2. What is the importance of stomata in photosynthesis?
3. What is the function of the guard cells?

During the lab activity, were more **stomata** observed on the upper surface of the leaf or the lower surface of the leaf? Explain why you think this distribution exists.

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1.10: DNA and Restriction Enzymes

Deoxyribonucleic acid (DNA) is located in the nucleus of eukaryotic cells (animals, plants, fungi, and protists). DNA contains information to direct the cell in the manufacture of proteins. Proteins control development, organ function, metabolism, enzymatic reactions, photosynthesis, muscle action, brain activity, and many other cellular processes. DNA is often referred to as the "blueprint for life".

DNA is a polymer composed of the nucleotide bases guanine (G), adenine (A), thymine (T), and cytosine (C), and two deoxyribose sugar/phosphate backbones (**Fig. 1**). Two DNA strands are twisted to form a double helix. DNA is wound tightly around histone proteins to form the thread-like structures called chromosomes. Cells of eukaryotic organisms contain multiple linear chromosomes. Prokaryotic organisms, such as single-celled bacteria like *Escherichia coli* (*E. coli*), contain a single circular chromosome.

A gene is a sequence of nucleotide bases (DNA) that codes for a specific protein. Every species has genes that code for proteins, but different species have varying numbers of genes. Human DNA, for example, contains about 20,000 genes, while the cells of the rice plant contain over 40,000 genes. The 3 billion nucleotide base pairs in the human genome are located on 46 chromosomes. The Human Genome Project has determined the order of the nucleotides on each chromosome, and thus the location of each gene. Despite the differences between the structure and number of chromosomes and genes in organisms, the DNA functions the same way in all organisms to encode proteins.

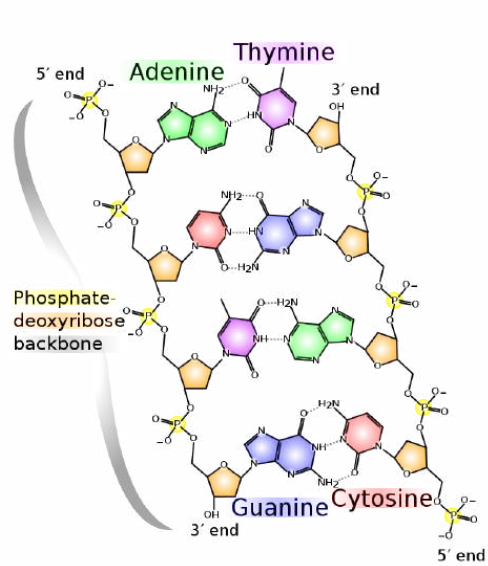

Figure 1. DNA Structure. ("Madprime (talk · contribs), Madeleine Price Ball, CC0, via Wikimedia Commons"

Exercise 1: DNA EXTRACTION

The first step in working with nucleic acids (DNA and RNA) is to remove the molecules from inside the cell. Different types of cells need to be processed differently in order to release nucleic acids. All cells have a **cell membrane**, a phospholipid bilayer that separates the internal environment of the cell from the external environment. In eukaryotes, DNA is housed inside the nucleus of the cell which is surrounded by the **nuclear membrane**, a second double-layered membrane, also composed largely of lipid molecules. When extracting DNA from plant cells, the **cell wall** must also be considered; some types of plant tissue require grinding or flash-freezing in order to break the tough cell wall.

In the DNA isolation procedure, plant cell walls and cell membranes are broken down by blending or mashing and heating the cells. **Detergent** in the extraction solution dissolves phospholipids in the cell membrane causing the cells to lyse. When cells undergo lysis, the cellular components, including the DNA, are released. The technique of filtration uses a medium, in this case, cheesecloth, to separate solids from liquids. The resultant material is referred to as filtrate. When cold **ethanol** is added to the filtrate, DNA precipitates at the water/ethanol interface. Although an individual DNA molecule is not visible with the naked eye, DNA isolated from large quantities of cells can be observed.

Strawberry fruit tissue is an excellent type of tissue to use for the demonstration of DNA extraction. First, ripe strawberries are soft and juicy; as the fruit matures, the cells fill up with water and sugar, which make the fruit so delicious! Second, as the strawberry ripens, a series of chemical reactions take place within the cells that lead to the breakdown of long-chain polysaccharides, like cellulose and pectin, that make the cell wall tough. Lastly, cultivated strawberries (*Fragaria* x *ananassa*) are the product of a hybridization between two other strawberry species, and they have an **octoploid** genome, meaning they have eight sets of chromosomes (**Fig. 2**) inside their cells and just under 1 billion DNA base pairs. Similarly, wheat (*Triticum aestivum)* is **hexaploid** but is likely the product of several hybridization events between three different related species and contains about 17 billion DNA base pairings! These translate to lots of molecules of DNA, which increases our yield and makes the DNA easier to visualize.

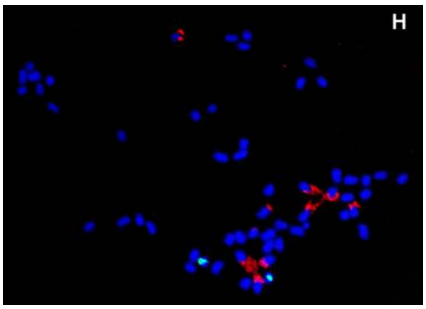

Figure 2. Image of strawberry chromosomes; Strawberries (*Fragaria* x *ananassa*) are **octoploid** (8N) N=number of chromosomes in one set. (image: https://bmcplantbiol.biomedcentral.com/articles/10.1186/1471-2229-11-157 CCBY)

Materials:

- Isopropyl alcohol 91% (rubbing alcohol) or 95% ethanol, Chilled in the freezer
- Graduated cylinders
- Salt
- DI Water
- Cheesecloth
- Dishwashing liquid (preferably, Dawn)
- Wide-mouth glass test tubes
- Funnel
- Disposable plastic cups
- Microcentrifuge tubes
- Wooden stirrer or glass rod
- Resealable plastic bag
- 100 or 200 mL beaker
- Ice buckets with ice
- A DNA source (about 1 cubic inch of food)
  - strawberries
  - bananas
  - ground flax seed
  - ground wheat germ
  - peas
  - broccoli
  - spinach

Preparation

- Have isopropyl alcohol (rubbing alcohol) or ethanol cooled in an ice bucket
- Prepare your food item if needed (i.e. remove green strawberry tops, peel banana, grind wheat germ, etc)
- If not already made, prepare DNA extraction (lysis) buffer by combining the following:

• 45 mL DI water

• 5mL liquid dish soap

• 0.75 g NaCl (table salt)

Procedure

1. Place the food item (i.e 2 large strawberries) into the plastic zipper bag. Seal it and gently smash the food with your hands for about 2 minutes. Completely crush the food (strawberries) to disrupt the cells.
2. Open the plastic zipper bag and add 10mL of the DNA extraction (lysis) buffer. Squeeze the bag to remove all air and seal the bag tightly.
3. Gently (to prevent over-sudsing or excessive foaming), but thoroughly, continue to crush the food (strawberries) inside the bag for about one minute or until it is a slushy consistency.
4. Completely line the funnel with a layer of cheesecloth. Place the funnel into the wide-mouth test tube.
5. Pour the food juice/DNA extraction buffer mixture into the funnel so the juice passes through the cheesecloth and into the test tube. Use the cheesecloth to strain the mixture so that only the juice flows into the tube and the pulp is retained in the cheesecloth.
6. Discard the cheesecloth and the pulp. Remove the funnel from the tube. The glass tube now contains a liquid called “filtrate”.
7. Carefully and slowly pipette an equal volume of ice-cold ethanol on top of the filtrate in the test tube using the plastic transfer pipette. The alcohol is less dense than the filtrate and will float as a layer on top of the filtrate. Do not mix or stir!
8. Hold the tube still at eye level and observe what happens at the interface of the alcohol and the filtrate. DNA will precipitate at the alcohol-lysis buffer interface. This means it will come out of solution into a “solid” form and appear as fluffy white cotton or cloudy material. Verify with your instructor that you have isolated DNA.
9. (optional) Use your wooden stirrer or glass rod to transfer your extracted DNA into a microcentrifuge tube. Add a small amount of ethanol to the tube to prevent your DNA from drying out.

Discussion

This protocol for extraction of DNA is based largely on the principle of **solubility**. Solubility refers to the ability of one substance (the solute) to dissolve in another substance (the solvent). Recall that polar substances dissolve easily in polar solvents, but do not dissolve easily in nonpolar solvents, a phenomenon commonly referred to as “like dissolves like.” Water is a polar solvent, and molecules that dissolve easily in water are referred to as **hydrophilic**. DNA molecules are hydrophilic because the sugar-phosphate backbone of the molecules is highly polar. This means that DNA dissolves in water, so in this experiment, the DNA that is released when the cells are crushed dissolves in the juice/extraction buffer mixture.

Remember, there are two key ingredients in the DNA extraction buffer aside from the water: **dish soap** and **salt**. The dish soap acts to break up the phospholipid molecules that form the cell membrane and the nuclear membrane, which lyses the cell and releases the cellular contents, including DNA. The salt has two functions in the extraction process. It helps to neutralize the charge on the sugar-phosphate backbone, making DNA *less soluble* in water and allows it to more easily precipitate when the alcohol is added. The salt also helps to remove the proteins that are bound to the DNA and to keep the proteins dissolved in the lysis solution.

Although the chemical reactions described above are all happening when you add the buffer and crush the food (strawberries), they are not visible with the naked eye. However, the addition of the cold ethanol caused a much more dramatic result! Ethanol is a nonpolar solvent, and when it is added to the juice extract, the DNA precipitates out of the solution. A precipitation reaction is a chemical reaction that causes a solid substance to emerge from a liquid solution. In this experiment, the addition of ethanol to the reaction forces the DNA to precipitate out of solution, which we can then spool onto the wooden stirrer or glass rod.

Exercise 2: Modeling of DNA Structure

The DNA molecule is a complex, yet simple molecule. Only four different nucleotide building blocks (adenine, thymine, guanine and cytosine) are used to form the DNA sequences that encode for all of the proteins in our bodies. Each nucleotide has three components: a negatively charged phosphate molecule, a deoxyribose sugar and a nitrogenous base (**Fig. 3**). On each strand of DNA the phosphate and deoxyribose sugar are arranged on the outside of the DNA molecule, forming a “backbone.” The nitrogenous bases are positioned in the interior of the double helix. Each strand of DNA is **complementary** to the other, meaning that the nitrogenous bases of the two strands are always paired in specific combinations, called base pairing. If there is an adenine

in one strand, then it is paired and directly across from a thymine in the other strand. If there is a cytosine in one strand then it is paired with a guanine in the other strand. Therefore, if you know the sequence of one of the strands of DNA, then you can deduce the sequence of the opposite (complementary) strand. Hydrogen bonds between the nitrogenous bases hold the two strands of DNA together.

The DNA strands also have directionality, referred to as the 5' (5 prime) and 3' (3 prime) ends. When looking at a strand of DNA, the end that has a free phosphate group is the 5' end. The end of the DNA strand that has a free -OH group on the 3' carbon of the deoxyribose sugar is referred to as the 3' end (**see Fig. 1**). The two DNA strands are **antiparallel** to one another, running in opposite directions like opposite lanes of a street.

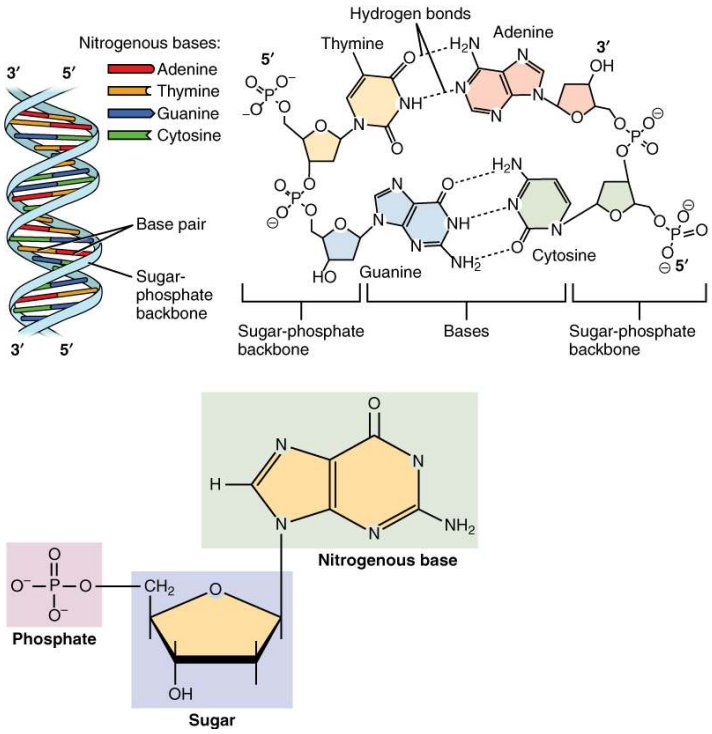

Figure 3. DNA and nucleotide structure. (CC BY 4.0; OpenStax via Wikimedia Commons.)

Materials:

This lab manual will utilize the Carolina BioKits: DNA Simulation Kit. Your instructor may provide you with an alternative DNA Simulation Kit and instructions. A simple modeling activity using candy is discussed here.

The Carolina DNA modeling kit uses different colored beads to represent the phosphate group, deoxyribose sugar and the four different nitrogenous bases. You will use the beads to build thymine, adenine, guanine and cytosine nucleotides. Then, you will link the individual nucleotides together to "build" a strand of DNA.

Procedure: (If using an alternative DNA simulation kit, follow the directions given by your instructor.)

1. Obtain from your instructor the DNA sequence that you are instructed to model. If you are provided with only the sequence of one strand, then you will need to determine the sequence of the complementary strand.
2. Begin assembling individual nucleotide bases, each consisting of one phosphate, one sugar and one nitrogenous base. Note which end of the nucleotide is the 5' end and which is the 3' end.
3. Link the nucleotide bases together in the correct sequence to build one of the strands of DNA. Pay close attention to orient the 5' end (with the free phosphate) in the correct direction and the 3' end in the correct direction.
4. Now, you will build the complementary strand of DNA. Link the nucleotides together in the correct sequence so that they are complementary to the other strand. Be sure that the nucleotides are oriented in the correct direction, with the 5' end and 3' end on the appropriate ends of the strand. Your two DNA strands should be antiparallel to one another.
5. Position the two strands together to check that base pairing is correct and that the strands are antiparallel.

6. Use the plastic connectors to attach the paired nitrogenous bases of the two strands together. The plastic connectors represent the hydrogen bonds that hold the two DNA strands together.
7. Twist the double stranded DNA model into a double helix. Check your model with your instructor.

Exercise 3: DNA Fingerprinting

DNA fingerprinting is routinely used today to establish paternity, to diagnose inherited disorders, and for use in criminal cases. DNA fingerprinting enables forensic investigators to determine whether two DNA samples originate from the same individual. Not all of the DNA present in a sample is used in an analysis. Restriction enzymes act as molecular scissors and are used to cleave DNA molecules at specific sequences of DNA. Over 2,500 different restriction enzymes have been identified. These enzymes are produced by bacteria and are used by the bacteria to destroy foreign DNA such as bacteriophages - viruses that infect and replicate within a bacterium. Each restriction enzyme cleaves DNA at a specific nucleotide sequence. For example, the restriction enzyme EcoR1, isolated from E. coli, cuts DNA at the sequence GAATTC (**Fig. 4**).

Figure 4. Recognition sequence of *EcoR*I.

The length and the number of the fragments produced depend upon the frequency and the distance between the recognition sites. This distinct pattern is known as restriction fragment length polymorphisms (RFLP's) which are unique to each individual, therefore forming a DNA fingerprint. After DNA samples are cut by restriction enzymes, the fragments are separated using gel electrophoresis. PCR, polymerase chain reaction, can be used to amplify trace amounts of DNA in a sample to levels that can be analyzed using restriction enzymes. The length of the segments analyzed is much smaller and the repeat sites are called microsatellites.

Materials:

- DNA sequences in Figure 6
- Highlighter
- Scissors

Procedure:

You will use DNA fingerprinting technology to determine which male dog is the real father of a puppy, Willy. DNA sequences from each dog are on the next page. In this DNA fingerprinting scenario, the restriction enzyme, *Haelll*, will be used to digest each DNA sequence. The recognition sequence and cut site of *Haelll* is shown below (**Fig. 5**).

Figure 5. Recognition sequence of *Hae*lll.

1. Cut the DNA sequence (Figure 6) paper into strips to separate the DNA sequences of each dog into one long strip. You will have 5 strips of DNA sequences (one for each dog).
2. Scan each sequence of DNA for the *Haelll* sequence. There may be more than one *Haelll* sequence in the DNA sequences. Be sure to scan the entirety of each DNA sequence. When you find the recognition sequence, use a highlighter to highlight the recognition sequence.
3. At each recognition site, use a pencil to draw a vertical line between both strands of DNA to indicate exactly where the restriction enzyme will cut the DNA, separating it into 2 pieces (refer to **Fig. 5**).

4. Use scissors to cut the DNA sequences at the indicated restriction sites you marked.
5. Count the number of nucleotides on one strand of each resulting fragment of DNA. Write the nucleotide length on each fragment.
6. For each dog, arrange the DNA fragments from largest to smallest to simulate how they would separate when analyzed via gel electrophoresis. Record those DNA fragment sizes in the table below.

Table 1. Restriction fragments from DNA fingerprinting

Size of DNA fragments	Mom	Willy	Sire X	Sire Y	Sire Z
Largest (60-51 bp)					
(50-41 bp)					
(40-31 bp)					
(30-21 bp)					
(20-11 bp)					
<10 bp					
smallest					

7. Compare the DNA fragments from Willy to the DNA fragments of the Mom and possible fathers. Every fragment in Willy's digest will be found in either the Mom or Sire's digest. This comparison will identify the real sire of Willy.

Copy and Paste
Image here.
Delete this
placeholder image

Figure 6. DNA sequences to be used for DNA fingerprinting

Questions for Review

1. How well do you understand the necessity of each step in the DNA extraction procedure? Match each of the procedures below with its function, by placing the appropriate letter on the line provided.

Procedure

1. Filter strawberry slurry through cheesecloth
2. Mix strawberry with salty/soapy solution
3. Initial smashing and grinding of strawberry
4. Addition of cold ethanol to filtered extract

Function

_____ To precipitate the DNA from solution

_____ Separate components of the cell

_____ Physically disrupt the cells

_____ Break up proteins and dissolve cell membranes

2. Briefly describe what precipitation means. Why was this step necessary?
3. Relate what you know about the chemical structure of DNA to what you observed during the precipitation step.
4. Briefly explain why strawberry fruit tissue is a great source for extracting DNA.

5. Why is it important for scientists to be able to extract DNA from living organisms? State at least 2 reasons.
6. What is the function of genomic DNA and where is it found in a eukaryotic cell?
7. You are given the DNA sequence below. Using the base-pairing rules, draw the complementary DNA strand in the space below. Make sure to designate the 5' and 3' ends appropriately.

5' - ATTCGCTCG - 3'

8. Why might DNA fingerprinting be more useful in identifying individuals than blood typing analysis?
9. What are the building blocks that compose DNA? What are the three specific components that make up these building blocks?
10. DNA sequences contain palindromes. Briefly explain what a palindrome is in DNA and give an example.
11. What biological molecules, which are isolated from different types of bacteria, act as molecular scissors to cut DNA at specific locations? What advantage do you think bacteria gain by having these particular molecules?

Practical Challenge Questions

1. For the DNA sequence shown below, use the base-pairing rules to draw the complementary DNA sequence. Be sure to designate the polarity of the resulting strand.

5' ATATCATGGAATTCGATCCTAG 3'

2. Now, "digest" your DNA molecule using EcoRl enzyme. The EcoRl recognition sequence is shown below. Draw a vertical line in your DNA sequence above to indicate specifically where the restriction enzyme would cleave the DNA.

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Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason. Foundations of Biology Lab Manual (Georgia Highlands College). "Lab Activity: DNA Extraction from Strawberries," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY https://oer.galileo.usg.edu/biology-textbooks/18

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1.11: Gel electrophoresis

In this exercise, gel electrophoresis (**Fig. 1**) of different electrophoretic dyes will be used to simulate the process of DNA fingerprinting (aka "DNA profiling"). DNA fingerprinting is a laboratory technique that forensic analysts use to compare a DNA sample collected at a crime scene with a DNA sample collected from a suspect. Even though 99.9% of the genome throughout the human population is the same, the remaining 0.1% of human DNA shows variation between individuals. These variable DNA sequences, called polymorphic markers, can be subjected to DNA gel electrophoresis to produce unique DNA banding patterns on an agarose gel. The DNA bands can then be used to differentiate or correlate individuals.

You will be tasked with analyzing the DNA of two individuals who are suspects in a crime scene from which human DNA samples (such as skin cells or hair) were recovered. Your goal is to match the DNA (in reality, this would be **DNA fragments** generated by **restriction enzymes**, explained below) from one of the two suspects to the DNA found at the crime scene.

If the DNA sample from a suspect matches the DNA at a crime scene, then that signifies that the suspect in question *was present at* the crime scene (although the suspect may not have committed the crime). If the DNA profiles from the crime scene do not match a suspect, then it can be concluded that the individual in question was not present at the crime scene.

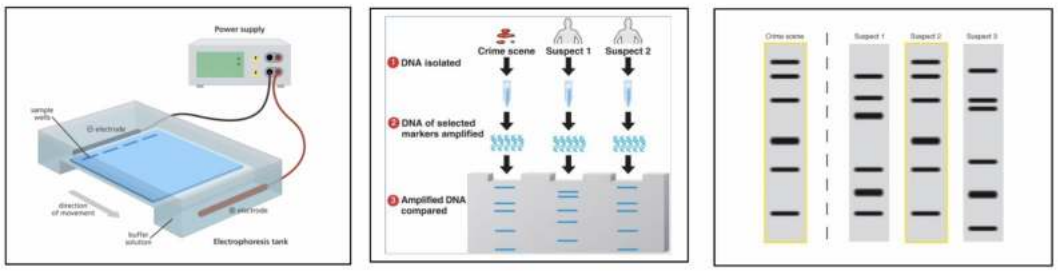


Fig. 1. Gel electrophoresis apparatus (left), (stylized) example of methodology used in this technique (center), and (stylized) example of gel from crime scene and three potential suspects (right). The point is to match characteristics of the DNA found at a crime scene with the DNA from potential suspects in the crime. In the right-most figure above, DNA from the crime scene matches DNA from Suspect 2.

DNA Fingerprinting:

DNA Fingerprinting (DNA profiling), similar to the exercise we are performing today, was first used in England in 1987, to help identify a murderer. This technique is now used routinely for identification purposes as diverse as the establishment or elimination of suspects in a crime, paternity suits, the verification of human remains after catastrophic events (e.g. plane crash), exoneration of the wrongly accused, or the establishment of family relations. Non-human DNA (such as that of endangered species, genetically modified plants, or disease-causing microorganisms such as *E. Coli* 0157:H7) can also be profiled.

Almost every cell in the human body contains DNA in the form of 23 chromosome pairs that collectively contain about 3 billion base pairs. On average, about 99.9% of the DNA in all humans is identical. However, the remaining 0.1%, which constitutes about 3 million base pairs, differs significantly enough among individuals (except identical twins) that it can be used to generate a unique genetic "fingerprint" for every person. Just like our physical fingerprints, "**DNA fingerprints**" are something we are born with and something unique to each person.

The unique 0.1% of our DNA contains **short, non-coding, sequences of repetitive DNA** that are 2-100 **base pairs (bp)** long. CTTG is an example of one such repeated unit (or simply **repeat**) that is 4 bp long. It might be repeated 3 to 100+ times as follows:

CTTGCTTGCTTGCTTGCTTGCTTG…..

Repeats are referred to by a variety of terms (sometimes confusing) depending on their size. For example, sequence repeats of 10 to 80 bp are called **minisatellites** or **variable number tandem repeats (VNTR)**. **Microsatellites**, also known as **short tandem repeats (STR)**, are smaller repeated units of 1 to 6 bp. Regardless of their size (number of base pairs) or names, DNA repeats show greater variation from one person to another than any other parts of our genome.

The number of times a given repeat (for example CTTG indicated above) occurs in any individual's DNA is a function of the DNA that a person received from his or her mother and father at conception. For example, three individuals (Mary, Jake, and Sue; **Fig. 2**) could exhibit the following variation in the length of a particular repeat sequence on the chromosomes they received from their parents.

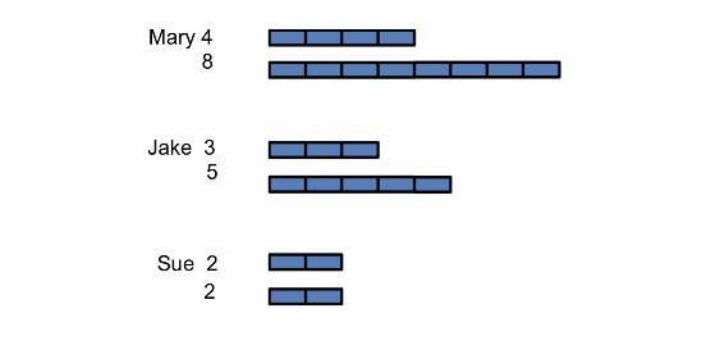


Fig. 2. Hypothetical variation in number of microsatellites in the DNA of 3 individuals. Blue rectangles represent microsatellite repeats on homologous chromosomes.

The process of DNA profiling uses molecular "scissors" called **restriction enzymes**, enzymes that cut DNA at specific nucleotide sequences. In this example, restriction enzymes would recognize particular nucleotide bases at the beginning and end of the repeating string of nucleotides (the **microsatellite region**). Consequently, one segment produced in this manner might be CTTGCTTG (2 repeats long) while another might be CTTGCTTGCTTGCTTGCTTGCTTG (6 repeats long). The DNA segments used in forensic investigations are, of course, much longer than this.

These DNA pieces of various lengths are separated using gel electrophoresis (see **Fig. 3** and text below).

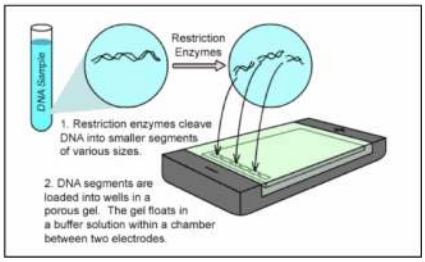


Fig. 3. How restriction enzymes are used in DNA fingerprinting.

Restriction Enzymes:

Restriction enzymes were first discovered in the 1970s. Restriction enzymes used in DNA profiling were developed from the 3,000 or more restriction enzymes (aka **restriction endonucleases**) that have been identified from bacteria and are a defense against the DNA of invading viruses. Specific bacterial restriction enzymes cut double-stranded viral DNA at specific locations (**base pair sequences**) into smaller non-infectious fragments (**Fig. 4**).

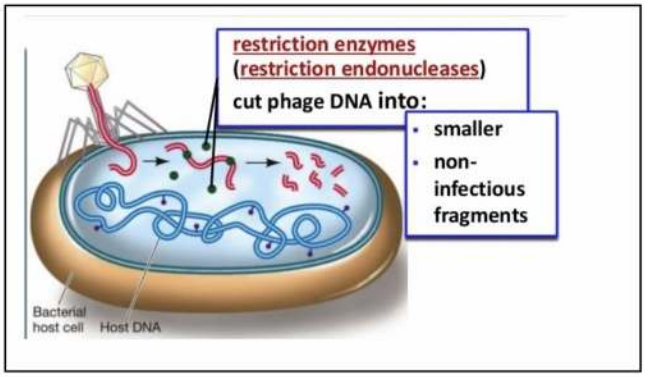


Fig. 4. How restriction enzymes function in bacteria.

When used in biotechnology, bacterial restriction enzymes act much as they do in bacteria. They locate and cut the DNA with which they are mixed (at specific restriction sites) to produce fragments.

Restriction enzymes are described by unique **acronyms** (abbreviations) that document the organism from which they were isolated. The first letter of the acronym is the first letter of the genus of the bacterium. The next two letters are the first two letters of the bacterium's species name. Additional letters and numerals indicate specific bacterial strains and their order of discovery. For example, *EcoR1* was the first restriction enzyme isolated from the RY13 strain of the bacterium *Escherichia coli.*

In the example below, the enzyme *EcoR1* has cleaved DNA between the G and neighboring A in the GAATTC recognition site (**Fig. 5, top**).

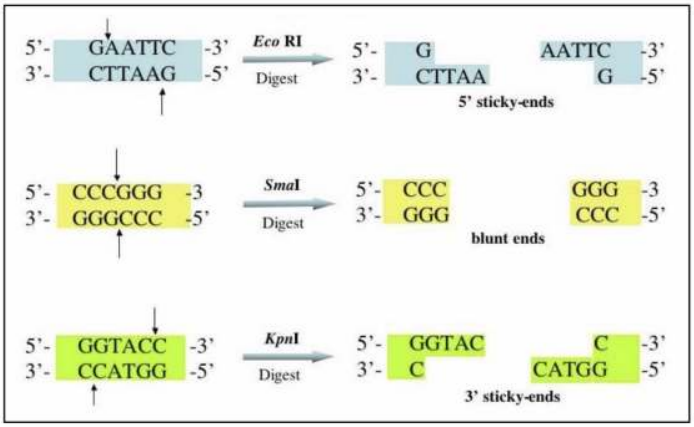


Fig. 5. Examples of how restriction enzymes can cut DNA.

It is important to note that the ends of the cleavage (cut) produced by *EcoR1* are staggered so that the resulting fragments project short overhangs of single-stranded DNA with complementary sequences. Such overhangs are referred to as "**sticky ends**" because the single strands produced can interact with (or stick to) other overhangs of single-stranded DNA with complementary sequences.

The discovery of restriction enzymes launched the **era of biotechnology** and has been a centerpiece for studies and advances in **molecular and gene cloning**, **DNA mapping**, **gene sequencing,** and various other endeavors including the DNA profiling discussed here.

Gel Electrophoresis:

Gel electrophoresis is a laboratory technique that allows macromolecules, such as DNA, or RNA fragments, or proteins, in a mixture to be separated according to their molecular size and/or charge. The molecules to be separated are placed in sample "wells" (depressions) in a thin porous gel slab (**Fig. 6**), which is then covered by a buffered solution and placed in a horizontal electrophoresis chamber (**Fig. 7**).

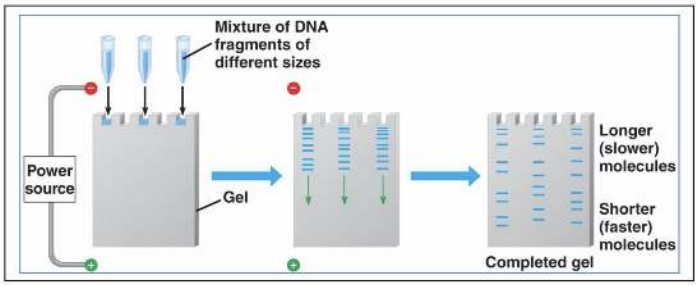


Fig. 6. The separation of DNA fragments in gel electrophoresis

The sugar-phosphate backbones of DNA are negatively charged. Consequently, if an electric current is passed through the chamber, **DNA fragments will migrate** through the pores in the gel, away from the **negative electrode** (where the wells are located) toward the **positive electrode**. Shorter DNA fragments move more quickly — and farther on the gel — than do larger fragments.

The different-sized DNA fragments that have migrated through the gel form distinct bands on the gel, which can be seen if they are stained with DNA-specific dye.

You will be given three samples that will simulate DNA from two suspects, as well as the investigator's DNA, that have been digested with a few restriction enzymes. (In reality, your samples contain electrophoretic dyes of different molecular sizes). Using agarose gel electrophoresis, these samples will form bands, which will then be compared to artificial DNA samples from a "crime scene" (that have also been digested with the same few restriction enzymes) and will run simultaneously in the same agarose gel.

The final step, following electrophoresis of the gel, is analyzing the suspect and investigator DNA sample profiles and comparing them for the presence or absence of particular bands in the crime scene sample profile. The more bands any given samples have in common, the more likely it is they came from the same person.

Components of the Electrophoresis Equipment:

Your instructor will explain and demonstrate how the gel electrophoresis chamber and its components function (**see Fig. 7 below**).

Power Supply: The high voltage power source (pictured below) connects to the electrophoresis chamber and sets up an electric field between the two electrodes — one positive and one negative. DNA-fragment samples (or in our case, electrophoretic dyes) loaded into the wells of an agarose gel are negatively charged and move through the gel toward the positive electrode as the agarose gel matrix separates the DNA molecules by size.

Electrophoresis power supplies typically have a variable output voltage allowing the user to set the output voltage for different size gel tanks and modify voltage for optimum results and convenience.

For our experiment, we will set the voltage on our power supply to **75 V**.

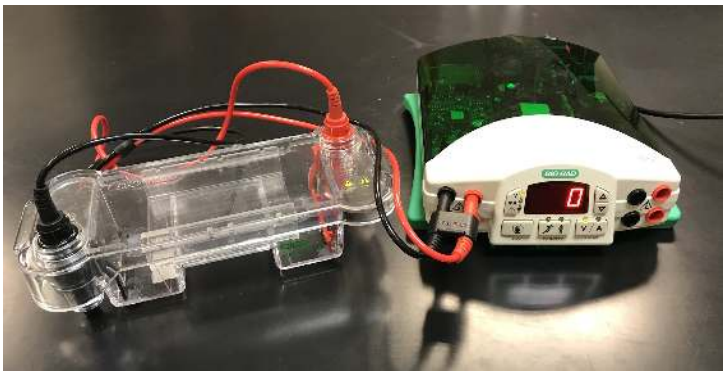


Fig. 7. Gel electrophoresis chamber and power supply (original photo)

Agarose, the main component of our gels, is a polysaccharide polymer extracted from seaweed. It is available as a powder, which is mixed with a buffered TBE solution (see below), heated until it dissolves, and then poured into molds where it solidifies (in

about 20 minutes) into a gel slab (having the consistency of finger jello). A serrated “comb” is placed in the mold before the agarose solidifies to create **sample wells** that form in the finished gel.

TBE (Tris/Borate/EDTA) Buffer is diluted from a 20x concentrate to a final concentration of 1X. It is used to cover the gel in the electrophoresis chamber and contains ions that carry the current through the apparatus. It also maintains a constant pH for the experiment.

Micropipettes (**Fig. 8**) are used to dispense all the samples in preparation for electrophoresis. These devices are designed to transfer small amounts of liquid (<1ml). The scale on micropipettes is in microliters (1000 µl = 1 ml). Your instructor will demonstrate how to set the pipette for a particular volume of liquid and how to properly dispense the calibrated volume.

Fig. 8. Micropipette (BioRad) (original photo)

Procedures

Lab Safety:

- Gloves and goggles should be worn throughout the lab.
- Exercise caution when using electrical equipment and any device (such as a water bath) that produces heat.
- Wash hands thoroughly with soap and water at the end of the lab.

Materials:

- For pipetting practice:
  - Petri dish with 1% agarose gel with wells (optional)
  - Beakers with colored practice solution
  - Micropipettes and tips
  - Empty beakers (in which to dispense practice solution)
- Gel electrophoresis apparatus:
  - Gel tray (mold) with ends taped
  - 8-well comb
  - Electrophoresis chamber
  - Power supply
  - 1% agarose prepared in advance and kept at 65 degrees Celsius in water bath
  - TBE (Tris base; boric acid; ethylenediaminetetracetic acid, or EDTA;NaOH), 20x to be diluted to 1x (or 1x buffer already diluted)
  - Microcentrifuge (helpful to spin down samples)
  - Electrophoresis samples in labeled microfuge tubes
    - DNA ladder (standard) labeled “L”
    - Crime scene DNA labeled “C”
    - Suspect 1 DNA sample labeled “S1”
    - Suspect 2 DNA sample labeled “S2”
    - Investigator DNA sample labeled “I”

Exercise 1 - Preparing the Agarose Gel:

Shortly after the lab starts, you will be instructed to pour your agarose gel.

1. Obtain a gel tray (in which the ends have been taped to prevent leaking).

2. Retrieve an Erlenmeyer flask containing 35 ml of the heated pre-mixed 1% agarose gel solution. (The gel solution was previously made by weighing out 0.35 g of agarose, dissolving it in 35 ml of 1X TBE buffer, and heating it until boiling in a microwave.)
3. Pour the heated gel solution into your gel casting mold. Once you have poured the gel into the mold, carefully place the 8-well comb into the gel and position as instructed.
4. The gel will solidify in approximately 20 minutes. It is ready for loading when it is firm and appears semi-opaque (cloudy).
5. While the gel is solidifying, go on to Exercise 2 and practice pipetting with the micropipette.

Exercise 2 - Practice Pipetting:

Micropipettes are molecular biology tools that are designed to dispense very small amounts of liquid. Different micropipettes can be utilized for a range of volumes, for example 2 µl to 20 µl.

1. Attach a plastic disposable pipette tip to the tapered end of the pipette and fit securely in place.
2. Examine your micropipette. What are the numbers designated on the plunger of the pipette? The smaller number represents the smallest volume that should be measured with the pipette. The larger number represents the largest volume that should be measured with the pipette.
3. Locate the window on the side of the pipette. This window displays the volume currently set for the pipette. Set the micropipette to the largest volume the pipette can measure. For example, if the largest number is 20 µl, then rotate the dial until the correct volume appears in the display window.
4. Obtain the colored practice solution.
5. Before placing the tip into the liquid, depress the pipette plunger with your thumb to the ***FIRST*** stop to eject any air.
6. Place the tip into the practice solution and slowly release the plunger, gently “sucking” the liquid into the tip. Your tip now contains the measured volume of liquid displayed in the window. Remove the tip from the liquid.
7. Move your hand so that the tip of the micropipette is over the empty beaker. Insert the pipette tip into the empty beaker so that the tip is close to the bottom of the beaker. Touch the tip to the side of the beaker. Slowly press the plunger down to the first stop and then continue to press the plunger ***ALL*** the way down to the ***SECOND*** stop in order to release all of the liquid from the tip. ***Don’t release the plunger yet!***
8. Pull the tip completely out of the beaker and away from the liquid, and then ***SLOWLY*** release the plunger back to the starting position.
9. Discard the tip, using the release button on the pipette. Use a new tip each time you use the micropipette.
10. Reset the volume in the display window to practice dispensing different volumes of practice solution.

Digested DNA Sample Simulation (Dyes)

Scenario:

DNA profiling may be used both to exonerate or convict criminal suspects. If a suspect’s DNA is not found at the crime scene, the suspect can be excluded or - if they had been falsely accused - exonerated. Conversely, if a suspect’s DNA is found at a crime scene that may or may not implicate them of the crime. In this case investigators must consider other factors, both biological (e.g. blood typing) and behavioral (e.g. motive and means). DNA alone is not sufficient evidence to convict, but it is sufficient evidence to exonerate.

In this activity you will play the role of investigator working a crime scene where you retrieved a sample of DNA. You suspect two different individuals of the crime and collected DNA samples from each of them. You send the samples to your analyst to conduct a DNA analysis. Per procedural protocol, you include a DNA sample of your own to rule out the possibility of DNA contamination at the crime scene. You assign a code to each sample to make sure the analyst conducts the analysis without bias. You code the samples as follows, with each code indicating the date of collection and a unique identifier. You ask the analyst to run a DNA profile for each of these samples hoping it will help you narrow your suspect pool.

Sample Code	Code KEY
1119_CS	Crime scene DNA
1119_BB	Suspect 1
1119_PG	Suspect 2
1119_MO	Investigator's DNA

The analyst receives your coded samples and proceeds with the analysis as follows.

Because of the difficulty involved in obtaining and storing stable DNA samples and the precision needed to perform a successful restriction digest, we will be simulating a DNA digestion using a mixture of dyes. Using dyes allows us to easily see the bands in the gel because of their different colors and because of how they separate on the gel. The table below shows information about the dyes we will be using.

Dye	color	Molecular weight (g/mol)	~DNA base pair equivalent movement	g/100ml*
xylene cyanol	blue	538	10,000	0.2
methyl orange	yellow/orange	327	4,500	0.2
Bromophenol blue	purple/blue	669	1,000	0.2
Ponceau G	pink	760	~100	0.2
*Each sample was made 0.2% by weighing out 0.2 g of dye and dissolving in 100 ml of 20% glycerol				

Exercise 3 - Loading, Running, and Analyzing the Gel:

Loading the Gel:

1. Retrieve your hardened gel.
2. Gently remove the tape from the edges. Gently remove the comb by lifting it slowly up out of the gel. Avoid tearing the gel. Leave the gel in the plastic mold.
3. Place the mold in the electrophoresis chamber. Place the gel so that the sample wells are toward the negative electrode (black).
4. Pour the 1X TBE Buffer into the chamber until the gel is completely covered.
5. Place the DNA samples into the microfuge and spin for 10 seconds. This will force all of the samples to the bottom of each tube.
6. Load 10 µl of each sample given to you by your instructor. Use the following table to run each sample in the appropriate lane. Make sure to use a clean tip for each sample!

Gel Lane (left to right)	Microfuge Tube	Contents (see key above)
1		
2	L	DNA Ladder (Standard)
3		
4	S1	1119_BB
5	S2	1119_PG
6	I	1119_MO
7	C	1119_CS
8		

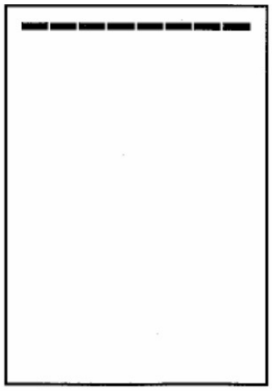
Running the Gel:

1. Place the lid on the electrophoresis chamber and connect the electrodes to the power supply, making sure you have “black to black” and “red to red”.
2. Set the power source to 75V and run the gel for approximately 60 minutes, or longer if possible.
3. Periodically check that the current is flowing correctly and the samples are migrating towards the positive electrode (red).

Analyzing the Gel:

You receive word that the DNA analysis is complete and rush to the lab to review the results. Working with the analyst you step through the results.

1. Lane 7 represents the Crime Scene DNA digested by restriction enzymes. It should yield distinct DNA banding patterns.
2. Lanes 4 and 5 represent the DNA samples from Suspect 1 and Suspect 2 respectively. Assume these DNA samples were digested with the same restriction enzymes used with the DNA in Lane 7.
3. Lane 6 represents your own DNA (called Investigator DNA). You ran your own DNA to ensure that you had not contaminated the DNA sample taken at the crime scene. Assume your DNA was digested with the same restriction enzymes used with the DNA in Lane 7.
4. To determine which suspect(s) was at the crime scene and which suspect(s) can be excluded, compare the banding patterns between each sample and Lane 7. A DNA sample that does not show any similarity to the pattern in Lane 7 can be excluded from your suspect pool. DNA samples showing even a partial similarity can not be excluded.
5. In the space below draw a representation of your gel. Use colored pencils to draw the results of the different colored fragments. Be sure to label each lane as well as the DNA standards (“Ladder”).



Investigator’s Report: After examining the gel you prepare your report. You include answers to the following questions in your report.

1. Based on the DNA analysis, which suspect(s) can be excluded from your suspect pool? Explain how you came to this conclusion.
2. Based on the DNA analysis, which suspect(s) can not be excluded from your suspect pool? Explain how you came to this conclusion.
3. For suspect(s) remaining in your suspect pool, is this evidence alone able to convict them of the crime? Explain your reasoning.
4. Did your DNA (Lane 6) match DNA at the crime scene? What steps can investigators take to make sure they do not contaminate a DNA sample taken at a crime scene?

Questions for Review:

1. Which lane contained a sample with the smallest DNA fragment? Explain.
2. What is the relationship between the migration distance and the size of the DNA fragment? Explain.
3. Why were the sample wells placed toward the negative (black) electrode?

4. If you were pouring your gel to run molecules that had both negative and positive charges, how would you position your comb?

Practical Challenge Question

1. If you look at the molecular weights of the dyes we used, they are not separating on the gel by molecular weight (e.g. Ponceau G is the heaviest but moves the furthest). What might explain this?

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 9: Gel Electrophoresis, Restriction Enzymes, & DNA Fingerprinting," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY https://oer.galileo.usg.edu/biology-textbooks/18

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