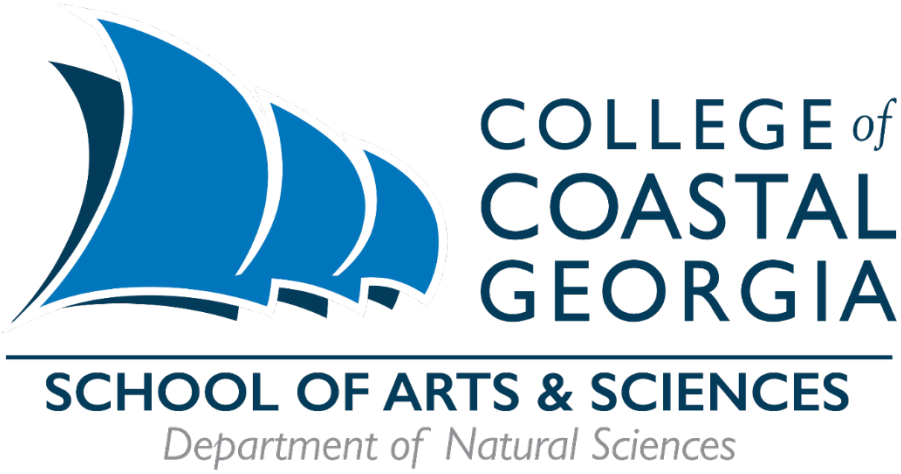
BIOL 1107L
Principles of Biology I
Laboratory Manual



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- Define important terminology associated with the scientific method
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Introduction

What is *science*? It is not simply a collection of facts to be memorized, although that is often required to establish a foundation of knowledge for scientists to build upon. Science is best understood as a *process* of discovery about the natural world. More specifically, science is a way of knowing based on **empirical (observable) evidence** and **scientific reasoning**, a kind of logic that is commonly portrayed as **"the scientific method"**.

The scientific method is often listed as a series of steps used to answer a question, however, in reality, the scientific process is not as rigid and often involves a lot of creativity. Nonetheless, the scientific method provides a helpful way to identify the basic components of the scientific process. Throughout this course, you will apply this process to biology, the scientific study of life. As you will see, conducting scientific research doesn't require an advanced degree; anyone can *do* science!

The Scientific Method

Science starts by **observing** nature. Scientists pay close attention to the world around them, using all their senses (be careful when tasting!), which can be extended by using modern scientific instruments such as microscopes. Further insight into natural phenomena can be attained through background reading and knowledge.

Most experiments are designed after an individual makes such an observation that causes them to ask questions or identify problems to be solved. The best questions to inspire scientific research are open-ended, *causal* questions (i.e., *How? Why? What causes?*), rather than simple yes/no questions, which can usually be reframed as hypotheses.

The individual will use the information they already know or have obtained through review of current literature about the scenario to make a **hypothesis or multiple hypotheses.** A hypothesis is an educated explanation or answer to the question. Note that the hypothesis is not a "guess" as it may have been previously described. The best hypotheses are the ones that can be tested through experimentation. Hypotheses can be supported, modified, or rejected after the experiments are conducted.

Example: I observe that a plant on one side of my house is growing better than the same type of plant on a different side of my house. I hypothesize that the plant that is growing better must be getting more sunlight than the other plant based on my knowledge that plants need sunlight to grow. What are some alternative hypotheses?

Scientists use different approaches to **test** hypotheses, including but not limited to observational studies and experiments. **Observational studies** test for predicted patterns or

correlations between naturally occurring variables, with minimal interference by the investigator. Only **experiments**, however, can demonstrate *cause-and-effect* relationships between variables.

In an experiment the investigator actively *manipulates* (i.e., intentionally alters) one or more factors that is known as the **independent variable(s)**. The independent variable(s) is related to the hypothesis being tested. The investigator then measures some outcome called the **dependent variable(s)** or response variable(s). If the hypothesis is correct, the dependent variable(s) will *depend on* or *respond to* the manipulation of the independent variable(s). In a "controlled" experiment, other factors sometimes referred to as **standardized variables** are "controlled for" or held constant so they do not affect the outcome of the experiment. Another important feature of experimental design is **replication**, or applying each experimental manipulation or treatment to multiple, independent units/subjects known as *replicates*. Replication reduces the likelihood that experimental results are due to chance or to unknown variables that were not controlled.

If I decide to test my hypothesis above using small plants, I will have one group of plants growing with no sunlight, one group growing with 4 hours of sunlight, and one group growing with 8 hours of sunlight. Each group would have ten plants. In the example experiment, the growth of the plant is the dependent variable, and the amount of sunlight would be the independent variable. All other variables such as water, plant food, and soil would be the same for all of the plants in this study. These would be considered standardized variables. Having ten plants per group allows for replicates to be observed during the same experiment.

Once the experimental design is complete, the individual will make a **prediction** about the outcome of the test if the hypothesis is correct. This should be completed before any actual data have been collected to prevent bias. Then, the individual can set up and conduct the actual experiment. Results, or **data**, should be recorded according to the predetermined plan. *In this example, plant growth might be measured daily or weekly depending on what is necessary for the experiment.* It is important to record the results accurately and to include all results. It is often recommended that researchers keep a notebook that details their experiment and the results as they are obtained. If an error is made, corrections should be recorded next to the initial value which should be marked through with a single line (i.e. ~~5 cm~~ 6 cm).

Data analysis will depend on the type of results that are collected. Many times, conclusions are drawn after statistical analyses (you will learn more about these later) are used to compare the observed results with the predicted results. If the actual results match the prediction, then the hypothesis is *supported*. If the actual results are inconsistent with the prediction, then the hypothesis is *rejected*. Note that hypotheses can never be *proved* absolutely true; scientific conclusions are tentative and can change if contradicting evidence is later observed.

In the example experiment, the growth of the plants would be measured in cm. The average height would be recorded for each of the plant groups so that comparisons could be made between the groups and a conclusion could be determined. In the example, there was a statistical difference between the height of the plants receiving the most sunlight and the other two groups. The hypothesis would be supported. What would have been a next step if there was not a statistical difference between the height of the plants in each group?

In the next part of this lab, you will read a scenario about two students performing an experiment for the science fair and will answer questions based on what you read.

To summarize, the steps to the scientific method are as follows:

- Step 1: Make an **observation** and ask a question.
- Step 2: Create **a hypothesis or hypotheses** for your experiment.
- Step 3: Design your **experiment**.
- Step 4: Conduct your experiment.
- Step 5: Collect and analyze your **results (data)**.
- Step 6: Draw a **conclusion** about your results.
- Step 7: **Accept, modify, or reject** your hypothesis.

Case Study and Questions

Trixx and Collin are 9th grade students at Westview High School and must do an experiment for the local science fair. They meet one day after school to discuss the project. Both of them are interested in microbiology and decide to do an experiment using the bacterium, *Escherichia coli*. This microbe was recently discussed in their biology class, and they learned that it is an important member of the digestive system in addition to causing disease. *E. coli* can be found in the large intestine and produces vitamin K for its human host. It is also associated with urinary tract infections. Trixx tells Collin that there is information in the library that says *E. coli* can grow using glucose as its main source of sugar but can also grow using lactose as a sugar source. Collin suggests that their project can be to test which sugar would produce the most growth in a culture of *E. coli.* Collin writes in their scientific notebook that their observation is that *E. coli* can grow using different sugars in their growth medium. They think that the *E. coli* will grow best using glucose as the sugar source but also want to test lactose, sucrose, and mannitol sugars.

1. What is their hypothesis?

2. What should Trixx and Collin do next if they are following the scientific method?

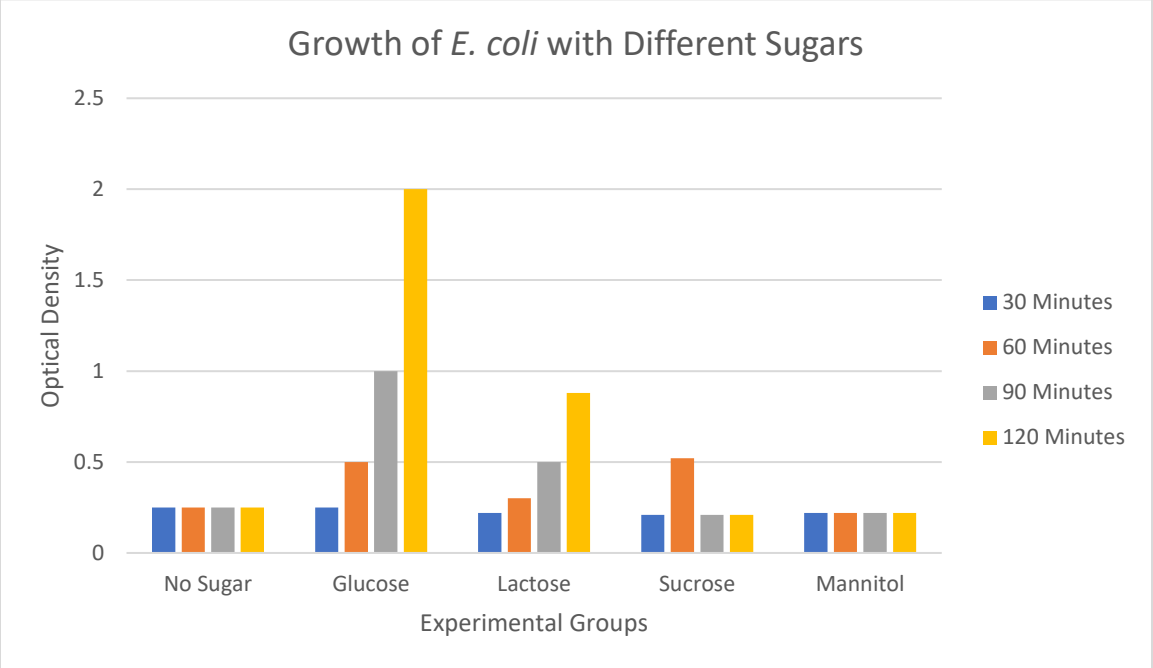
The biology teacher gives Trixx and Collin approval to move forward with their science fair project. The next part of their assignment is to design their experiment and to identify what their controls and variables will be for the process. They decide to grow the *E. coli* at 37 ºC (approximately body temperature) in test tubes containing 10 ml of nutrient broth (a common growth medium used for bacteria). They will have 5 tubes for each growth condition, including one set of tubes with bacteria but no added sugars as their control group. The growth of the bacteria will be determined every 15 minutes using a spectrophotometer that measures the optical density (cloudiness) of the broth. Once the settings are entered, Trixx and Collin will just need to insert each tube into the machine and write down the digital readout. See the table below for their setup of tubes and growth conditions.

Number of tubes	Temperature	Volume	Sugar
5	37 ºC	10 ml	None
5	37 ºC	10 ml	Glucose
5	37 ºC	10 ml	Lactose
5	37 ºC	10 ml	Sucrose
5	37 ºC	10 ml	Mannitol

3. Why is it important that all of the growth conditions are the same besides the sugar being tested?

4. As part of their experimental design, Trixx and Collin submit that their independent variable would be the growth of the bacteria and the dependent variable is the sugar being used. Are they correct in their submission? Why or why not?

Trixx and Collin set up their experiment by adding nutrient broth, *E. coli*, and the appropriate sugars to the test tubes. They incubate the tubes for a total of two hours taking measurements of growth every 30 minutes. They recorded their results for each of the tubes, took an average of growth values for each time, and created the following graph to represent the data for each set of tubes.



5. Was their hypothesis supported? Why or why not?

6. What could have happened with the sucrose tubes to explain the results that are shown in the graph?

7. After reviewing the data with Collin, Trixx realized that the data point at 60 minutes for sucrose (0.25) was accidentally entered as 0.52. What should they do to correct this error?

Trixx and Collin concluded that *E. coli* grew the best with glucose but could also grow with lactose. They concluded that sucrose and mannitol would not be good sugars to use to increase the growth of the bacteria. Their project won a ribbon at the local science fair, and they earned an A on the assignment.

Design an Experiment

In this part of the lab, you will work with your partner to design an experiment using the terminology and case study above to guide you. You will only be expected to design the experiment, determine the controls and variables, and plan how you would collect the results. This experiment is theory only; you will not be doing this experiment in the lab.

1. Write a question based on your observation(s) that could be answered through experimentation.
2. What would be your hypothesis?
3. What would be your control group?
4. What would be your experimental group or groups?
5. Define your dependent and independent variables.
6. Explain how you would collect your results.
7. Make predictions about the possible outcomes.

Now that you have designed your experiment, discuss your plan with another group in the class. Have the other group provide you with feedback about your experimental design. Give them feedback about their experimental design. Remember to be constructive in this process, there is no need to be mean.

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Define hypothesis, independent variable, and dependent variable.

2. Write a hypothesis for Trixx and Collin that would have been rejected based on the results that they obtained.

3. Trixx and Collin were invited to do the science fair the next year as 10^{th} grade students. What could they do as the next experiment to build on their original results?

4. You and your lab partner are collecting results of plant height for an experiment in class and get the following data: 4 cm, 4.5 cm, 5.5 cm, 4.2 cm, and 10 cm. The last data

point is much larger than the others, so you recheck the measurement. It is correct. When doing your analysis, your partner says you should ignore that plant height since it is different from the rest. Do you agree or disagree that you should ignore the 10 cm value? Explain.

5. Scientific results can cause controversy from time to time. What are some things you should always consider when reviewing scientific results?

References

Portions of this lab were adapted and/or modified from:

Burran, S, DesRochers, D. 2015. Principles of Biology I Lab Manual. Biological Sciences Open Textbooks. 3. https://oer.galileo.usg.edu/biology-textbooks/3. CC BY-SA 4.0.

Measurements

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

Objectives

- Define important terminology associated with the metric system, biological measurements, significant figures, and statistical analysis
- Practice metric conversions
- Take measurements of length, mass, volume, and temperature using appropriate equipment

Introduction

Measure the length of an object using your hand as the measuring device. Then have your lab partner measure the same item using their hand. Do you get the same number? Probably not. This was a common problem prior to the 18th century when different areas utilized different units of measurement, sometimes even within the same country. Imagine how confusing that would be to everyone involved. The **metric system** is based on units of ten and was created around 1790 as a way to standardize measurements from one area to another. It is still used in most parts of the world for measuring length, mass, volume, and temperature.

The United States, Liberia, and Myanmar use the **Imperial System** that includes inches, feet, miles, ounces, cups, quarts, and pounds. It would be costly and time consuming for the United States to convert everything to the metric system so a joint system has been adopted. This is why in scientific reporting you most often see the metric system being used.

In this activity, you will practice measuring length, mass, volume, and temperature in metric units. You will also work on conversion problems to gain an understanding of how to move the decimal between units. Observe the prefixes in Figure 1. When working conversion problems, the first step is to decide if you are converting from left-to-right or right-to-left. This will tell you which way to move the decimal. The next step is to count how many units are between the units you are converting. This will tell you how many places to move your decimal.

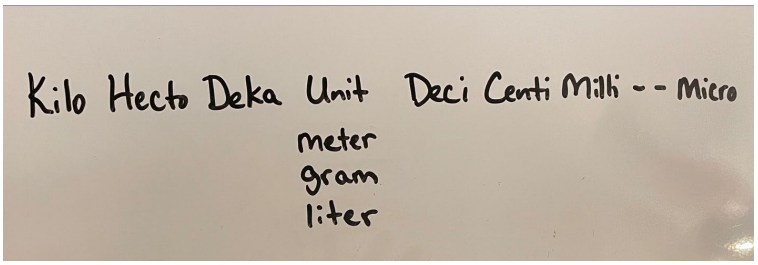


Figure 1. Units of Measurement

Example 1: Convert 5.87 milligrams (mg) to kilograms (kg).

The first step is to figure out using your units, which direction the decimal will move. Since milli is to the right of kilo, this should tell you that the decimal needs to move from the right to left. Then you must figure out how many places to move the decimal. Milli to Centi is 1 place; Milli to Deci is 2 places; Milli to Gram is 3 places; Milli to Deka is 4 places; Milli to Hecto is 5 places; and Milli to Kilo is 6 places (see Figure 2 below). This means you must move the decimal 6 places to the left. Because the number 5.87 becomes 0.587 when you move the decimal one place, you just keep adding zeros until you can move the correct number of spaces. So, 5.87 mg = 0.00000587 kg. If you want to double check that you are correct, you can move the decimal back to the right 6 places and you should get 5.87. Practice your conversions.

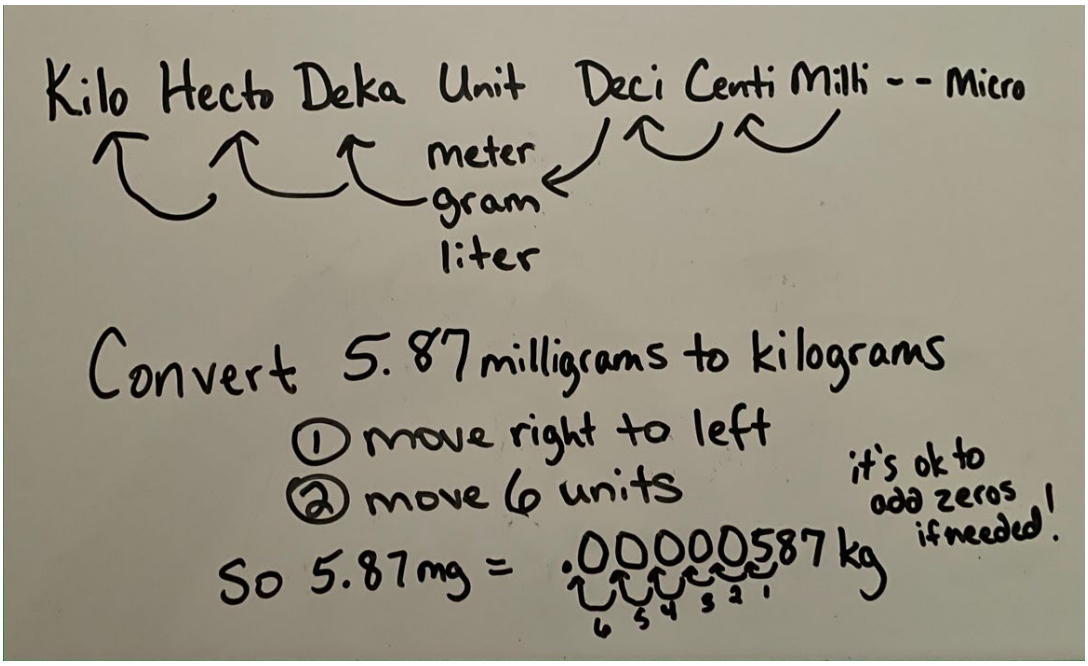


Figure 2. Example 1: Moving the Decimal Right to Left

Example 2: Convert 1.72 meters to centimeters.

Using the same technique that you used for example 1, you can use the units to determine which direction the decimal will move. In this example, it would be from left to right. Then determine how many places are between meter and centimeter. It is two places so, you move the decimal two places to the right. 1.72 m = 172 cm. (See Figure 3 below.)

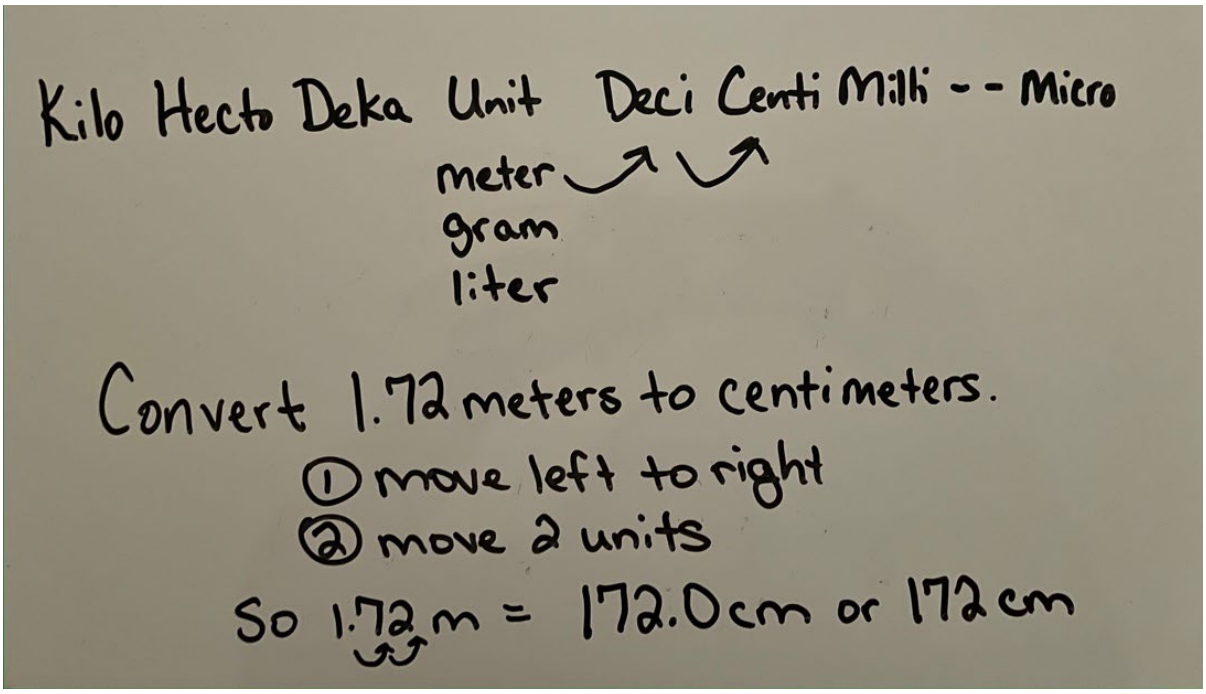


Figure 3. Example 2: Moving the Decimal from Left to Right

Measurements

In this part of the activity, you will learn about measuring length, mass, volume, and temperature, and the instruments that are used for obtaining them.

A. Length

The **meter (m)** is the standard unit of length or distance.

Conversion Factors
1 meter =39.4 inches = 1 yard = 3 feet
1 kilometer = approximately 0.6 miles
1 inch = 2.54 centimeters

A micrometer (μm), about the size of one *E. coli* bacterial cell, is one thousandth of a millimeter and is too small to be seen with the naked eye. You will learn how to use microscopes in a later lab.

1. Compare a ruler and a meter stick. Note the difference on the ruler between the metric side and the inches side.
2. Use the ruler to measure and record the length and width of one notecard in cm. Use those numbers to calculate area in cm^2 if Area = length x width.
    a. Length =
    b. Width =
    c. Area =

3. Use the meter stick to measure and record your height in cm. Then use that number to calculate your height in inches if 1 inch = 2.54 cm.
    a. Height in cm =
    b. Height in inches =
    c. Does your calculation match what you thought your height was in inches?

B. Mass

The **kilogram (kg)** is the standard unit of measure for mass which represents the amount of matter in an object. It is also acceptable to report smaller objects' mass in grams (g). Mass should not be confused with weight which takes gravity into account. Your mass on Earth is the same as your mass on the moon where your weight would be higher on Earth than on the moon because of the effect of gravity. The mass of an object is typically determined using a digital balance in the laboratory. Your instructor will demonstrate how to use the balance correctly.

4. Place a weigh boat on the balance and follow the instructions given by your professor to zero out the digital balance before and between measuring the objects.
5. Find the mass in grams of the following objects:
    a. Marble =
    b. Binder clip =
    c. Penny =

C. Volume

The **liter (L)** is the standard unit of measure for volume. Volume is a three-dimensional space occupied by a solid, liquid, or a gas. The volume of a solid can be measured using a process called **displacement**. This is done by putting the solid object in a known volume of water and measuring how much the water is displaced (changes) due to the object.

The volume of a liquid is measured using a graduated cylinder. It is important to measure the lowest point of the curve (when looking at eye level) that forms in the graduated cylinder. This point is called the **meniscus**. Some volumes are too small to be measured accurately using the graduated cylinder. If you are measuring less than five milliliters you may want to use a glass pipette. For measuring volumes in the microliter (µl) range, you would want to use a micropipette instead. Your instructor will demonstrate the proper use of the micropipette. You will have an opportunity to practice with one during this lab.

6. Use the graduated cylinder at your station to measure the volume of the marble, binder clip, and penny from the mass activity. Start with 30 ml of water and measure the displacement.
    a. Marble =
    b. Binder clip =
    c. Penny =
    d. **Density =** mass/volume. Calculate the density of each of these items. Water has a density of 1 g/ml. Items with higher density will sink while items of lower density will float. Will the marble, binder clip, and penny sink or float?

7. Use the graduated cylinders provided at your station or by the sink to measure the volume of water needed to fill one coffee cup and the volume of water that fits in a gallon milk jug.
    e. Coffee cup =
    f. Gallon milk jug =
8. Practice using the micropipette by moving water from a beaker to a strip of parafilm and from the parafilm back to the beaker.

D. Temperature

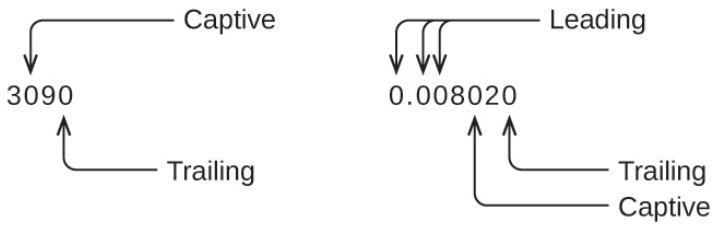
The metric unit of temperature is **degrees Celsius (ºC).** Body temperature is 37ºC. The conversion to Fahrenheit (F) is done using ºC=5 (ºF-32)/9. Other important temperatures include the boiling point of water (100ºC) and the freezing point of water (0ºC).

9. Use the digital probes at the station on the lab bench to determine the temperature of each of the following in ºC.
    a. Room Temperature Water =
    b. Cold Water from the Tap =
    c. Water on the Hot Plate =
    d. Water with Ice =
10. Convert each of the temperatures above to Fahrenheit using the formula above.
    e. Room Temperature Water =
    f. Cold Water from the Tap =
    g. Water on the Hot Plate =
    h. Water with Ice =
11. If you have a fever of 100ºF, what would that be in Celsius?

Significant Figures

The numbers of measured quantities, unlike defined or directly counted quantities, are not exact. If you place a quarter on a standard electronic balance, you may obtain a reading of 6.72 g. The digits 6 and 7 are certain, and the 2 indicates that the mass of the quarter is likely between 6.71 and 6.73 grams. The quarter weighs *about* 6.72 grams, with a nominal uncertainty in the measurement of ± 0.01 gram. If we weigh the quarter on a more sensitive balance, we may find that its mass is 6.723 g. This means its mass lies between 6.722 and 6.724 grams, an uncertainty of 0.001 gram. Every measurement has some uncertainty, which depends on the device used (and the user's ability). All of the digits in a measurement, including the uncertain last digit, are called **significant figures or significant digits**. Note that zero may be a measured value; for example, if you stand on a scale that shows weight to the nearest pound and it shows "120," then the 1 (hundreds), 2 (tens) and 0 (ones) are all significant (measured) values.

Whenever you make a measurement properly, all the digits in the result are significant. But what if you were analyzing a reported value and trying to determine what is significant and what is not? Well, for starters, all nonzero digits are significant, and it is only zeros that require some thought. We will use the terms "leading," "trailing," and "captive" for the zeros and will consider how to deal with them.

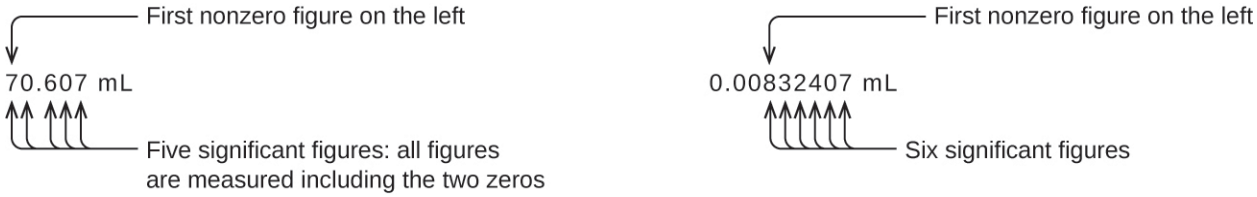


The left diagram uses the example of 3090. The zero in the hundreds place is labeled "captive" and the zero in the ones place is labeled trailing. The right diagram uses the example 0.008020. The three zeros in the ones, tenths, and hundredths places are labeled "leading." The zero in the ten-thousandths place is labeled "captive" and the zero in the millionths place is labeled "trailing."

Starting with the first nonzero digit on the left, count this digit and all remaining digits to the right. This is the number of significant figures in the measurement unless the last digit is a trailing zero lying to the left of the decimal point.

The left diagram uses the example of 1267 meters. The number 1 is the first nonzero figure on the left. 1267 has 4 significant figures in total. The right diagram uses the example of 55.0 grams. The number 5 in the tens place is the first nonzero figure on the left. 55.0 has 3 significant figures. Note that the 0 is to the right of the decimal point and therefore is a significant figure.

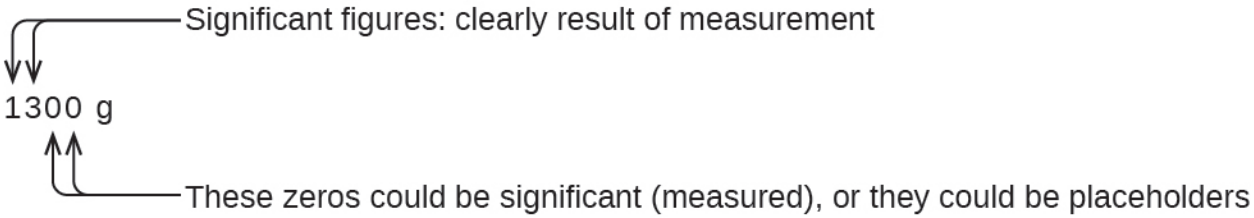
Captive zeros result from measurement and are therefore always significant. Leading zeros, however, are never significant—they merely tell us where the decimal point is located.



The left diagram uses the example of 70.607 milliliters. The number 7 is the first nonzero figure on the left. 70.607 has 5 significant figures in total, as all figures are measured including the 2 zeros. The right diagram uses the example of 0.00832407 M L. The number 8 is the first nonzero figure on the left. 0.00832407 has 6 significant figures.

The leading zeros in this example are not significant. We could use exponential notation and express the number as 8.32407×10^{-3}; then the number 8.32407 contains all of the significant figures, and 10^{-3} locates the decimal point.

The number of significant figures is uncertain in a number that ends with a zero to the left of the decimal point location. The zeros in the measurement 1,300 grams could be significant or they could simply indicate where the decimal point is located. The ambiguity can be resolved with the use of exponential notation: 1.3×10^3 (two significant figures), 1.30×10^3 (three significant figures, if the tens place was measured), or 1.300×10^3 (four significant figures, if the ones place was also measured). In cases where only the decimal-formatted number is available, it is prudent to assume that all trailing zeros are not significant.



This figure uses the example of 1300 grams. The one and the 3 are significant figures as they are clearly the result of measurement. The 2 zeros could be significant if they were measured or they could be placeholders.

When determining significant figures, be sure to pay attention to reported values and think about the measurement and significant figures in terms of what is reasonable or likely—that is, whether the value makes sense. For example, the official January 2014 census reported the resident population of the US as 317,297,725. Do you think the US population was correctly determined to the reported nine significant figures, that is, to the exact number of people? People are constantly being born, dying, or moving into or out of the country, and assumptions are made to account for the large number of people who are not actually counted. Because of these uncertainties, it might be more reasonable to expect that we know the population to within perhaps a million or so, in which case the population should be reported as 317 million, or 3.17×10^8 people. Keep significant figures in mind when doing statistical analyses.

A second important principle of uncertainty is that results calculated from a measurement are at least as uncertain as the measurement itself. We must take the uncertainty in our measurements into account to avoid misrepresenting the uncertainty in calculated results. One way to do this is to report the result of a calculation with the correct number of significant figures, which is determined by the following three rules for rounding numbers:

1. When we add or subtract numbers, we should round the result to the same number of decimal places as the number with the least number of decimal places (the least precise value in terms of addition and subtraction).
2. When we multiply or divide numbers, we should round the result to the same number of digits as the number with the least number of significant figures (the least precise value in terms of multiplication and division).
3. If the digit to be dropped (the one immediately to the right of the digit to be retained) is less than 5, we "round down" and leave the retained digit unchanged; if it is more than 5, we "round up" and increase the retained digit by 1; if the dropped digit *is* 5, we round up or down, whichever yields an even value for the retained digit. (The last part of this rule may strike you as a bit odd, but it's based on reliable statistics and is aimed at

avoiding any bias when dropping the digit "5," since it is equally close to both possible values of the retained digit.)

The following examples illustrate the application of this rule in rounding a few different numbers to three significant figures:

- 0.028675 rounds "up" to 0.0287 (the dropped digit, 7, is greater than 5)
- 18.3384 rounds "down" to 18.3 (the dropped digit, 3, is less than 5)
- 6.8752 rounds "up" to 6.88 (the dropped digit is 5, and the retained digit is even)
- 92.85 rounds "down" to 92.8 (the dropped digit is 5, and the retained digit is even)

Statistical Analysis

Many times, scientific data are validated and compared using the results of statistical analyses. The statistics allow inferences to be made about a larger group of data points from a smaller subset of those data points. Often times in biology, the most important value from a set of numbers is the **mean,** or the average of the data points. This is calculated by getting the sum of the data points and dividing by the number of data points. You will sometimes also hear about a **median** data point which is the middle data point when the entire set of data points are organized from smallest to largest. If there is an even set of data points, the median is simply the average of the two in the middle.

12. Determine the mean and median of the following data points:
    a. 1, 3, 7, 9, 12
    b. 15, 29, 13, 2, 10, 7, 11
    c. 75, 43, 38, 22, 18, 74
    d. 87, 100, 35, 66, 72, 43, 59, 99, 22, 87

In addition to finding the mean and median, another value that is often calculated is the **standard deviation (s.d.)**. The standard deviation gives the person doing the analysis an idea of how much each data point varies in relation to the mean. If the standard deviation is low, it indicates that the data points are all relatively close to the mean. If the standard deviation is high, the data points are farther away from each other and the mean. Final calculations are usually written as the mean ± standard deviation. To calculate the standard deviation, you would use the following formula:

$$\text{s.d.} = \sqrt{\frac{\Sigma(x_i - \mu)^2}{N}}$$

N = population size

x_i = each value from the population

μ = the population mean

Example problem: We want to find the mean and standard deviation for a small set of plant heights. The measurements are 3cm, 5cm, and 10cm. To find the mean, we would sum the numbers and divide by 3.

(3cm + 5cm + 10cm)/3 = 6 cm would be the mean

To find the standard deviation, use the chart below:

Measurement (x_i)	Mean (μ)	Measurement - Mean	Square of M-M $((x_i - \mu)^2)$
3cm	6cm	-3cm	9*
5cm	6cm	-1cm	1*
10cm	6cm	4cm	16
			Sum $(\Sigma(x_i - \mu)^2) = 26$

Note that it doesn't matter if the subtraction is negative because it will become positive when squared

s.d. = $\sqrt{\frac{26}{3}}$ = 2.9cm

We would then report the results as 6.0 ± 2.9 cm.

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Use significant figures and complete the following conversions:
    a. 12.56 milliliters = _____ liters
    b. 0.875 kilograms = _____ decigrams
    c. 453.21 centimeters = _____ meters
    d. 0.212 hectograms = _____ kilograms
    e. 42ºC = _____ ºF

2. Why is it important for scientists to report their results using the metric system?

3. Which of the units would be best for reporting the length of a race for charity?

4. Why are the units used for large soda bottles listed in liters instead of kiloliters or milliliters?

5. What instrument/method would you use to measure the volume of a small rock?

6. Why is it important to read a meniscus at eye-level?

7. If your body temperature is 36ºC, do you have a fever? Why or why not?

8. Why does a digital balance need to be zeroed with the weigh boat before using it to get the mass of an object?

9. Which race is longer, a half-marathon (13.1 miles) or a 10K? Explain your answer.

10. What would you need to score on your fourth exam to have an average (mean) of 90 on the exams in your lecture class, if your first three exam grades are 85, 94, and 89?

References

Portions of this lab were adapted or modified from:

Flowers P, Theopold K, Langley R, and Robinson W. 2019. Chemistry 2e. OpenStax. https://openstax.org/details/books/chemistry-2e CC BY 4.0.

Genovesi E, Blinderman L, Natale P. 2019. Unfolding the Mystery of Life, Biology Lab Manual for Non-Science Majors. Open Textbook Library. https://open.umn.edu/opentextbooks/textbooks/736 CC BY 4.0.

pH and Biological Buffers

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

Objectives

- Understand the basis of the pH scale and how pH is measured
- Understand the importance of buffers in living organisms
- Use the scientific method to develop a testable hypothesis and experiment aimed at determining which over-the-counter antacids are most effective at neutralizing stomach acid

Introduction

In living organisms, maintaining homeostasis is important for survival. This stable equilibrium includes maintaining a constant pH. pH is a measure of the concentration of hydrogen ions in solution and is calculated as pH = -log [H^+].

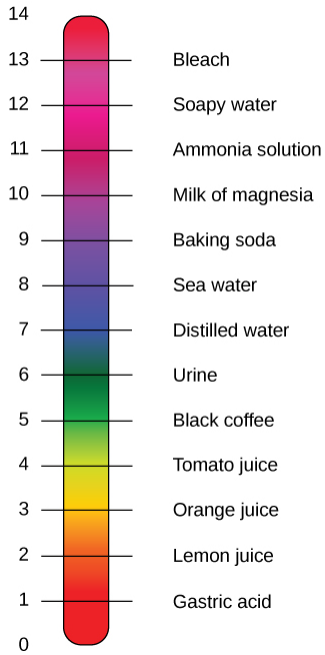


Figure 1. The pH scale ranges from 0 to 14, with common solutions and their pH listed. This work has been released to the public domain by its authors Zedalis J, Eggebrecht J.

Solutions with a pH less than 7 are acidic and solutions with a pH greater than 7 are basic. A pH of 7 is considered neutral (Figure 1).

Using the equation for pH above, answer the following questions:

1. What is the concentration of H^+ in orange juice?

2. What is the concentration of H^+ in soapy water?

3. Which has a higher concentration of H^+, tomato juice or lemon juice?

Extremes in pH in either direction from 7.0 are usually considered inhospitable to life. The pH inside cells (6.8) and the pH in the blood (7.4) are both very close to neutral. However, the environment in the stomach is highly acidic, with a pH of 1 to 2. So how do the cells of the stomach survive in such an acidic environment? How do they maintain the near neutral pH inside them? The answer is that they cannot do it and are constantly dying. New stomach cells are constantly produced to replace dead ones, which are digested by stomach acids. It is estimated that the lining of the human stomach is completely replaced every seven to ten days.

So how can organisms whose bodies require a near-neutral pH ingest acidic and basic substances (a human drinking orange juice, for example) and survive? Buffers are the key. Buffers readily absorb excess H^+ or OH^-, keeping the pH of the body carefully maintained in the narrow range required for survival.

Maintaining a constant blood pH is critical to a person's well-being. The buffer maintaining the pH of human blood involves carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-), and carbon dioxide (CO_2). When bicarbonate ions combine with free hydrogen ions and become carbonic acid, hydrogen ions are removed, moderating pH changes. Similarly, as shown in Figure 2, excess carbonic acid can be converted to carbon dioxide gas and exhaled through the lungs. This prevents too many free hydrogen ions from building up in the blood and dangerously reducing the blood's pH. Likewise, if too much OH^- is introduced into the system, carbonic acid will combine with it to create bicarbonate, lowering the pH. Without this buffer system, the body's pH would fluctuate enough to put survival in jeopardy.

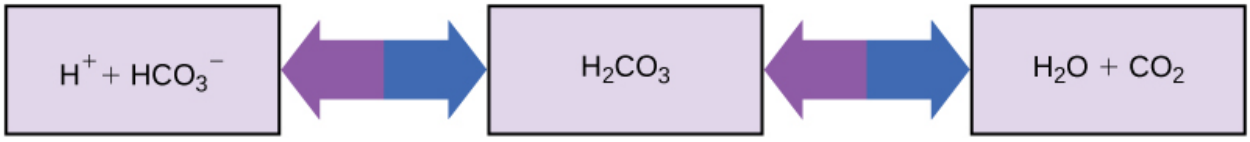


Figure 2. This diagram shows the body's buffering of blood pH levels. The blue arrows show the process of raising pH as more CO_2 is made. The purple arrows indicate the reverse process: the lowering of pH as more bicarbonate is created. This work has been released to the public domain by its authors Zedalis J, Eggebrecht J.

Experiment 1: Testing Buffering Capacity of Various Solutions

In this experiment, you will test four solutions to determine which has the greatest buffering capacity or resistance to a change in pH. You will do this by observing the change in pH after adding a dilute hydrochloric acid solution (0.1 M HCl) to each solution.

Procedure:

1. Obtain 5 mL of the four solutions listed in **Table 1** in four test tubes. Label each tube.
2. Measure the initial pH of each solution and record this in **Table 1** under 'Initial pH'.
3. Add 5 drops of 0.1 M HCl to the first tube, mix gently by swirling or inverting with parafilm.
4. Measure the pH after adding acid and record this in **Table 1** under 'pH after acid'.
5. Repeat steps 3 and 4 for the remaining three tubes.
6. Which solution had the greatest buffering capacity, and how do you know?

Table 1. Testing the buffering capacity of various solutions

Solution	**Initial pH**	**pH after acid**
Water		
0.1 M NaCl		
Milk		
0.1 M phosphate buffer		

You can also test the buffering capacity of various solutions by using a pH indicator like bromcresol purple, which will indicate a change in pH between 5.2 (yellow) and 6.8 (purple). This can help you determine when a solution has reached its capacity to resist a change in pH by becoming slightly acidic, changing from purple to yellow.

Experiment 2: Testing the efficacy of antacids

Many commercial antacids help treat indigestion and heartburn by neutralizing stomach acid. The effective ingredients in these antacids act as buffers described above, as they absorb excess H^+ in the stomach. The buffers commonly found in these antacids include:

- Sodium bicarbonate ($NaHCO_3$)
- Calcium carbonate ($CaCO_3$)
- Magnesium hydroxide ($Mg(OH)_2$)
- Aluminum hydroxide ($Al(OH)_3$)

Another class of antacids, called H_2 blockers, works to treat heartburn in a different way. Ingredients in this class of antacids include ranitidine, famotidine, nizatidine and cimetidine. These ingredients work by inhibiting the acid-secreting cells in your stomach from responding to histamine, which is a compound produced in response to inflammation. H_2 blockers, while effective for long-term relief of heartburn and indigestion, do not act as buffers.

As a class, guided by your instructor, your task is to:

1. Develop a testable hypothesis regarding which antacid available in class will be the most effective at neutralizing stomach acid by resisting a change in pH
2. Design an experiment to test your hypothesis, using various antacid tablets, a 0.1 M HCl solution and the pH indicator bromocresol purple to determine when your solution has reached its buffering capacity by changing color from purple (basic) to yellow (acidic).

As you work in groups, some things to keep in mind:

- Note the ingredients in each of the antacids provided in lab, remembering which are buffers and which are H_2 blockers. Which do you think will work better to neutralize acid, or resist a change in pH?
- To test each antacid in a similar manner, note the specified dose for each antacid provided.
- As more acid (0.1 M HCl) is added to your antacid solutions, the bromocresol purple will stay purple as long as H^+ is still being absorbed. However, once the color changes and remains a dull yellow after mixing, the solution becomes acidic and the antacid has reached its buffering capacity.
- Remember to include both a positive and negative control in your experimental design. Your instructor can provide you with a buffer for the positive control.
- Make a table to record your data, and help you determine which antacid is the most effective buffer.

Testable Hypothesis:

Experimental Procedure:

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Which antacid did you expect to be the most effective at neutralizing stomach acid, and why?

2. After carrying out your experiment, did your results support your hypothesis? If not, what could be a possible explanation?

3. Designing a controlled experiment that tests a specific hypothesis is hard (you likely found this out!) The scientific method is an iterative process, in which you are constantly refining your questions, hypotheses and methods of testing. What worked well in your experiment, and what would you change moving forward?

4. What follow-up questions do you have after this experiment, and what kinds of new hypotheses could you develop and test?

Macromolecules

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

Objectives

- Define and discuss important terminology associated with the four groups of macromolecules: carbohydrates, proteins, lipids, and nucleic acids
- Identify carbohydrates, proteins, and lipids using specific reagents
- Isolate DNA from strawberries
- Determine the identities of unknown solutions
- Report results in a written format

Introduction

Macromolecules include carbohydrates, proteins, lipids, and nucleic acids. They are important for cells because they provide substrates that can be used to make energy and provide building blocks for cellular structures. When looking at the groups of macromolecules, it is often helpful to think about the **monomers** (single subunits) that make up the **polymers** (many subunits) of each group.

A. Carbohydrates

What do you think of when you hear the word carbohydrate? Carbohydrates are the most abundant macromolecule on the planet and can be used immediately for energy through a process called cellular respiration which you will learn about later. They have the molecular formula $(CH_20)_n$ meaning that they always have equal amounts of Carbon and Oxygen and twice as many Hydrogens. Carbohydrates are divided into three groups based on the number of sugar subunits that make them up: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides (mono = one) such as glucose and its isomers (fructose and galactose), are made up of one sugar subunit and are referred to as simple sugars. Glucose has the molecular formula $C_6H_{12}O_6$ and is broken down to make ATP energy during the process of glycolysis. It is what diabetics measure in their blood when they take a blood glucose reading. Simple sugars can also be found as parts of other macromolecules. **Disaccharides** (di = two) such as sucrose and maltose are made up of two sugar subunits. Sucrose is made from combining glucose and fructose while maltose is made up of two units of glucose. You may be familiar with sucrose as table sugar and fructose from hearing about high fructose corn syrup. These sugars are part of our diet and are broken down into their subunits for energy production. **Polysaccharides** (poly = many) such as glycogen and starch are made up of more than two subunits of sugar and are typically stored in the cell to use later as an energy source. Glycogen is typically found in animal cells, particularly in the liver, while starch is typically found in plants such as potatoes. Plants and bacteria also use the polysaccharides cellulose and peptidoglycan respectively for structural support in their cell walls. See Figure 1 for carbohydrate examples.

In this activity, you will use Benedict's Reagent to test for the presence of simple sugars/monosaccharides in several solutions. The Benedict's Reagent (blue) contains copper ions that will be reduced by the simple sugar to produce copper (I) oxide when heated and will produce a green color (small amount of sugar) or an orange color (large amount of sugar). This reaction does not occur with more complex disaccharides or polysaccharides. You will use Iodine in another set of tubes to test the same solutions for the presence of starch. Iodine (yellow brown) reacts with the amylose enzyme in starch to form a dark purple-black color.

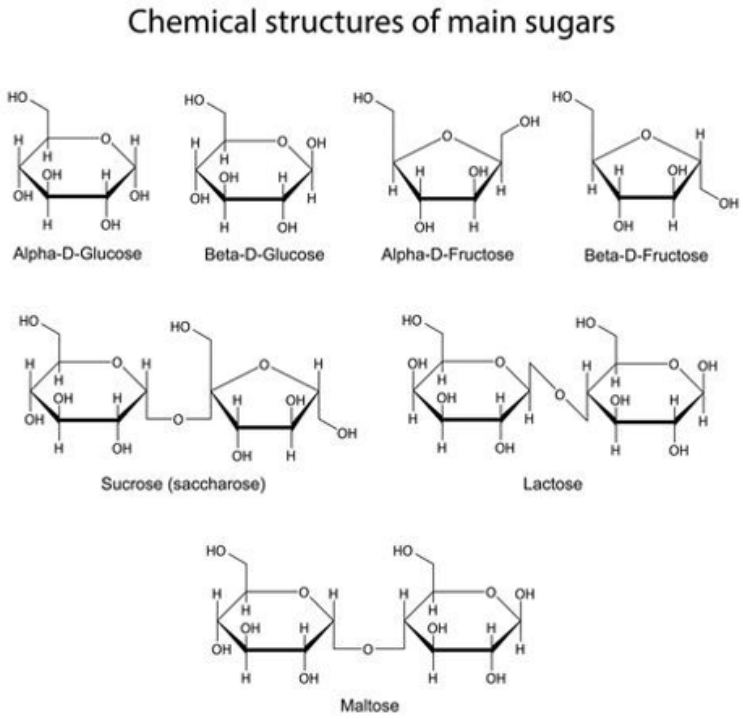


Figure 1. Examples of Sugars

B. Proteins

What do you think of when you hear the word protein? Proteins can have multiple functions within a cell. They can work as enzymes, transporters, structural components, regulators, and even in defense against foreign invaders as antibodies.

Proteins are made up of monomers called **amino acids** joined by peptide bonds. Amino acids are made up of an amino group, a carboxyl group, and a side-chain or R-group (Figure 2). The R-group will determine how the amino acids will interact with each other in a single protein or how the protein will interact with other molecules. The interactions between amino acids also determine the structure associated with a protein. The structure is hierarchal and is classified as primary, secondary, tertiary, and quaternary (Figure 3). The **primary structure** is the sequence of amino acids joined by peptide bonds. The **secondary structure** occurs when the amino acids begin to form interactions and fold to form either alpha-helices or beta-sheets. The **tertiary structure** forms when the secondary structures interact to form a three-dimensional

structure. Some proteins stop here but others will form a **quaternary structure** when multiple three-dimensional protein subunits come together to form a completely functional protein.

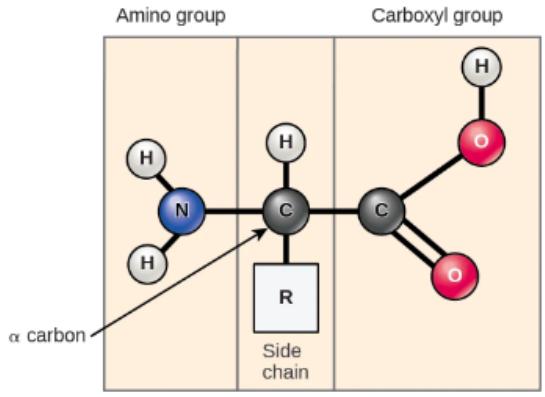


Figure 2. Amino Acid Structure
Figure from https://openstax.org/books/biology-2e/pages/3-4-proteins

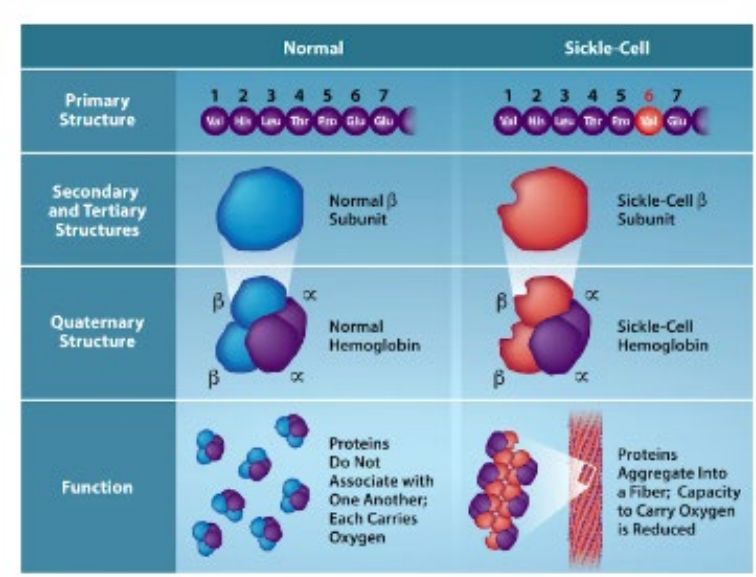


Figure 3. Levels of Protein
Figure from https://openstax.org/books/biology-2e/pages/3-4-proteins

In this activity, you will use Biuret Reagent to test for the presence of proteins in a solution. Biuret Reagent reacts with peptide bonds to form a purple color.

C. Lipids

What do you think of when you hear the word lipid? Do you think of fats and oils? If so, these are two examples of lipids. Other examples include steroids and phospholipids. These macromolecules do not follow the structure of monomers and polymers that we have seen in the other groups of macromolecules. Instead, **fats and oils** are triglycerides made up of a **glycerol** molecule associated with three **fatty acids,** or long chains of hydrocarbons containing a carboxyl group at the end. Due to these structures, lipids are nonpolar, do not dissolve in water, and are labeled as hydrophobic (water fearing). Fatty acids are also classified as being

either **saturated** (containing as many hydrogen bonds as possible) or **unsaturated** (not containing as many hydrogen bonds as possible, allows for double bonds between carbons). Saturated fats are often solid at room temperature and examples include animal fats such as bacon grease or butter. Unsaturated fats are often liquid at room temperature and examples include plant sources such as vegetable, canola, or olive oils. Figure 4 shows a saturated and an unsaturated fat.

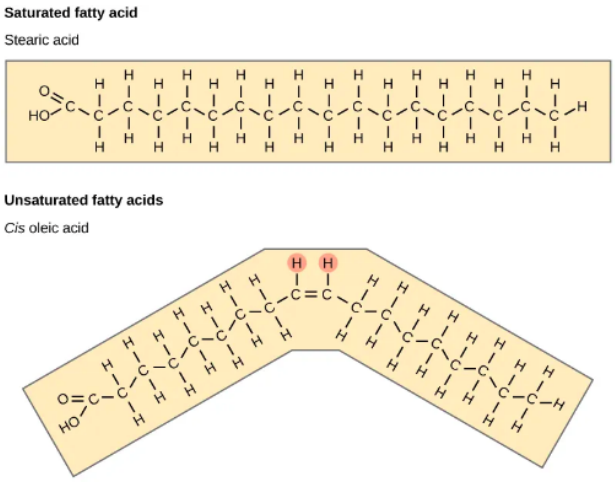


Figure 4. Examples of Saturated and Unsaturated Fats
Figure from https://openstax.org/books/biology-2e/pages/3-3-lipids

The group of **steroids** includes molecules of cholesterol, estrogen, and testosterone and all are made of fused hydrocarbon rings. Cholesterol is important for cells as it provides structural support for membranes and is a precursor to other steroid molecules. Estrogen and testosterone are important hormones produced by animals.

Phospholipids are modified triglycerides that contain glycerol, two fatty acids, and a phosphate group. Phospholipids are important structures that make up cellular membranes and are typically found as a bilayer. The phosphate head is polar and hydrophilic (water loving) while the tails are nonpolar and hydrophobic. When the bilayer forms, the polar heads will align to the outside of the layer with the nonpolar tails aligning in the middle. Figure 5 shows a phospholipid bilayer.

In this activity, you will use Sudan IV Reagent to test for lipids in a solution. Sudan IV binds to the lipids and will show a concentrated red color if lipids are present.

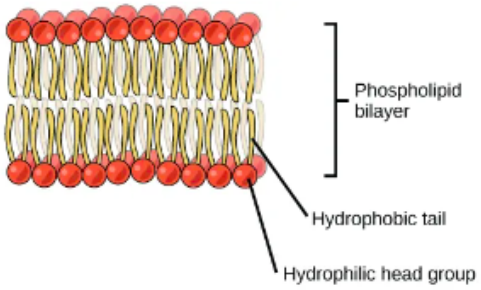


Figure 5. Phospholipid bilayer
Figure from https://openstax.org/books/biology-2e/pages/3-3-lipids

D. Nucleic Acids

What do you think of when you hear the words nucleic acids? Nucleic acids include **deoxyribonucleic acid** (DNA), **ribonucleic acid** (RNA), and **adenosine triphosphate** (ATP). You are probably familiar with DNA since it is important **genetic material** for passing on information from one generation to the next and also for giving the cells instructions for all activities. DNA is composed of subunits called nucleotides that contain the sugar deoxyribose, a phosphate group, and one of the following nitrogenous bases (adenine, guanine, cytosine, or thymine). The primary structure of DNA is the strand of nucleotides connected by phosphodiester bonds. The secondary structure is formed when two strands of DNA form hydrogen bonds with each other to form a double helix. In this structure, adenine (A) and thymine (T) bond with each other and cytosine (C) and guanine (G) bond with each other. Figure 6 shows the double helix of DNA.

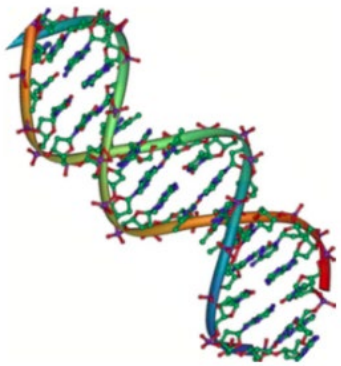


Figure 6. DNA Double Helix
Figure from https://openstax.org/books/biology-2e/pages/3-5-nucleic-acids

RNA is composed of subunits called nucleotides that contain the sugar ribose, a phosphate group, and one of the following nitrogenous bases (adenine, guanine, cytosine, and uracil). Note that while the names of the bases are the same, adenine, guanine, and cytosine in RNA have a different sugar than the same molecules in DNA. The nucleotides are held together via phosphodiester bonds and only form a single strand. RNA molecules are created via a process called transcription and function in the **synthesis of proteins** in a process called translation. You will learn about these in more detail later.

ATP is composed of an adenosine molecule and three phosphates. It is the main source of energy for cells due to the high amounts of energy contained within the phosphate bonds.

In this activity, you will isolate DNA from a strawberry and will visualize it with your naked eye. It will be a viscous material. You will not be able to identify the individual nucleotides because they are too small to be seen with the naked eye or the compound microscopes that are in the lab. They can only be seen with specialized electron microscopes.

Week 1: Experiments with Known Solutions

Experiment 1: Simple Sugars

In this activity, you will be using Benedict's Reagent to test each solution for the presence of simple sugars. If simple sugars are present, the reagent will turn green to orange after being heated. Be sure to identify which tubes are your control tubes. The negative control does not contain simple sugars and will not turn green to orange. The positive control contains simple sugars and will turn green to orange.

1. Collect and label 6 test tubes with the numbers 1 – 7.
2. Using a wax pencil and a ruler, mark each tube at the 1 cm and the 2 cm mark.
3. Using separate plastic pipettes, add the following solutions to the 1cm mark in each tube
    a. Tube 1 – water
    b. Tube 2 – sugar solution
    c. Tube 3 – starch solution
    d. Tube 4 – orange juice
    e. Tube 5 – potato juice
    f. Tube 6 – regular soda
    g. Tube 7 – diet soda
4. Using a new plastic pipette or the dropper bottle of solution, add Benedict's Reagent to the 2 cm mark in each tube. Avoid touching the solution in the tube with the pipette to prevent cross contamination.
5. Place the tubes in a beaker of boiling water for 10 minutes. Remove the tubes carefully using test tube holders. **Do not touch the glass**. It will be HOT!
6. Record the color of the solutions in the tubes.
7. After recording your results, wash the tubes as instructed.

Tube Number	Solution	Initial Color	Final Color	Conclusion
1	Water			
2	Sugar			
3	Starch			
4	Orange Juice			
5	Potato Juice			
6	Regular Soda			
7	Diet Soda			

Experiment 2: Starches

In this activity, you will be using Iodine Reagent to test each solution for the presence of starch. In the presence of starch, iodine will turn a dark purple-black color. Be sure to identify which tubes are your control tubes. The negative control does not contain starch and will not turn dark purple-black. The positive control contains starch and will turn dark purple-black.

1. Collect and label 6 test tubes with the numbers 1 – 7.
2. Using a wax pencil and a ruler, mark each tube at the 1 cm and the 2 cm mark.
3. Using separate plastic pipettes, add the following solutions to the 1cm mark in each tube
    a. Tube 1 – water
    b. Tube 2 – sugar solution
    c. Tube 3 – starch solution
    d. Tube 4 – orange juice
    e. Tube 5 – potato juice
    f. Tube 6 – regular soda
    g. Tube 7 – diet soda
4. Using a new plastic pipette or the dropper bottle, add Iodine Reagent to the 2 cm mark in each tube. Avoid touching the solution in the tube with the pipette to prevent cross contamination.
5. Gently mix the contents of the tubes as demonstrated by your instructor.
6. Record the color of the solutions in the tubes.
7. After recording your results, wash the tubes as instructed.

Tube Number	Solution	Initial Color	Final Color	Conclusion
1	Water			
2	Sugar			
3	Starch			
4	Orange Juice			
5	Potato Juice			
6	Regular Soda			
7	Diet Soda			

Experiment 3: Proteins

In this activity, you will be using Biuret Reagent to test each solution for the presence of peptide bonds/proteins. In the presence of proteins, Biuret will turn purple. Be sure to identify which tubes are your control tubes. The negative control does not contain protein and will not turn purple. The positive control contains protein and will turn purple.

1. Collect and label 6 test tubes with the numbers 1 – 7.
2. Using a wax pencil and a ruler, mark each tube at the 1 cm and the 2 cm mark.

3. Using separate plastic pipettes, add the following solutions to the 1cm mark in each tube
    a. Tube 1 – water
    b. Tube 2 – protein solution
    c. Tube 3 – glucose
    d. Tube 4 – albumin
    e. Tube 5 – amino acid solution
    f. Tube 6 – regular soda
    g. Tube 7 – diet soda
4. Using a new plastic pipette or the dropper bottle, add Biuret Reagent to the 2 cm mark in each tube. Avoid touching the solution in the tube with the pipette to prevent cross contamination.
5. Gently mix the contents of the tubes as demonstrated by your instructor.
6. Record the color of the solutions in the tubes.
7. After recording your results, wash the tubes as instructed.

Tube Number	Solution	Initial Color	Final Color	Conclusion
1	Water			
2	Protein			
3	Glucose			
4	Albumin			
5	Amino Acid			
6	Regular Soda			
7	Diet Soda			

Experiment 4: Lipids

In this activity, you will be using Sudan IV Reagent to test each solution for the presence of lipids. In the presence of lipids, Sudan IV will be a concentrated red. Be sure to identify which tubes are your control tubes. The negative control does not contain lipids and will not turn red. The positive control contains lipids and will turn red.

1. Collect and label 6 test tubes with the numbers 1 – 5.
2. Using a wax pencil and a ruler, mark each tube at the 1 cm and the 2 cm mark.
3. Using separate plastic pipettes, add the following solutions to the 1cm mark in each tube
    a. Tube 1 – water
    b. Tube 2 – vegetable oil
    c. Tube 3 – honey
    d. Tube 4 – albumin
    e. Tube 5 – melted butter
4. Using a new plastic pipette or the dropper bottle, add Sudan IV Reagent to the 2 cm mark in each tube. Avoid touching the solution in the tube with the pipette to prevent cross contamination.

5. Gently mix the contents of the tubes as demonstrated by your instructor.
6. Record the color of the solutions in the tubes.
7. After recording your results, wash the tubes as instructed.
8. Another test you can do for lipids is called the **grease-spot test**. In this test, you place a small amount of solution on a piece of brown paper and allow it to dry. If lipids are present, there will be a translucent sheen on the paper when dried. (Think about the bottom of a pizza box). If lipids are not present, the translucent sheen will not appear. Conduct a grease-spot test for each of the solutions above and record your results in the chart.

Tube Number	Solution	Initial Color	Final Color	Conclusion	Grease Spot
1	Water				
2	Vegetable Oil				
3	Honey				
4	Albumin				
5	Melted Butter				

Experiment 5: Nucleic Acids (DNA Isolation)

1. Collect a strawberry and a plastic sandwich bag.
2. Mash the strawberry to physically break open the cells of the strawberry.
3. Add 5 ml of lysis buffer to the bag.
4. Continue to mash the strawberry until it is completely mashed.
5. Pour the mixture into a funnel containing cheese cloth.
6. Strain all the bits and keep the juice.
7. Using a wax pencil and a ruler, mark a glass tube at the 5 cm and the 8 cm mark.
8. Add the strawberry/buffer juice to the 5 cm mark on the tube.
9. Add ICE COLD ethanol to the 8 cm mark on the tube.
10. Let the DNA precipitate to the top of the tube. Pull the DNA out of the tube using a glass stir rod or plastic stick.
11. Clean your station by throwing away the bag and the cheese cloth and washing the rest of the materials.

Week 2: Identifying Unknown Solutions

Design an Experiment

In this activity, you will be given three unknown solutions: one sugar, one lipid, and one protein. Design an experiment using the information you learned last week to identify which solution is which using the materials you have previously used in experiments.

- Outline of Experimental Procedures

- What are your controls for each part of your experiment?

- Draw a chart to record your results.

- Write down your conclusions for each solution.

- Complete your writing assignment based on this activity. Your professor will provide you with more details on this assignment in lab.

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Why is it important to not confuse your reagents when doing the experiments in this activity?

2. What are the monomers of carbohydrates called?

3. Why are starch and glycogen considered polymers?

4. What is the difference between glucose and sucrose?

5. What reagent tests for starch?

6. What are the monomers of proteins called?

7. What type of bond forms between amino acids?

8. Why was a tube of water included in each of the experiments?

9. What were the positive controls for the sugar, starch, protein, and lipid experiments?

10. What other cells could you use in the DNA isolation experiment?

References

Portions of this lab were adapted and/or modified from:

Burran, S, DesRochers, D. 2015. Principles of Biology I Lab Manual. Biological Sciences Open Textbooks. 3. https://oer.galileo.usg.edu/biology-textbooks/3. CC BY-SA 4.0.

Genovesi E, Blinderman L, Natale P. 2019. Unfolding the Mystery of Life, Biology Lab Manual for Non-Science Majors. Open Textbook Library.
https://open.umn.edu/opentextbooks/textbooks/736 CC BY 4.0.

Microscopy and the Cell

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

Objectives

- Properly carry and maintain a compound light microscope.
- Identify parts of a compound light microscope and their functions.
- Focus a specimen through all objectives of a compound light microscope.
- Define magnification and resolution.
- Compare and contrast prokaryotic and eukaryotic cells under the compound light microscope.
- Differentiate between animal cells and plant cells using the compound light microscope.
- Identify various cellular components and their associated functions.
- Properly prepare and view wet mount slides under the compound light microscope.

Exercise 1 – Use and Care of a Compound Light Microscope

Introduction Part A: The Compound Light Microscope

The microscope is a vital tool used in the biological field; in laboratories, the compound light microscope allows for the visualization of specimens not visible to the naked eye by utilizing a light source to view the specimen and two sets of lenses to enlarge it for observations. In addition to magnifying a specimen, the microscope also provides resolution, which allows the viewer to distinguish between two points.

Parts of the Compound Light Microscope

This list begins at the top of the microscope and moves down the instrument. Follow along with *Figure 1* and label the parts of the microscope being described.

A. Attached to the head of the microscope are the **ocular lenses** (also called the eyepieces), which allow the user to view the specimen. The microscopes used in our laboratories are binocular microscopes because they have two ocular lenses (microscopes with only one are called monocular). Each ocular lens magnifies the image 10X. When observing a specimen, the user should adjust these eyepieces, so they see one complete background (this is also called the **field of view**).
B. The **arm** connects the binocular head to the rest of the microscope. This is one location to place hands when carrying the microscope from one location to another.
C. A revolving **nosepiece** holds the objective lenses. When observing a specimen, the user can rotate this nosepiece to change the total magnification of their view.
D. Attached to the nosepiece are the **objective lenses**. Our microscopes are equipped with four different objective lenses with varying degrees of magnification. Total magnification of a specimen is calculated by multiplying the magnification of the ocular

lens with the magnification of the objective lens being used. The four objective lenses of our compound microscopes are:

i. **Scanning lens**: This lens has a red band around it and magnifies an image 4X. This lens is to be used when centering a specimen in the field of view. Total magnification on this lens is (10X)(4X) = 40X.
ii. **Low power lens**: This lens has a yellow band and magnifies an image 10X. Total magnification is (10X)(10X) = 100X.
iii. **High power lens**: This lens has a blue band and magnifies the specimen 40X. Total magnification is (10X)(40X) = 400X.
iv. **Oil immersion lens**: This lens has a silver or white band and magnifies the specimen 100X. This lens can only be clicked into place once immersion oil has been placed on the slide. Total magnification is (10X)(100X) = 1000X.

E. The **stage** is the location where a slide containing the specimen will be placed for viewing. The stage is equipped with a **stage clip** to ensure the slide stays in place when moving the stage to center the image through the ocular lenses. There are two adjustment knobs attached to the stage called **stage controls**. The top control adjusts the stage forward/backwards while the bottom control moves the stage clip (and slide) left/right. These microscopes are **parcentered**, meaning the specimen will stay mostly in the center between objective lenses (though some slight adjustments may need to be made after increasing the magnification).
F. Underneath the stage is the **condenser**, which functions to collect the light beams being emitted from the light source and concentrate them on the specimen through the lens in the middle of the stage.
G. Attached to the condenser is the **iris diaphragm**. This adjustable lever allows the user to adjust the diameter of light being focused on the specimen at one time.
H. Two adjustments knobs are imbedded in one another and serve to raise or lower the stage to bring the specimen closer to the objective lens. These knobs have a set limit with how far they can adjust (***never force knobs*** to move as this could damage the microscope and/or the specimen). Utilizing these knobs correctly is critical to not only viewing a specimen appropriately, but also being able to view different depths. Adjusting these knobs allow the user to determine the **depth of field**. This refers to how thick a specimen is or the arrangement of specimen structures (for instance, which piece of the specimen is on top or bottom).
    i. The **coarse adjustment knob** is the outermost knob and will move the stage up or down in large increments. This adjustment knob is to only be used when the scanning lens is in place.
    ii. The **fine adjustment knob** is the innermost knob and moves the stage in very small increments. This knob is used to refocus the specimen after the next objective lens has been clicked into place. These microscopes are **parfocal**, meaning they will remain mostly in focus once the next objective lens is clicked into place.
I. The **light source** is found in the base of the instrument and will shine light upward to illuminate the specimen on the stage.
J. Below the light source is the **light adjustment knob**. This is used to control the brightness of the light coming out of the light source. It is recommended to have this at

the lowest setting when using the scanning lens. The brightness will need to be adjusted as the viewer moves up in magnification.

K. The **base** of the microscope is the bottommost section of the instrument. This is where a hand should be placed when carrying the microscope from one location to another.

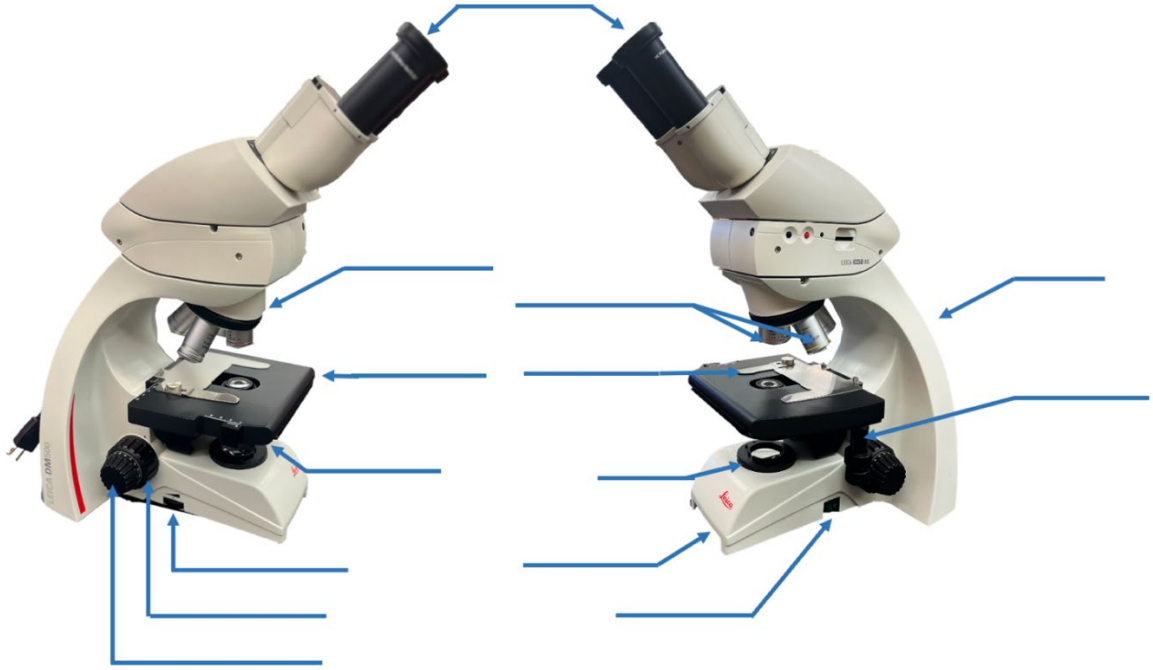


Figure 1. Label the parts of the microscope.
Photo Credit: Deanna Helphrey, CCGA DNS. 2023.

Introduction Part B: The Compound Light Microscope

The compound light microscope is a precision instrument fundamental to all biological laboratories. These instruments are delicate and must be well cared for and properly maintained.

Proper handling of the Compound Light Microscope

Appropriate measures must be taken when transporting the microscope to ensure the user does not drop the microscope, which has the potential to cause harm to both the microscope and the individual carrying it. When holding the microscope, two hands are to be used **at all times**: one hand must be firmly wrapped around the neck of the microscope with the other hand placed under the base for support.

Cleaning and care of the Compound Light Microscope

Our microscopes are for communal use, meaning they are utilized by multiple students daily. To avoid contamination and degradation of microscope quality, the instruments must be properly cleaned after each use. We will now cover how to properly clean and store the microscopes. When the microscope is no longer needed, lower the brightness, and switch the microscope off. Unplug the microscope and wind the power cable appropriately.

An alcohol prep pad should be used on the rubber pieces surrounding the ocular lenses. This removes particles from the eyepieces and ensures they are cleared prior to the next individual using the instrument. The glass portion of the oculars are then wiped with lens paper dampened with a special lens cleaning solution to remove any dust from the lenses.

The objective lenses are also wiped with lens paper dampened with lens cleaning solutions. *It is imperative that only lens paper is used*, rather than a paper towel or a Kim wipe, as these are harsher materials and have the potential to scratch the lenses. When preparing to store the microscope, the scanning objective lens should be clicked into place.

Specimen/slides are to be removed from the stage area and the stage must be free of any liquids or other materials. The stage must be lowered as far as it can go prior to storage (do not force knobs to go further than designed). After confirming the stage has been lowered and the scanning lens is in place, cover the microscope with its dust jacket. Place the microscope in the cabinet or on the designated bench top per instructions from the lab instructor (be sure to handle the microscope appropriately).

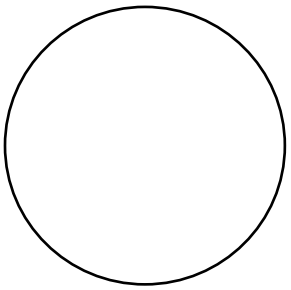
Activity 1 – Viewing a Specimen Using a Compound Microscope

The following materials are needed for this activity:

- A compound light microscope
- A letter *e* slide

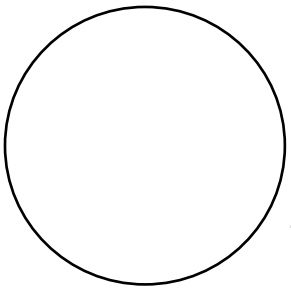
Procedure 1

1. Collect the microscope, remove the dust jacket, and plug it in. Use a clean piece of lens paper to gently wipe the ocular lenses, the objective lenses, the condenser, and light source to remove dust. Ensure the scanning lens (4X) is currently in place and the stage is all the way lowered, then power on the microscope.
2. Collect one prepared slide of the letter *e*. In the circle below, draw how this appears on the slide (NOT on the microscope).



3. Place the microscope slide on the stage, securing it in place with the stage clip. Adjust the position of the slide using the stage controls to ensure the specimen (the letter *e*) is positioned over the condenser lens in the middle of the stage. While looking through the ocular lenses, begin to raise the stage using the coarse adjustment knob, bringing the microscope slide physically closer to the objective lens.

4. Once the specimen has become visible through the ocular lenses, use the fine adjustment knob to bring it into focus.
5. In the circle below, draw how the letter *e* appears through the ocular lenses.



Total Magnification: _____

6. Answer the following questions based on your observations.
    a. Does the letter *e* appear different when observed through the microscope? If so, how?

    b. While looking through the ocular lenses, use the stage controls to move the stage away from you. Which direction did this move the letter *e* in your field of view?

    c. What about when you moved the slide to the right?
7. On the microscope, rotate the nosepiece to click the low power objective (10X) lens into place. Use the fine focus adjustment knob to bring the letter *e* back into focus.
    a. What is the term for this property of the microscope?

8. Use the nosepiece to bring click the high-power objective (40X) lens into place. Use the fine adjustment knob to bring the letter *e* back into focus.
    a. Are you able to bring the entire letter *e* into clear focus on this objective lens, or is the center of the image in focus while the outside edge is unclear?

Exercise 2 – The Cell

Introduction Part C: The Cell

The **cell** is the fundamental unit of life and comes in many shapes, sizes, and arrangements. In this lab, the differences between cellular types and structures will be observed using the compound light microscope, since cells are much too small to be seen with the naked eye. There are two major categories of cells, prokaryotes and eukaryotes.

Organisms belonging to the domains **Archaea** and **Bacteria** are **prokaryotes**. These organisms are single-celled and do not have typical membrane-bound organelles, such as mitochondria or chloroplasts, making them much smaller than their eukaryotic counterparts.

Organisms within the domain Eukarya are eukaryotes (plants, animals, fungi, and protists). There is a lot of variability within the eukaryotes: some are single-celled, and many are multicellular organisms, some have cell walls while others do not.

Cells of all types have the following structures:

1. The **plasma membrane** (also known as the cytoplasmic membrane) is the selectively permeable barrier that keeps everything needed to sustain the life of the cell inside of it, while keeping everything else out. These membranes are comprised of two layers of phospholipids (and are also known as phospholipid bilayers).
2. The plasma membrane keeps the **cytoplasm** inside the cell. Cytoplasm is the fluid inside of the cell that allows intracellular components to free float around the cell.
3. **Ribosomes** can be found in the cytoplasm of all cells. These structures are comprised of RNA (ribonucleic acid) and function to synthesize proteins necessary for cell survival.
4. All cells have genetic material in the form of **DNA (deoxyribonucleic acid)**. In prokaryotic cells, DNA is found in a space called the **nucleoid region** inside of the cytoplasm. In eukaryotic cells, DNA is housed in a special structure called the **nucleus**.
5. Cells of most prokaryotic species contain a **cell wall**, while certain groups of eukaryotic species possess one (such as plants, fungi, and some protists). This feature is found outside of the plasma membrane and provides the cell with structural support and protection from external forces. The cell wall of bacteria is comprised of a structural polysaccharide called **peptidoglycan**, while plant cell walls have **cellulose**. Fungal cell walls are made of **chitin**, which can also be found in crustaceans.

Other cellular structures:

6. Prokaryotic cells, and some eukaryotic cells, can have external features, such as **flagella** (or a singular flagellum) for motility. These are strands of protein extending outward from the cell and anchored to the cell wall. Prokaryotic flagella spin like a propeller in either clockwise or counterclockwise directions to propel the cell around its environment while eukaryotic flagella tend to move in a wavelike fashion.
7. **Fimbriae** are short, needle-like projection extending out of the cell wall that prokaryotic cells use for attachment to surfaces and other cells.
8. Eukaryotic cells can also have external structures for motility, and the structure type depends on the species.
    a. An *amoeba* uses **pseudopodia** for movement and grabbing nutrients from the environment. Pseudopodia are extensions of the cytoplasmic membrane that allow the cell to pull themselves in the direction of a stimulus.
    b. **Cilia** is used by various species, such as *Paramecium*, to move around their environment. These are small projections from the cytoplasmic membrane. They are found all over the cell and allow the organism to 'swim'.
9. Internally, eukaryotic cells have a variety of **membrane-bound organelles** that serve specific functions. The organelles work together, like a factory, to supply the cell with some function/product to promote survival.
    a. The **endoplasmic reticulum** can be found just outside of the nucleus and is separated into two sections with unique functions. The **rough endoplasmic reticulum** (**RER**) gets its name from its textured appearance because of numerous ribosomes being embedded in it. The RER serves as a site of protein synthesis. The **smooth endoplasmic reticulum** (**SER**) does not have ribosomes, so it is not a site of protein synthesis. However, the breakdown and

creation of necessary lipids (cholesterol, phospholipids) happens in this organelle.

b. Located near the RER is the **Golgi body**. Incoming proteins from the RER are modified, tagged, and packaged into vesicles in the Golgi. From the Golgi, these proteins are sent to their final destination either inside or outside of the cell.
c. Some eukaryotes (plants and protists) contain **chloroplasts**, which are special organelles (called **plastids**) that capture light energy and convert it into sugars. Another example of a plastid is an **amyloplast**, which functions to store starch.
d. The sugars created by the chloroplasts can then be used by the **mitochondria** to create the necessary energy molecules (ATP) for the cell.
e. **Lysosomes** are small vesicles in animal cells that are filled with digestive enzymes. These vesicles function to recycle old cellular components, or they can aid in defense of the cell by breaking down foreign material/organisms.
f. Plant cells have a **large central vacuole** that stores materials such as water, waste, and pigments. Vacuoles help the plant cell keep proper osmotic pressure inside the cell.

Activity 2 – Viewing Cells on the Compound Light Microscope

In this activity, you will utilize the compound light microscope to view bacteria, plant cells, animal cells, fungi, and protists. These cells will be viewed using a combination of prepared ('fixed') slides and living specimen.

Materials needed for this activity:

- Compound light microscope
- Prepared slides of bacteria
- 2 glass slides
- 2 glass coverslips
- Water
- *Elodea* leaf
- 1 toothpick
- Methylene blue stain
- Prepared slides of *Amoeba*
- Prepared slide of *Paramecium*

Observe prepared slides of Bacteria

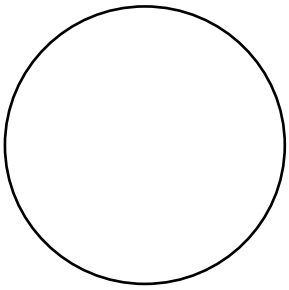
As mentioned previously, bacteria are prokaryotic cells, which are much smaller than eukaryotic; viewing them under the microscope can be more of a challenge. These bacterial cells will need to be viewed using the high-power objective lens. With these samples, you can compare the cell walls and shapes of the bacteria. Your instructor may also ask you to try viewing the bacteria using the oil immersion and oil immersion objective lens instead.

Procedure 2

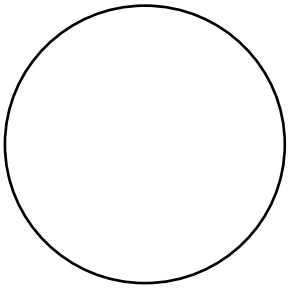
1. If not already set up from the previous activity, collect a compound light microscope and place it on the bench. Set it up according to Activity 1 above.
2. Place the first prepared bacteria slide on the microscope's stage using the stage clip to hold it in place. Follow the steps in Activity 1 to bring the specimen into focus on the scanning objective lens.
3. Once you have located a cell, bring it into focus using the fine focus adjustment. Click the low power lens into place and refocus. *You will only use the fine focus adjustment to refocus the microscope.* Click the high-power lens into place and refocus.

   Bacterial cells have three main morphologies:
   i. Coccus (spherical shaped)
   ii. Bacillus (rod-shaped)
   iii. Spirillum (spirals)

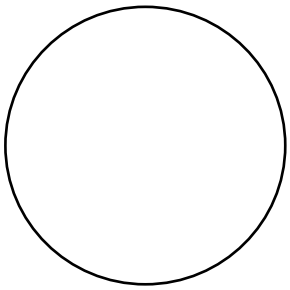
4. For the bacteria on your slides, sketch the cells at high power in the circles provided below.



Shape: _____
Total Mag: _____



Shape: _____
Total Mag: _____



Shape: _____
Total Mag: _____

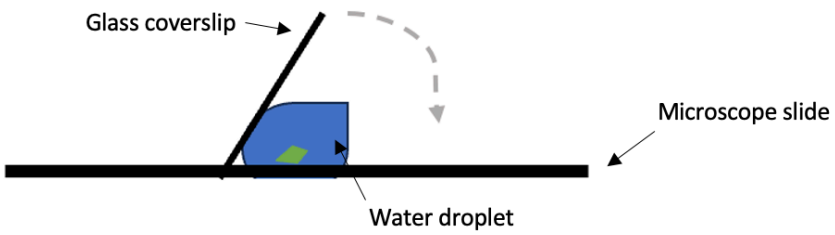
Steps to prepare a wet mount slide

Wet mounts are one of the most common preparations of specimens to be viewed under the microscope. In this activity alone, you will be performing three of them. The following steps are to be taken when performing a wet mount preparation (see Fig. 2 for reference).

1. Obtain a clean glass slide and coverslip (**note**: the coverslips are very thin and made of glass. They can break easily and cut through gloves and skin. Handle these with extreme caution).
2. If you are viewing a specimen that is not already suspended in a liquid, place this specimen on the slide first.
3. Use a pipet to gather liquid (either water or stain) and place a **single** drop onto the slide (if you placed a specimen down first, the drop goes onto it).
4. Hold the coverslip by the edges and place one edge on slide, so you are holding the coverslip at an angle. Slowly bring the edge of the coverslip into contact with the liquid

until you can visibly see the water coating that part of the coverslip. Carefully lower the coverslip down over the sample.

5. If prepared correctly, minimal air bubbles should be present under the coverslip. If any large bubbles are visible, carefully push on the coverslip with an eraser to guide them out of the coverslip.
6. The single drop of liquid placed on the slide is sufficient to keep the coverslip in place. If there is too much liquid, the coverslip will move around the slide. If too much liquid was used, gently touch the corner of a paper towel to the side of the coverslip to draw up some of the excess liquid.
7. View prepared specimen under the microscope (ensure the bottom of the slide and the top of the coverslip are dry prior to placing it on the stage).



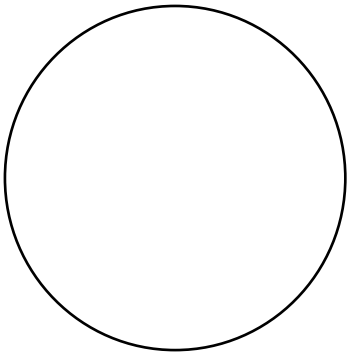
Prepare a wet mount of Elodea

Plant cells are similar to bacterial cells in that they have a cell wall. In this procedure, you will be creating a wet mount of an *Elodea* leaf. These are eukaryotic cells and are large enough to see structures (such as the cell wall and membrane) on the low power lens, but the high-power lens will provide more detail. The microscope will also allow you to view the inside of these cells, where you could potentially see organelles, such as numerous amounts of **chloroplasts** or a **large central vacuole**.

Procedure 3

1. Prepare a wet mount of an *Elodea* leaf by carefully cutting one leaf from the stem. Place this leaf flat on a clean microscope slide. If the *Elodea* plant is placed in a water bowl, use a pipet to place a single drop of water on top of the *Elodea* leaf. Place a coverslip on the slide as outlined in the above section.
2. Ensuring the top and bottom of the slide is dry, place the wet mount on the microscope stage and bring the specimen into focus, starting with the scanning lens. Observe how your specimen looks at this magnification. Note the shapes of the cells and the cell walls.
3. Proceed to the low power lens and bring the specimen into focus. Observe how the view of the cell changes. Rotate the fine focus lens to begin seeing the internal structures of the cell.

4. If you are able, view the slide at high power to see more of these internal structures.
    a. In the circle below, draw the view of the Elodea specimen you can see on the highest power you can focus.
    b. Label the following cell components: nucleus, cell wall, cell membrane, cytoplasm, chloroplast, and any other structure you can see.



Plant Cell

Total Magnification: _____

5. Once you are finished with your observations, dispose of the *Elodea* leaf and place the coverslip and slide in the appropriate containers as outlined by your instructor.

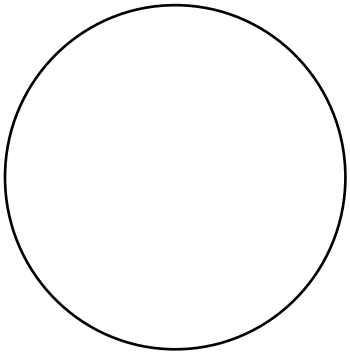
Prepare a wet mount of animal cells

Your cheeks contain multiple layers of cells that are removed constantly through eating and drinking. These cells are tightly packed and layered to provide protection and support to the underlying tissues, while also being replenished frequently. These cells are animal cells; for this activity, you will be collecting cells from your cheeks, staining them to provide contrast, and observing them under the microscope.

Procedure 4

1. Obtain a clean slide, a coverslip, and a toothpick.
2. Place one drop of methylene blue stain on the microscope slide.
3. Use the toothpick to gently rub the inside of your cheek to collect cells (note: this is not a scraping; you can roll the toothpick along the inside of your cheek instead).
4. Smear the end of the toothpick (with the collected cells) through the methylene blue stain. Discard the toothpick into the Sharps container.
5. Apply the coverslip to the slide. Place the slide on the microscope's stage and bring into focus using the scanning objective lens.
6. Once the cells have been located, click the low power lens into place and refocus using the fine focus adjustment knob. These may still look like small, dark-blue blobs. Click the high-power lens into place and refocus using the fine focus adjustment knob.
    a. In the circle provided below, draw what you see under the highest power you can focus.

b. Label the following structures: nucleus, cytoplasm, cell membrane, and any other organelles you can see in your view.



Animal Cell
Total Magnification: _____

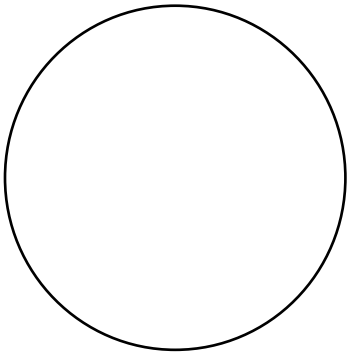
7. When your observations are complete, rinse the coverslip and microscope slide in the sink and place them in the appropriate containers provided by the instructor.

Observe protists under the compound light microscope

Protists are single-celled eukaryotic organisms that don't conform to one structure type. You will be observing prepared slides of two common protists that can be found worldwide: *Amoeba* and *Paramecium*. Protists are classified based on their methods of locomotion; *Amoeba* use pseudopodia ('fake feet') and *Paramecium* have cilia. *Amoeba* extend their membranes to pull themselves around their environment and to capture food particles. *Paramecium* beat their cilia to swim to and away from stimuli.

Procedure 5

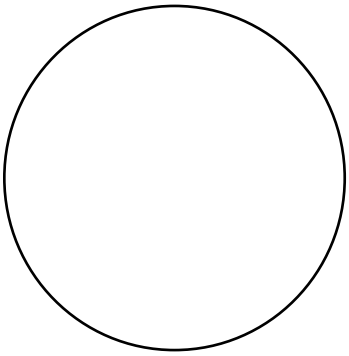
1. Obtain a prepared slide of *Amoeba* and place it on the stage of the microscope. Bring this specimen into focus using the scanning lens.
2. Once you have located a cell, click the low power lens into place and refocus, followed by the high-power lens. Remember to refocus using only the fine focus adjustment knob.
    a. In the circle provided, draw what you can see on the highest power you are able to focus.
    b. Label the following structures: Nucleus, cytoplasm, plasma membrane, pseudopodia, vacuoles, and any other organelles you can see.



Amoeba

Total Magnification: _____

3. Lower the stage and reset the microscope. Remove the prepared *Amoeba* slide and replace it with a prepared *Paramecium* slide.
4. Repeat step 2 for the *Paramecium* slide.
    a. In the circle provided, draw what you can see on the highest power you are able to focus.
    b. Label the following structures: nucleus, cytoplasm, plasma membrane, cilia, and any other organelles you can see.



Paramecium

Total magnification: _____

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. In the chart provided, describe the function of each microscope part.

Oculars	
Condenser	
Iris Diaphragm	
Coarse Adjustment Knob	
Fine Adjustment Knob	

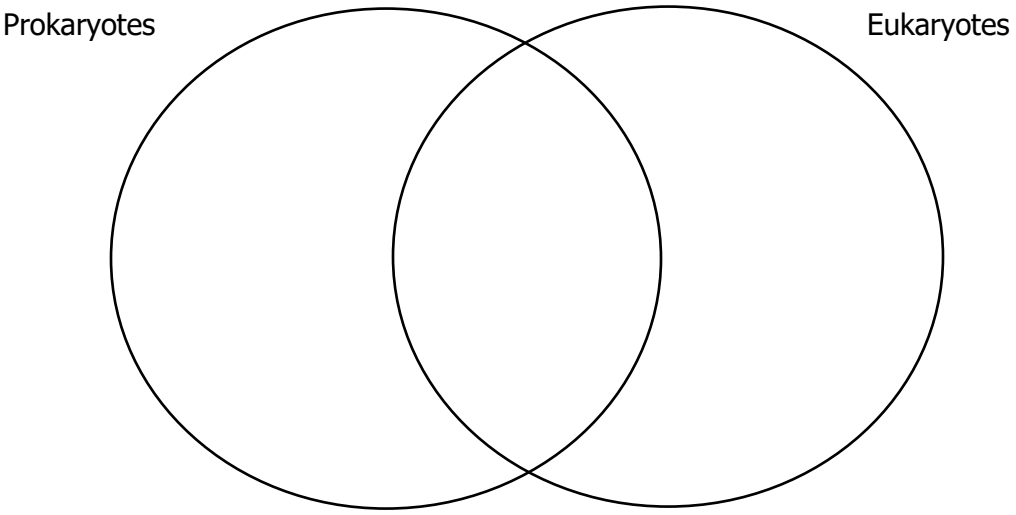
2. In the chart provided, determine the total magnification for viewing a specimen through each objective lens.

Ocular lens magnification	Objective lens magnification	Total magnification

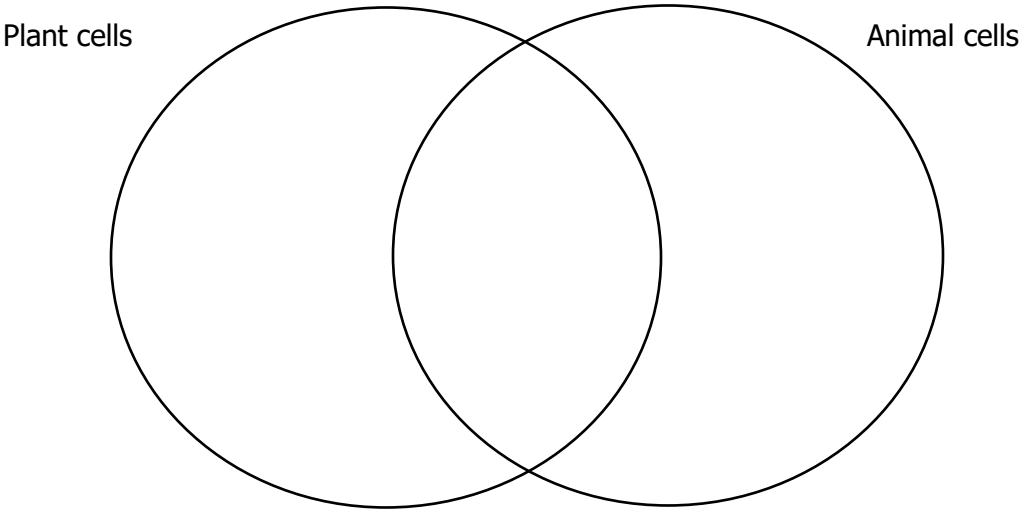
3. On which objective lens(es) is the coarse adjustment knob to be used?

4. What is the purpose of using a stain on the cheek cells prior to viewing them under the microscope?

5. In the Venn diagram provided, compare and contrast cellular structures found in prokaryotic cells and eukaryotic cells.



6. In the Venn diagram provided, compare and contrast cellular structures found in plant cells and animal cells.



7. In your own words, describe how to prepare a wet mount slide.

8. What organelles could you observe in your *Elodea* specimen? Why were there so many chloroplasts?

9. Compare and contrast the structural differences between animal and plant cells.

10. Does *Amoeba* have a cell wall? How can you tell?

References

Portions of this lab exercise were adopted and/or modified from:

Mukhopapdhyay, S., Davis, C., and Wilson, C. 2020. Fundamentals of Biology Lab Manual. Open ALG Ancillary Materials. 3. https://alg.manifoldapp.org/projects/fundamentals-of-biology-lab-manual. CC BY 4.0.

Enzymes

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

Objectives

- Identify different sources of an enzyme
- Distinguish between the enzyme, the substrate, and the product of a reaction
- Understand the effects of temperature, pH, and concentration on a reaction

Introduction

Enzymes are proteins that function as catalysts to speed up chemical reactions. They are essential to cellular functions such as biosynthesis and energy production. Each enzyme has a specific three-dimensional shape with an **active site** that binds to a substrate. The substrate is specific to the enzyme like a key is specific to a lock. During a reaction, the substrate and enzyme bind together in an enzyme-substrate complex. The enzyme lowers the **activation energy,** or amount of energy needed for the reaction to occur and can break bonds within the substrate to form the product or products of the reaction. The enzyme is not used up during the reaction and can be recycled multiple times as long as it maintains its shape or conformation. High heat or chemicals can cause an enzyme to **denature or** lose its three-dimensional shape which causes it to become nonfunctional. A generic enzymatic reaction can be written like this:

$$E + S \rightarrow ES \rightarrow P + E$$

In this activity, you will be using the enzyme catalase for your experiments. Catalase enzymes are found in a number of plant and animal cells and function as a form of protection against oxide radicals for the cell. In your experiment, you will use hydrogen peroxide (H_2O_2) as the substrate for the catalase. The products that will be formed will be water (H_2O) and oxygen (O_2) which will be observed as bubbles. To plug this information into the formula, it would look like this:

$$\text{Catalase} + \text{Hydrogen Peroxide} \rightarrow \text{Water} + \text{Oxygen} + \text{Catalase}$$

In the first experiment, you will be using plant, animal, and yeast cells as sources of the enzyme catalase to find the best source. In the second experiment, you will test the effects of temperature on the ability of the catalase to break down the hydrogen peroxide. In the third experiment, you will test the effects of pH on the ability of the catalase to break down the hydrogen peroxide. Finally, in the last experiment, you will test the effects of concentration on the function of the catalase. Before you begin, make predictions for the results of each of the experiments.

Experiment 1: Sources of Catalase

1. Collect and label 5 test tubes with the numbers 1 – 5.
2. Using a wax pencil and a ruler, mark each tube at the 1 cm mark.
3. Using separate plastic pipettes, add the following solutions to the 1cm mark in each tube
    a. Tube 1 – water
    b. Tube 2 – apple solution
    c. Tube 3 – potato solution
    d. Tube 4 – yeast solution
    e. Tube 5 – liver solution
4. Using a new plastic pipette or the dropper bottle, add 10 drops of hydrogen peroxide in each tube. Avoid touching the solution in the tube with the pipette to prevent cross contamination.
5. Determine the amount of bubbles that form in each tube using (0) as no bubbling, (+) as minor bubbling, and (++) as major bubbling and record the results in the chart below.
6. After measuring the bubbles, wash the tubes as instructed.

Tube Number	Source of Enzyme	Amount of Bubbles (0, +, ++)
1	water	
2		
3		
4		
5		

- Which source provided the most catalase activity?

- Was your prediction correct? Explain your answer.

Experiment 2: Effects of Temperature on Catalase Activity

1. Collect and label 4 test tubes with the numbers 1-4.
2. Using a wax pencil and a ruler, mark each tube at the 1 cm mark.
3. Using a plastic pipette, add your source of catalase solution to the 1 cm mark in each of the 4 tubes.
4. Place the tubes at the following temperatures for 5 minutes:
    a. Test tube 1 – boiling water bath
    b. Test tube 2 - 37ºC incubator or bead bath
    c. Test tube 3 – room temperature
    d. Test tube 4 – ice water bath

5. After incubating the tubes at the appropriate temperatures, use a new plastic pipette or the dropper bottle to add 10 drops of cold Hydrogen peroxide to each tube. Avoid touching the solution in the tube with the pipette to prevent cross contamination.
6. Determine the amount of bubbles that form in each tube using (0) as no bubbling, (+) as minor bubbling, and (++) as major bubbling, and record the results in the chart below.
7. After measuring the bubbles, wash the tubes as instructed.

Tube Number	Temperature	Amount of Bubbles (0, +, ++)
1		
2		
3		
4		

- What was your hypothesis for this experiment? Was it supported or rejected?

- What is the dependent variable in this activity?

- What is the independent variable in this activity?

- Which temperature is the best for the function of the catalase?

- Did you get bubbles in the tube that was boiled? Why or why not?

Experiment 3: Effects of pH on Catalase Activity

1. Collect and label 3 test tubes with the numbers 1-3.
2. Using a wax pencil and a ruler, mark each tube at the 1 cm and 2 cm marks.
3. Using a plastic pipette, add your source of catalase solution to the 1 cm mark in each of the 3 tubes.
4. Using separate plastic pipettes or dropper bottles, add the following solutions to the 2 cm mark and then incubate at room temperature for 5 minutes:
    a. Test tube 1 – 1 M hydrochloric acid as demonstrated by the instructor
    b. Test tube 2 – distilled water
    c. Test tube 3 – 1 M NaOH as demonstrated by the instructor

5. After incubating the tubes with the appropriate solution, use a new plastic pipette or dropper bottle to add 10 drops of cold Hydrogen peroxide to each tube. Avoid touching the solution in the tube with the pipette to prevent cross contamination.
6. Determine the amount of bubbles that form in each tube using (0) as no bubbling, (+) as minor bubbling, and (++) as major bubbling, and record the results in the chart below.
7. After measuring the bubbles, wash the tubes as instructed.

Tube Number	pH	Amount of Bubbles (0, +, ++)
1		
2		
3		

- What was your hypothesis for this experiment? Was it supported or rejected?

- What is the dependent variable in this activity?

- What is the independent variable in this activity?

- Which pH is the best for the function of the catalase?

Experiment 4: Effects of Concentration on Catalase Activity

1. Collect and label 3 test tubes with the numbers 1-3.
2. Using a wax pencil and a ruler, mark each tube as follows:
    a. Test tube 1 at the 0.5 cm mark
    b. Test tube 2 at the 1 cm mark
    c. Test tube 3 at the 2 cm mark
3. Using a plastic pipette, add your source of catalase solution to the first mark in each of the 3 tubes.
4. Using a new plastic pipette or the dropper bottle, add 10 drops of Hydrogen peroxide to each tube. Avoid touching the solution in the tube with the pipette to prevent cross contamination.
5. Determine the amount of bubbles that form in each tube using (0) as no bubbling, (+) as minor bubbling, and (++) as major bubbling, and record the results in the chart below.
6. After measuring the bubbles, wash the tubes as instructed.

Tube Number	Contents of Tube	Amount of Bubbles (0, +, ++)
1		
2		
3		

- How does concentration of the enzyme and the substrate compare in each tube?

- What conclusion can you make about the importance of concentration in the enzymatic reaction with catalase and hydrogen peroxide?

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Write the equation for the catalase chemical reaction that was used in this activity.

2. Why didn't the sources used in the first experiment have the same amount of catalase activity?

3. Name one additional source of catalase that could have been used in this study.

4. Using your results from the temperature activity, draw a bar graph to represent your results. Be sure to label your axes.

5. Another molecule, amylase, is produced by humans in the oral cavity via saliva and in the small intestine. Amylase breaks starch into simple sugars. Identify the enzyme and its substrate.

6. Design an experiment to test the optimal temperature for amylase activity. Be sure to identify your controls.

7. What does it mean for an enzyme to denature? Did you experience denaturation of your enzyme in any of the experiments for this lab?

8. The enzyme, pepsin, works to break down proteins in the stomach. What pH would you expect to give the best results?

9. Design an experiment to test the optimal pH of pepsin. Be clear on what your control group and standardized variables would be.

10. Identify one source of error that may have occurred during your experiment, and explain why it could be considered a source of error.

References

Portions of this lab were adapted and/or modified from:

Burran, S, DesRochers, D. 2015. Principles of Biology I Lab Manual. Biological Sciences Open Textbooks. 3. https://oer.galileo.usg.edu/biology-textbooks/3. CC BY-SA 4.0.

Genovesi E, Blinderman L, Natale P. 2019. Unfolding the Mystery of Life, Biology Lab Manual for Non-Science Majors. Open Textbook Library.
https://open.umn.edu/opentextbooks/textbooks/736 CC BY 4.0.

Photosynthesis and Cellular Respiration

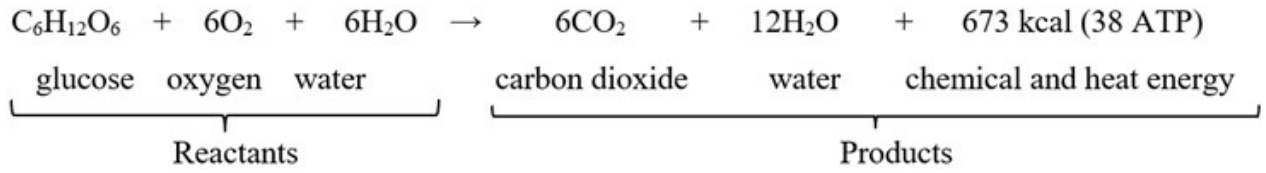
Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

Objectives

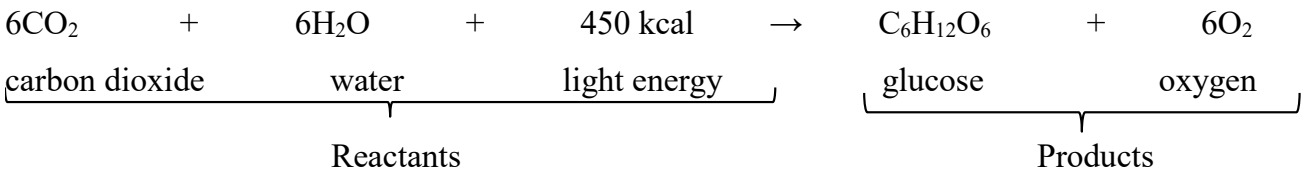
- Understand the reactants and products for the chemical reactions for photosynthesis and cellular respiration.
- Be able to use paper chromatography and calculate R_f values to isolate and identify plant pigments.
- Be able to use a spectrophotometer to identify which light wavelengths are involved in photosynthesis and how it can vary depending on plant pigments.
- Observe the effects of exercise on cellular respiration and identifying the role carbon dioxide production, breathing rate, and heart rate in determining the rate of cellular respiration.
- Determine which factors may contribute to altered rates of photosynthesis and respiration.

Introduction

In order for organisms to function, survive, and reproduce, they must be able to utilize energy. This energy, as we learned previously, cannot be created; it can only be transformed through the process of metabolism. As many organisms consume food, the food is metabolized through a process of **cellular respiration** that also requires the uptake of oxygen molecules and the release of carbon dioxide molecules. The chemical reaction for cellular respiration is provided below:



However, the process does not stop there. Organisms known as autotrophs are able to produce carbohydrates from carbon dioxide in a process called **photosynthesis**. This process requires energy in the form of light energy and can be defined as a type of an anabolic reaction. Not only that, but one of the products of photosynthesis is oxygen: something we, as well as, plants need in order to undergo cellular respiration. The chemical reaction for photosynthesis is provided below:



Photosynthesis occurs in cellular organelles called chloroplasts, while cellular respiration occurs in mitochondria. Both organelles can be found in plant and algae cells. For plant photosynthesis, light energy is required. Visible light is composed of many different wavelengths (Figure 1), but not all is used for plant photosynthesis. Plants have several types of pigments to absorb different wavelengths to use for photosynthesis, attracting pollinators, etc. White light contains all of the wavelengths of the visible spectrum from violet (approximately 400 nm) to red (approximately 700 nm) and which are absorbed and which are transmitted depend on the type of pigment.

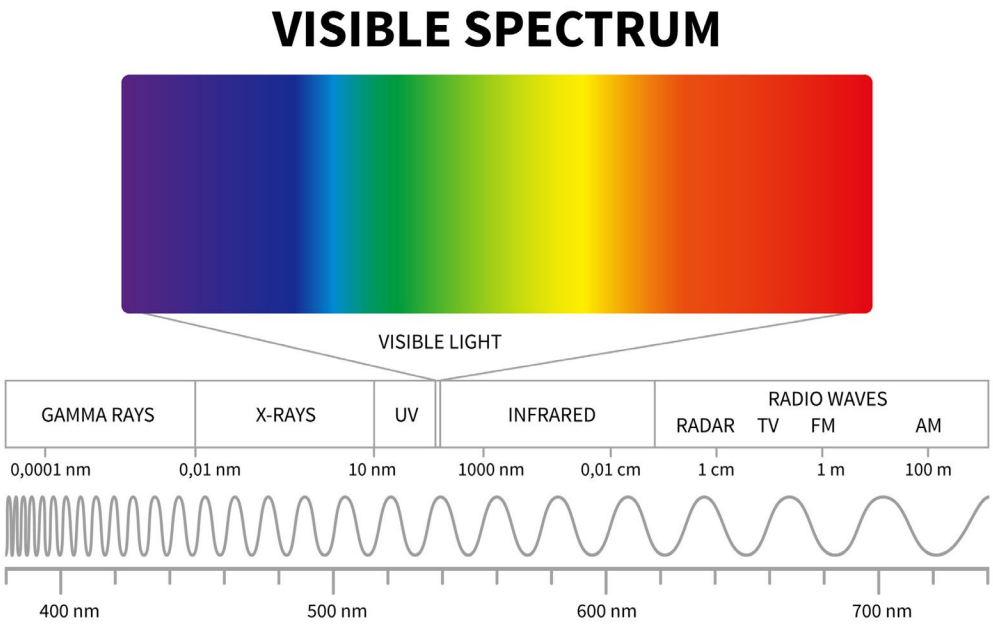


Figure 1. Visible light spectrum showing light wave frequency in the electromagnetic spectrum.

The main light-harvesting pigments for photosynthesis are chlorophylls, carotenoids, xanthophyll, and anthocyanins. Each pigment is best suited to absorb particular wavelengths of light and as a result produces the color of the reflected wavelength. Chlorophylls (a and b) absorb the most light for photosynthesis, while carotenoids, xanthophylls, and anthocyanins also play a role in protecting chlorophylls against photooxidation (Figure 2).

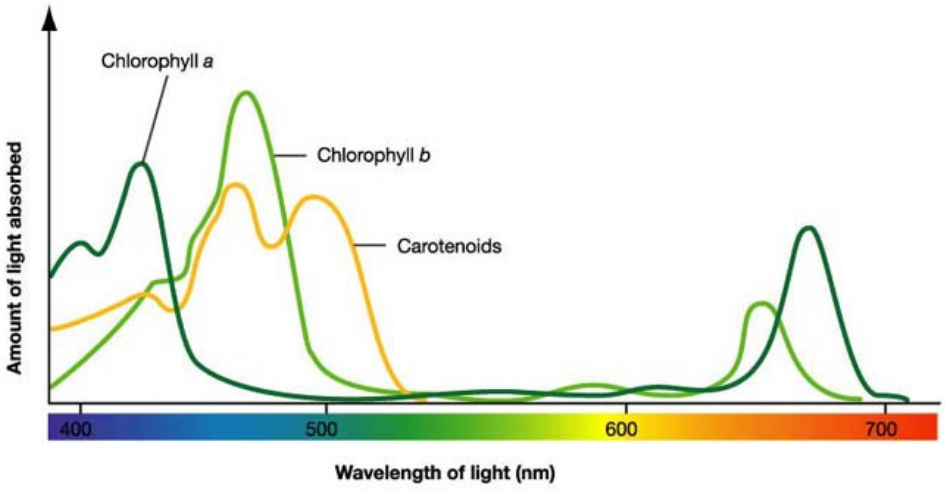


Figure 2. Light absorption spectrum for chlorophyll and carotenoid pigments.

Plants also undergo cellular respiration; however, it is easier to measure at night when photosynthesis is not occurring. Plant cells require ATP which they obtain from the breakdown of glucose created from photosynthesis. Oxygen is still required for cellular respiration to occur, and the plant releases carbon dioxide.

Methods

In the first experiment, you will be using paper chromatography to determine what pigments are found in a prepared spinach extract and two other plant extract which you will prepare. **Paper chromatography** is a process that uses filter paper and a solvent to separate pigments in a solution. Because each pigment molecule varies in its chemical qualities, different pigments will travel up the chromatography paper at varying rates. Students are welcome to bring their own leaf samples, but they need to make sure that their leaves have been stored in a quart plastic bag in a refrigerator for no more than 24 hours.

In the second experiment, you will use a **spectrophotometer** to determine which wavelengths of light are absorbed and transmitted when a beam of white light is passed through a solution in your three samples. **Transmission** is the fraction of light at a given wavelength that passes through the solution. **Absorbance** is the negative of the log of the transmittance. Since absorbance is a linear function with respect to the concentration of the solution, scientists usually work with absorbance rather than transmittance.

In the third experiment, you will see how oxygen and carbon dioxide levels vary in plant leaves exposed to light (photosynthesis) verses dark (cellular respiration). You will use Lab Quest O_2 gas sensors and CO_2 gas sensors to measure oxygen and carbon dioxide production. You may use the leaves you brought for the previous experiment as long the leaves have been properly stored. *It is recommended that you set up this experiment at the beginning of lab to allow time for the leaves to acclimate in the respiration chamber*.

For each experiment, remember that everyone in the group is required to wear googles and gloves at all times when handling any chemicals or chromatography paper.

Experiment 1: Plant Pigment Chromatography

First you will need to prepare an extraction for your leaf sample. The spinach leaf extract is already prepared. To prepare your extract, you need to do the following:

1. Tear your leaf into small pieces (avoid the main vein) and place the tissue in a mortar for grinding.
2. Add 10 mL of acetone to the sample and grind with the pestle until only tiny specks of leaf tissue remain. You may need to add an additional 5 mL of acetone to the mortar depending on the thickness of your leaf.
3. Filter this liquid into a labeled beaker through a funnel lined with filter paper.
4. Dispose of the filter in the designated trash bin and cover your sample beaker with Parafilm or a rubber cork to avoid acetone evaporation.
5. Thoroughly clean your mortar before starting the next procedure.

Once you've obtained your extracts, you will need to prepare a sheet of chromatography paper for your leaf extract and a provided concentrated spinach extract.

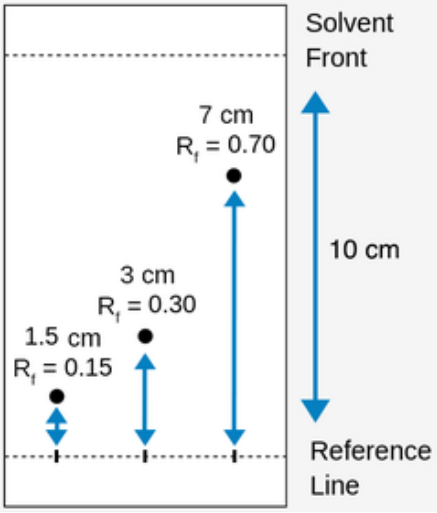
1. Obtain a piece of chromatography filter paper, making sure to wear gloves as you handle it so that oils from your fingers do not interfere with your experiment. Cut the filter paper to 15 cm by 3 cm. You will need two strips. Using a pencil, on the top of the sheet indicate which sample you will be applying to the paper.
2. Wear your goggles and use the paintbrush to apply one of the plant extracts to the filter paper approximately 3 cm from the bottom edge. You want to be sure that when you place the paper in the solvent, the solvent doesn't directly touch the extract.
3. Paint a line of extract on the filter paper at least 15 times. Try to paint the line as straight as you can. Repeat this with the other strip and concentrated spinach extract with a clean paintbrush.
4. Place each filter paper in a jar of solvent (ether-acetone) with the pigment side down. The ether-acetone will move through the paper via capillary action and will carry the pigments with it to the opposite end.

SAFETY NOTE: You will want to limit breathing the vapors of ether-acetone so wear a mask. Also, make sure to open the jar only when you are ready to place the strips and close the jar quickly.

5. Close the jar and leave it sitting on the side desk while the solvent moves up the filter paper. Stop the experiment when the solvent is about 3-5 cm from the top edge of the filter paper.
6. With your gloves and googles on, remove the paper carefully from the jar and quickly recap the jar of solvent. Place the filter paper on a paper towel, mark the top edge of the solvent front with a pencil, and let it dry. Draw each strip using colored pencils in the space provided in the results section.
7. Once dry, use a ruler to determine the distance traveled by each of the pigments. Using the formula below, calculate the R_f values (retention factor) for each of the pigments from the spinach extract. Do this again with your sample extract. This number will allow you to determine the solubility of your pigment in the solvent being used.

You calculate the R_f value using the following equation:

$$R_f = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent front}}$$



Pigments that travel short distances along the chromatography are less soluble than pigments that travels a further distance along the paper. The closer your R_f value is to 1, the more soluble your pigment is in that particular solvent.

Using the colored pencils, sketch the filter paper for each extract after the experiment in the space below and label the solvent line and pigments. Make sure to label each pigment based on your calculated R_f values.

Type of Extract:

Here are the theoretical R_f values for the most common plant pigments:

R_f for carotene =1
R_f for xanthophyll =0.615
R_f for chlorophyll *a* =0.340
R_f for chlorophyll *b* =0.231

In the table below, provide your calculated Rf value and identify each pigment using the theoretical R_f values. Do this for each extract.

Concentrated Spinach Extract			
R_f Value	**Pigment**	**R_f Value**	**Pigment**

Consider the following questions as you interpret your results:

- What is paper chromatography? How does it work?

- How do you calculate Rf value? What does it tell you about solubility?

- Did each of your extracts have the same types of pigments? Why or why not?

- What could be some potential sources of error in your experiment? Why?

Experiment 2: Plant Pigment Light Absorption and Transmittance

For this portion of the experiment, you will need the spinach extract and the extracts from your two leaf samples. You will also need 3 glass cuvettes: one filled with acetone, one filled with the diluted spinach extract, and the third with your leaf extract. Make sure you wear gloves and googles as you carefully handle these cuvettes.

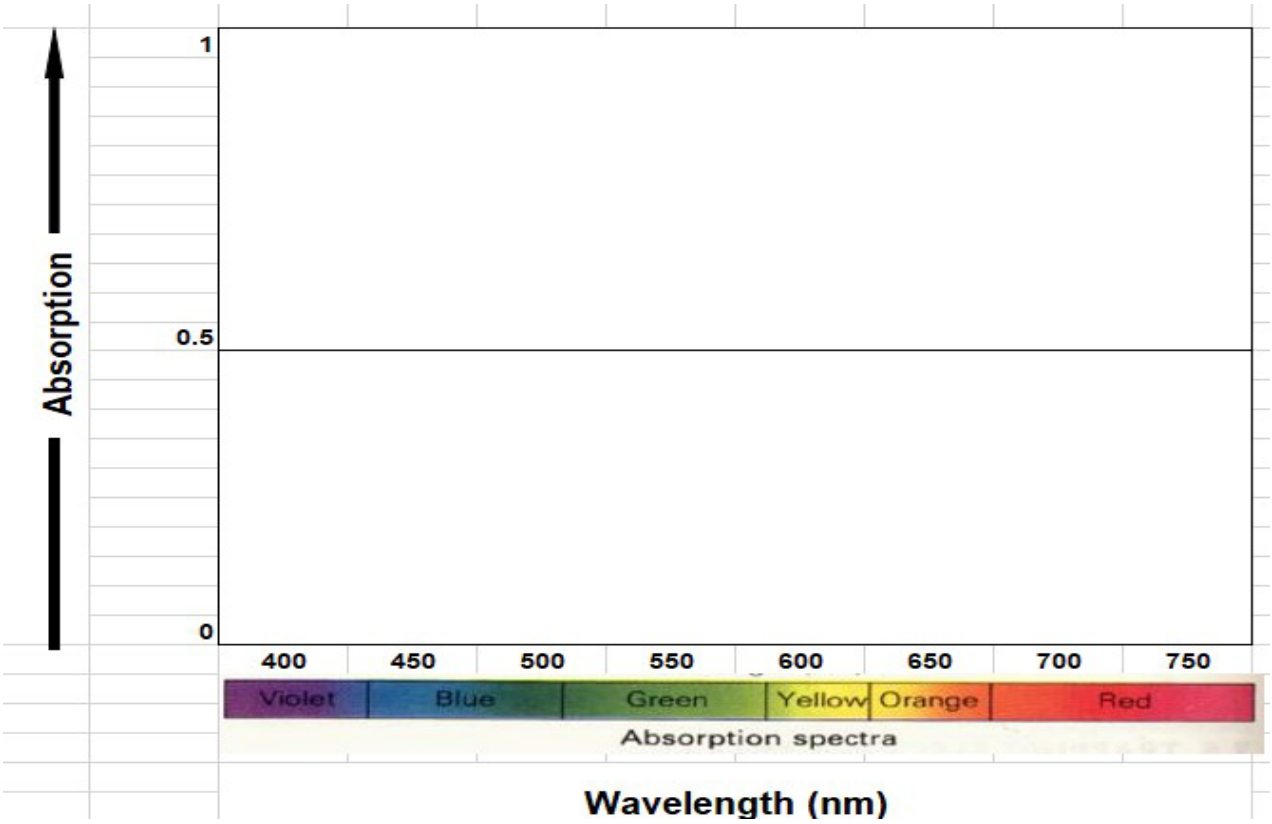
Follow these instructions to set up your LabQuest and SpectroVis Plus:

1. Turn on LabQuest and attach the SpectroVis Plus to it.
2. On the meter screen, use the stylus to select 'Sensors' and then select 'Calibrate USB:Spectrometer'. Wait for the lamp to warm up.
3. Use a cuvette that is three quarters of the way full with acetone (this is called the blank). The extracts were diluted with acetone so to figure out what the absorbance of the extract alone will be, you must subtract the acetone. You will do this by using acetone as your blank.
4. Wipe the clear sides of the cuvette with a Kimwipe. Place the blank cuvette in the SpectroVis Plus slot with the clear sides aligned with the white arrow and white lamp icon.
5. On the LabQuest, when warmup is complete, select 'Finish calibration'. Once calibration is completed, select 'OK'.
6. Remove the acetone blank cuvette and empty the acetone in the container provided by the laboratory coordinator.
7. Fill a cuvette three quarters of the way full with the dilute spinach extract.
8. Wipe the sides of the cuvette and place it in cuvette slot on the SpectroVis Plus slot.
9. Select the green arrow play button. If the following message appears: 'This collection will overwrite the latest run. Do you wish to store or discard the latest run?', then select 'Discard'.
10. Wait approximately 30 seconds, and select the red square stop button.
11. In the table provided in the results section, record the absorbance at each wavelength between 400nm and 700 nm in 50 nm increments. The LabQuest display includes a visible spectrum over the absorbance spectrum. The wavelength of the maximum absorbance is displayed in the lower right corner of the screen. If you want to see a different wavelength, touch the stylus to the screen where you want to know the wavelength and that wavelength will be displayed.
12. When finished discard the spinach extract as instructed by your professor and carefully rinse the cuvette well with distilled water. Place used cuvette on designated tray.
13. Repeat the procedures 7-12 for your leaf extract. If you are having difficulties getting a reading, you may need to add another 5 mL of acetone to your extract. Complete the results portion for this experiment.

Record the absorbance maximum wavelength (nm) data from the SpectroVis for each extract in the table below.

Wavelength (nm)	Diluted Spinach Extract	
400 nm		
450 nm		
500 nm		
550 nm		
600 nm		
650 nm		
700 nm		

Using colored pencils, sketch the absorption spectrum for each extract in the space below. Be sure to include a legend.



Consider the following questions as you interpret your results:

- Why was a blank used before the extract measurements were taken?

- What is an absorption spectrum graph? What does it tell you about a sample?

- Did each of your extracts produce the same absorption spectrum graph? Why or why not?

- Why did your samples show two peaks on the absorption spectrum graph? What can you conclude about the best wavelengths for photosynthesis for each extract?

- What could be some potential sources of error in your experiment? Why?

Experiment 3: Photosynthesis and Cellular Respiration

For this portion of the experiment, you will need freshly picked leaves from the same plant. You will also need a respiration chamber BioChamber 2000, aluminum foil, and a light source. Make sure you wear gloves as you handle the respiration chambers and leaves.

Before you start this experiment, place your dry leaves inside the respiration chamber, making sure the leaves are flat on the bottom of the chamber. Cover the two holes, and sit it under a light source for at least 15 minutes. If fact, the longer under the light source, the better so set this up at the beginning of lab!

As you wait, complete this portion before you start your experiment:

- Create a hypothesis for your experiment:

- Create a prediction for your experiment:

- What would be your independent variable(s) for your experiment?

- What would be your dependent variable(s) for your experiment?

- What would be your standardized (control) variables for your experiment?

Follow these instructions to set up your LabQuest and gas sensors:

1. Attach the Vernier O_2 gas sensor to one port, and the Vernier CO_2 gas sensor to the other port on the right of the LabQuest2 tablet. Carefully insert each sensor through one of the two holes in the lid. Be careful to not damage the chamber or the sensors, and also make sure the sensors are sitting straight up and not horizontally.

2. Turn on the tablet, and allow the sensors to warm up for at least 90 seconds. You should see a blue screen and a red screen with a green arrow at the lower left. If you don't, you may need to restart your LabQuest2 so it will recognize the sensors.

3. Wait 5 minutes to allow the sensors to get a base reading before using the stylus to click on the gray box to the right that has 'Mode, Rate, and Duration'. You will need to change the duration from 300 seconds to 15 minutes. This will also change the rate to 1 sample per minute.

4. Once you changed the duration, click on the green arrow at the bottom to start data collection. You will collect data for 15 minutes. The system will stop automatically.

5. When data collection has finished, use the CO_2 graph and O_2 graphs to determine the rate of CO_2 production and O_2 production for leaves in the light. Click on 'Analyze' and then 'Curve Fit' to pick on which graph you wish to work on first.
    a. Under 'Fit Equation', chose 'Linear' to perform a linear regression.
    b. Under "Coefficients', you will see the components for the equation for calculating a straight line. You only want the value for the slope, *m*.
    c. Record this value in the table provided and repeat the steps for the next graph. Also note the overall pattern of the line graph.
    d. The slope angle indicates production verse consumption. So, a negative slope suggests a decline of gases inside the chamber (plant consumption) and a positive slope suggests an increase in gases inside the chamber (plant production).
    e. Make sure to repeat these steps for the other sensor graph.

6. After you've collected the data, wrap the respiration chamber with aluminum foil so no light can reach the leaves. Make sure the leave the sensors attached. Wait 15 minutes before starting using the stylus to click on the green arrow to start data collection.

7. Collect data for 15 minutes and follow the steps in #5 again. When data collection has finished, use the CO_2 graph and O_2 graphs to determine the rate of CO_2 production and O_2 production for leaves in the dark. Record these values in table provided.

8. When complete unplug both sensors and remove them from the chamber before opening the chamber to remove the leaves. Make sure to keep the Vernier O_2 gas sensor upright. Then you will carefully wipe and dry the chamber before putting it away.

9. When all lab groups are done, pool the data together to get the overall class average and record the averages in the second table.

NOTE: One problem is that CO_2 is measured in ppm while O_2 is a percentage. Therefore, you can still compare the patterns, but not the actual numbers between CO_2 and O_2. However, if you feel ambitious, here is how you can convert % to ppm:

Percent to ppm conversion: 1% = 1/100. 1ppm = 1/1000000. ...

Example. Convert 1.7% to ppm: x(ppm) = 10000 · 1.7% = 17000ppm.

Record the slope value (m) for carbon dioxide rate of production/consumption (ppt/min) and the oxygen rate of production/consumption (%) for each part in the table below. Also include a rough sketch of your line for each measurement.

Leaves	**CO_2 Rate of Production/Consumption (ppt/min)**	**O_2 Rate of Production/Consumption (%)**
In the Dark		
In the Light		

Now pool together the slope data from all tables and provide the average into the table below.

Leaves	**CO_2 Rate of Production/Consumption (ppt/min)**	**O_2 Rate of Production/Consumption (%)**
In the Dark		
In the Light		

Consider the following questions as you interpret your results:

- What can you conclude about the rate values for CO_2 between leaves in the dark verses leaves in the light? What is the biological significance of this?

- What can you conclude about the rate values for O_2 between leaves in the dark verses leaves in the light? What is the biological significance of this?

- Do you have evidence that cellular respiration occurred in the leaves? Why or why not?

- Do you have evidence that photosynthesis occurred in the leaves? Why or why not?

- Did the overall results support or reject your hypothesis? Why or why not?

- What could be some potential sources of error in your experiment? Why?

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. How did your two plant extracts compare in relation to types of pigments and light absorption spectrums? What were the variations between the two plant extracts?

2. Despite leaves having similar functions, they can vary in types of pigments depending on where leaves are located on a plant or where a plant is growing. Why do you think leaves would require different types of pigments?

3. In general, plants will consume more carbon dioxide in light conditions and release more carbon dioxide in dark conditions. Why do you think this would be the case? Did you see a similar pattern with your leaves? Explain why you did or did not see any differences.

4. With oxygen production, plants tend to release more oxygen in light conditions while consuming more oxygen in dark conditions. Why do you think this would be the case? Did you see a similar pattern with your leaves? Explain why you did or did not see any differences.

5. Provide at least one new idea or suggestion for improvement to either experiments and explain why the suggestion would be useful.

References

Portions of this lab exercise were adopted and/or modified from:

CISER: Center for the Integration of STEM Education & Research [Internet]. 2020. Lubbock, TX: Texas Tech University. Shared Sources - Plant Traveling. Available from: https://www.depts.ttu.edu/ciser/

Cell Division: Mitosis and Meiosis

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

Objectives

- Understand the purpose and outcome of mitosis and meiosis
- Understand the cell cycle and when cell division occurs
- Be able to recognize the distinct stages of mitosis and meiosis based on chromosome arrangement
- Understand what karyotypes are and how they can be used to determine various chromosomal abnormalities

Introduction

All cells come from preexisting cells, and eukaryotic cells must undergo mitosis to form new cells. A cell's replication is part of the overall cell cycle (Figure 1) composed of interphase and M phase (mitotic phase). M phase, which consists of mitosis and cytokinesis, is the portion of the cell cycle where the cell divides, reproducing itself. **Mitosis** is the division of the nucleus and its contents.

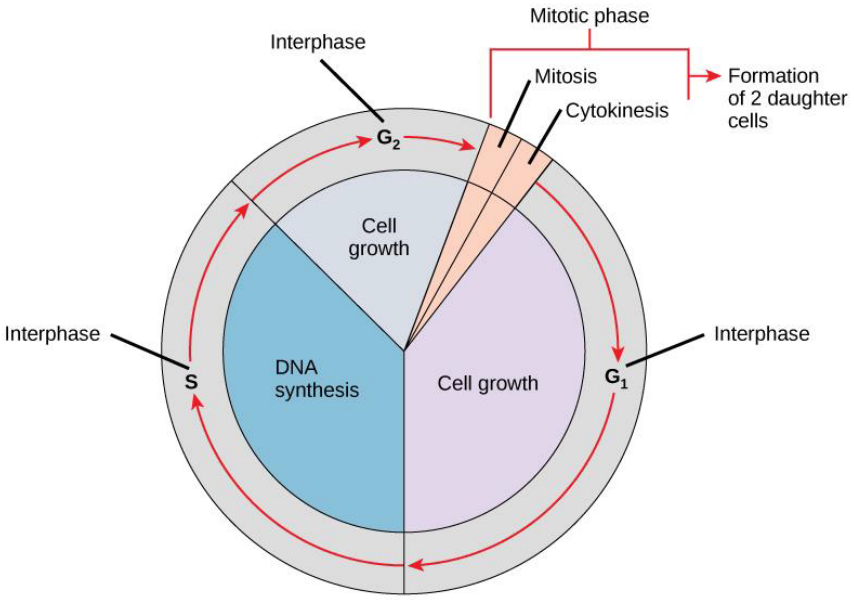


Figure 1. The cell cycle. This work has been released into the public domain by its authors Clark MA, Douglas M, Choi J.

In mitosis, DNA (deoxyribonucleic acid) which has been copied in S phase of interphase is separated into two individual copies. Each copy will end up in its own cell at the end of M phase. Mitosis has several steps: **prophase, prometaphase, metaphase, anaphase**, and

telophase (Figure 2). The spindle fibers, which are formed by the cell as mitosis progresses, are used to attach to chromosomes, align them down the middle of the cell, and pull chromosomes apart into their identical individual chromatids which will end up in separate cells.

As mitosis is nearing its end and the cell is in telophase, the cytoplasm also divides so that both new cells will have their own fluid, organelles, etc. This division of the cytoplasm is called **cytokinesis**. Mitosis and cytokinesis can be viewed under a microscope and the mitotic index can be calculated.

The **mitotic index** is the ratio of the number of cells undergoing mitosis to the total number of cells viewed. This number tells you about the cells' ability to divide and the rate at which the cells are dividing. This can be helpful when trying to diagnose certain medical conditions such as cancer.

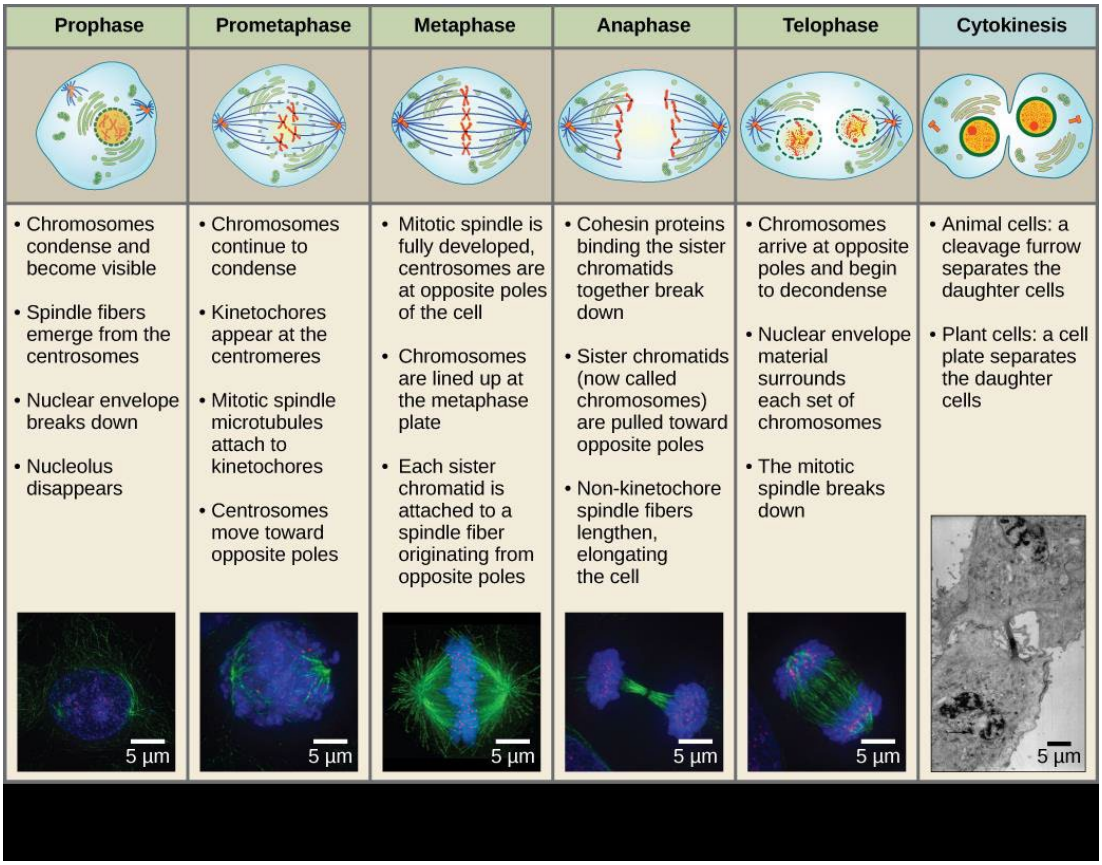


Figure 2. Stages of M phase. This work has been released into the public domain by its authors Clark MA, Douglas M, Choi J.

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

Two events that contribute to this genetic diversity during meiosis are **crossing over** (Prophase I) and **independent assortment** (Metaphase I). Cells that divide by meiosis prepare for division (during interphase) much like every other cell. They double their contents so that every chromosome replicates to form sister chromatids. Meiosis also progresses through the same phases as mitosis (**prophase, anaphase, metaphase, telophase and cytokinesis**). However, unlike mitosis, meiosis involves two divisions (meiosis I and meiosis II, see below), which reduces the chromosomal number in daughter cells by half (Figure 3).

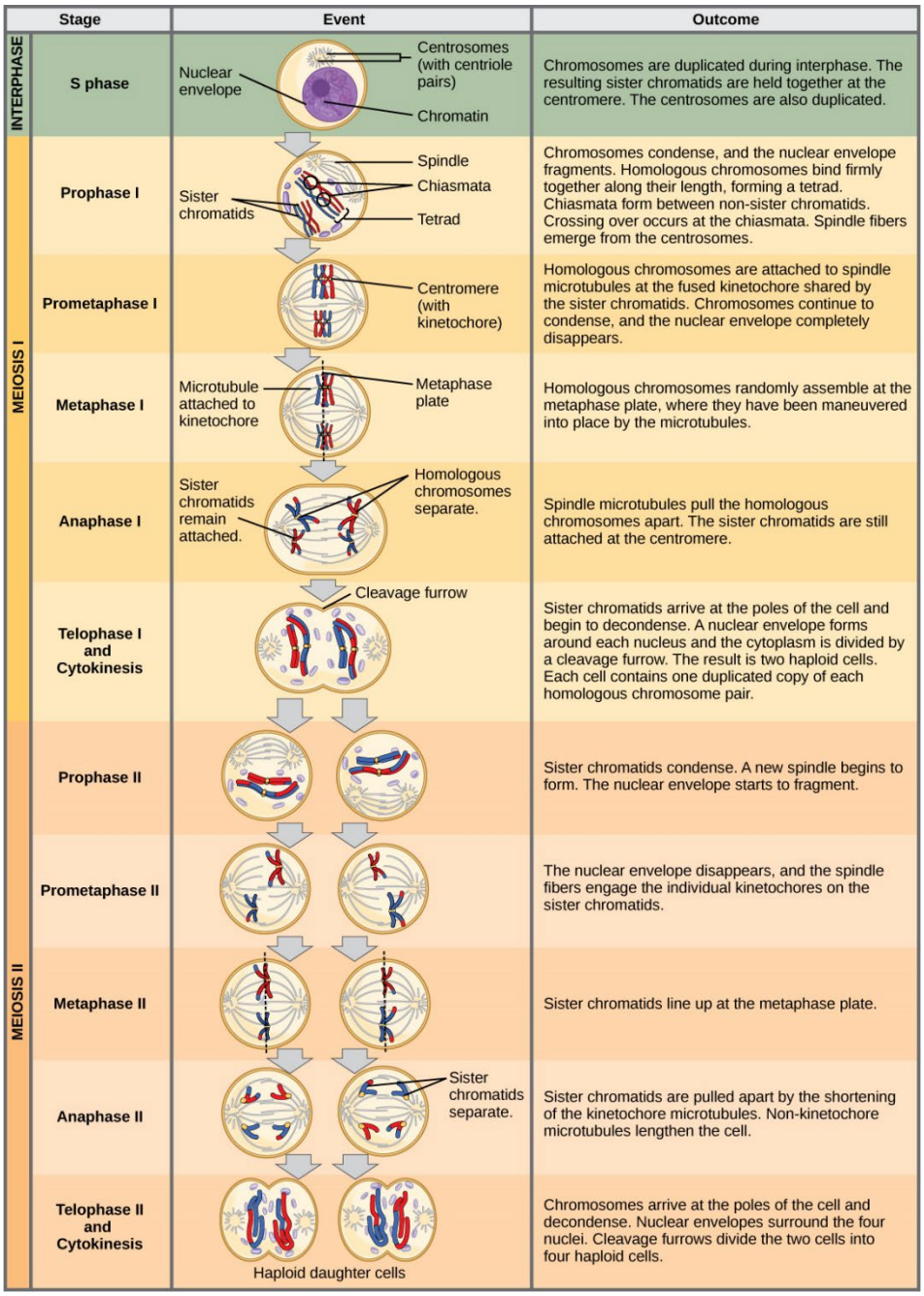


Figure 3. An animal cell with a diploid number of four ($2n = 4$) proceeds through the stages of meiosis to form four haploid daughter cells. This work has been released into the public domain by its authors Clark MA, Douglas M, Choi J.

Exercise 1: Mitosis of Onion Root Tip

- Obtain a prepared slide of onion root tip.
- Examine the slide under the microscope.
- Calculate the Mitotic Index (ratio of cells undergoing cell division over the total number of cells).
- Identify a cell in each stage of mitosis and draw a cell in each stage, making sure to focus on the chromosomes and their position.

Mitotic index _____

Interphase

Prophase

Metaphase

Anaphase

Telophase and Cytokinesis

Exercise 2: Mitosis of Whitefish Blastula

- Obtain a prepared slide of a whitefish blastula.
- Examine the slide under the microscope.
- Calculate the Mitotic Index (ratio of cells undergoing cell division over the total number of cells).
- Identify a cell in each stage of mitosis and draw a cell in each stage, making sure to focus on the chromosomes and their position.

Mitotic index _____

Interphase

Prophase

Metaphase

Anaphase

Telophase and Cytokinesis

Exercise 3: Meiosis in animal cells

- After your instructor has gone over the stages of Meiosis with you, use **Figure 3** (above) to draw an animal cell with a diploid number of 2n = 6 in each stage of Meiosis, specifically paying attention to what the chromosomes are doing in the nucleus. Include both Meiosis I and Meiosis II and use red and blue to indicate maternal and paternal chromosomes.

Meiosis I

Prophase I

Metaphase I

Anaphase I

Telophase I and Cytokinesis

Meiosis II

Prophase II

Metaphase II

Anaphase II

Telophase II and Cytokinesis

Exercise 4: Karyotypes and Non-disjunction

Failure of chromosomes to separate during mitosis or meiosis will result in an incorrect number of chromosomes in daughter cells. This occurrence is known as **non-disjunction**, and it is often triggered by a lapse during a mitotic checkpoint. Should non-disjunction occur during meiosis, the resulting egg or sperm cell may have an incorrect number of chromosomes; if this sex cell is then fertilized, the fetus will have a chromosomal abnormality. The term given for having an incorrect number of chromosomes is **aneuploidy**.

A common type of aneuploidy is trisomy, which is when there are 3 copies of a particular chromosome instead of 2. The most common trisomy that a human can survive is Down syndrome, which occurs at chromosome 21. To diagnose a chromosomal abnormality, doctors use a map of the chromosomes known as a **karyotype**. Each chromosome pair is laid out side-by-side, so it is relatively easy to determine if there are any irregularities. Referring to the karyotype below (Figure 4), it is clear that each chromosome pair is present and of relatively equal length. Note that the last chromosome pair (23) is labeled X/Y; these are the sex chromosomes are the only 2 that do not exactly match.

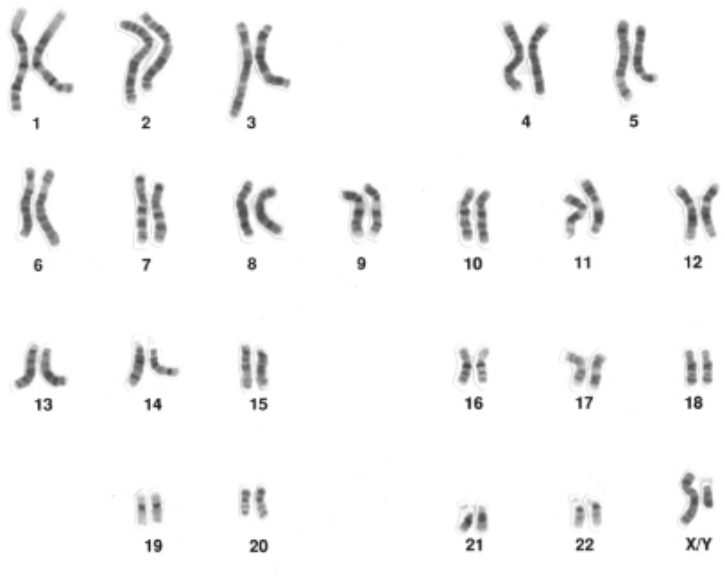


Figure 4. "Human male karyotype" by National Human Genome Research Institute is licensed under Public Domain via Wikimedia Commons

Work with your lab partner to determine the human chromosomal abnormality exhibited by the karyotype you have been assigned:

- Match each homologous pair and align them in order from 1 to 23 pairs.
- Tape homologs in order (1-23) onto mat provided by your instructor.
- Identify which chromosomal condition you have using the table provided by your instructor.

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. How many daughter cells are produced by mitosis?
2. Are these daughter cells genetically identical to the parent cell and each other?
3. How many daughter cells are produced my meiosis?
4. Are these daughter cells genetically identical to the parent cell and each other? If not, why?
5. When during the cell cycle does DNA replication occur?
6. What is the purpose of meiosis?
7. What two events occur during meiosis that greatly contribute to genetic variation in sexually reproducing organisms?
8. When does crossing over occur?
9. When does independent assortment occur?
10. What is non-disjunction?
11. What is aneuploidy?
12. Draw a cell with a diploid number of 8 (2n = 8) in metaphase of mitosis.
13. Draw a cell with a diploid number of 8 in metaphase I of meiosis.
14. Draw the same cell above in metaphase II of meiosis.

References

Portions of this lab exercise were modified from OERs:

Belwood J, Rogers B, Christian J. 2019. Foundations of Biology Lab Manual. Biological Sciences Open Textbooks. https://alg.manifoldapp.org/projects/foundations-of-biology-lab-manual-ghc.

Burran S, DesRochers D. 2015. Principles of Biology I Lab Manual. Biological Sciences Open Textbooks. Book 3. http://oer.galileo.usg.edu/biology-textbooks/3.

Clark MA, Douglas M, Choi J. 2018. Biology 2e. OpenStax. https://openstax.org/details/books/biology-2e. CC BY-SA 4.0

Mendelian Inheritance

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

Objectives

- Understand how events in meiosis result in predicted Mendelian inheritance ratios
- Apply Punnett squares to predict outcomes of monohybrid and dihybrid crosses
- Understand the inheritance of sex-linked traits
- Understand the concept of codominance and how this applies to the ABO blood groups in humans

Introduction

Genetics is the study of heredity, or the passing of traits from parents to offspring. Genes (specific segment of a DNA molecule that holds the information for one specific protein) come in different versions, or alleles. Humans are called diploid organisms because they have two **alleles** at each genetic locus (location), with one allele inherited from each parent. Each pair of alleles represents the (**genotype**, combination of alleles) of a specific gene. Genotypes are described as **homozygous** if there are two identical alleles at a particular locus and as **heterozygous** if the two alleles differ. A **dominant** allele hides a **recessive** allele and determines the organism's appearance (**phenotype**, set of observable traits). In other words, an organism only needs one dominant allele to express a dominant trait but would require two recessive alleles to express a recessive trait. Dominant alleles are represented with a capital letter (R), while recessive alleles are represented with a lowercase letter (r).

Gregor Mendel's **Principle of Segregation** states that pairs of genes segregate during the formation of gametes (meiosis), so that each gamete has one of each gene pair but not both. The **Principle of Independent Assortment** states that each gene pair is distributed (assorts) independently of other gene pairs during the formation of gametes (meiosis). These principles laid the foundation for the study of inheritance that continues today.

Scientists use a grid-like tool (**Punnett Square**) to make predictions about various genetic problems. The Punnett Square shows only the probability (the chance of something occurring) of what might occur and not the actual results. For example, if one wants to flip a coin 100 times, since there are 2 sides to the coin, one would expect 50 heads and 50 tails. If one flips the coin 100 times, one may actually get 60 heads and 40 tails. The Punnett Square only shows the chances of what might occur each time the event is undertaken.

Exercise 1: Simulating a monohybrid cross

Procedure:

1. Each group will pick up two paper bags filled with 30 red (R) beans and 30 white (r) beans. This represents two heterozygous parents (Rr x Rr).
2. At the same time, each student will reach into their bag and pull out one of the beans. The only possibilities that can be made from this selection are RR (homozygous red), Rr (heterozygous red) and rr (homozygous white). Mark the resulting genotypes and phenotypes in the table below.
3. Return the beans back to the bags and repeat the process 9 more times (for a total of 10 times).

Trial	Offspring Genotype	Offspring Phenotype
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Questions:

1. What are the genotypes and phenotypes of the two parents in this simulation?

2. Draw a Punnett square for this cross of these two parents. What are the genotypic ratios expected?

3. What are the phenotypic ratios expected?

4. Were the results of your simulation close to these expectations?

Exercise 2: Dihybrid Crosses Involving Two Traits

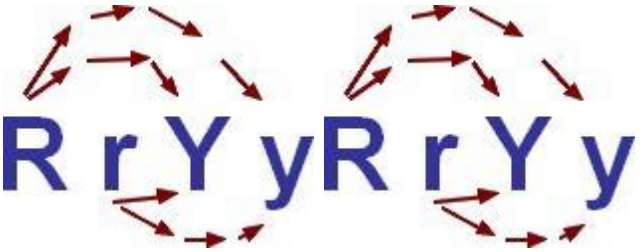
These can be more challenging than monohybrid crosses to set up, and the square you create will be 4x4. This simple guide will walk you through the steps of solving a typical dihybrid cross common in genetics. The method can also work for any cross that involves two traits.

Consider the following cross: A pea plant that is heterozygous for round, yellow seeds is self-fertilized; what are the phenotypic ratios of the resulting offspring?

Step 1: Determine the parental genotypes from the text above, the word "heteroyzous" is the most important clue, and you would also need to understand that self-fertilized means you just cross it with itself. When choosing letters to represent your genes, you'll need to choose one letter per characteristic. If it is dominant, it will be capitalized and if it is recessive it will be lower case.

RrYy x RrYy

Step 2: Determine the gametes. This might feel a little like the FOIL method you learned in math class. Combine the R's and Ys of each parent to represent sperm and egg. Do this for both parents:



Gametes after "FOIL": **RY, Ry, rY, ry** (parent 1) and **RY, Ry, rY, ry** (parent 2). These gametes will appear in your Punnett square (see below). This work has been released into the public domain by the authors.

Step 3: Set up a large 4x4 Punnet square, place one gamete set from the parent on the top, and the other on the side. Refer to the figure on the following page.

Step 4: Write the genotypes of the offspring in each box and determine how many of each phenotype you have. In this case, you will have 9 round, yellow; 3 round, green; 3 wrinkled, yellow; and 1 wrinkled green.

Gametes	RY	Ry	rY	ry
RY				
Ry				
rY				
ry				

Some Shortcuts: In any case where the parents are heterozygous for both traits (AaBb x AaBb) you will always get a 9:3:3:1 ratio. 9 is the number for the two dominant traits, 3 is the number for a dominant/recessive combination, and only 1 individual will display both recessive traits.

Another way to determine the ratios is to do it mathematically, using the addition and multiplication rules of probability; 3/4 of all the offspring will have round seeds AND 3/4 of all the offspring will have yellow seeds 3/4 x 3/4 = 9/16 will have round, yellow seeds. Where you use AND you multiply. Where you use OR you add.

Additional Dihybrid Exercises

In rabbits, grey hair is dominant to white hair. Also in rabbits, black eyes are dominant to red eyes.

GG = gray hair
Gg = gray hair
gg = white hair

BB = black eyes
Bb = black eyes
bb = red eyes

1. What are phenotypes of rabbits with the following genotypes: Ggbb, ggBB, ggbb, and GgBb?

2. A male rabbit with genotype GGbb is crossed with a female rabbit with genotype ggBb. Draw a Punnett square to illustrate the proportions of phenotypes expected from this cross.

3. How many out of 16 have grey fur and black eyes?

4. How many out of 16 have grey fur and red eyes?

5. How many out of 16 have white fur and black eyes?

6. How many out of 16 have white fur and red eyes?

7. A male rabbit has the genotype GgBb. Determine the gametes produced by this rabbit?

8. Set up a Punnett square representing the cross between the male from Question 7 and a female rabbit with the same genotype. What are the phenotypic ratios of offspring produced?

Exercise 3: Sex-linked or X-linked Inheritance

In many organisms, the determination of sex involves a pair of chromosomes that differ in length and genetic content - for example, the XY system used in humans and other mammals.

The X chromosome carries hundreds of genes, and many of these are not connected with the determination of sex. The smaller Y chromosome contains genes responsible for the initiation and maintenance of maleness, but it lacks copies of most of the genes that are found on the X chromosome. As a result, the genes located on the X chromosome display a characteristic pattern of inheritance referred to as **sex-linkage** or **X-linkage**.

Females (XX) have two copies of each gene on the X chromosome, so they can be heterozygous or homozygous for a given allele. However, males (XY) will express all the alleles present on the single X chromosome that they receive from their mother, and concepts such as 'dominant' or 'recessive' are irrelevant.

A number of medical conditions in humans are associated with genes on the X chromosome, including hemophilia, muscular dystrophy and some forms of color blindness.

Use the above information on sex-linked traits to answer the following questions.

1. In fruit flies eye color is a sex-linked trait. Red (R) is dominant to white (r). What are the sexes and eye color of the flies with the following genotypes: X^RX^r, X^RY, X^rX^r, X^RX^R, X^rY?
2. What are the genotypes of the following flies: white eyed male, heterozygous red eyed female, white eyed female, red eyed male?

3. Draw a Punnett square to show the cross between a white eyed female and a red eyed male. Are there any males with red eyes produced?

4. Draw a Punnett square to show the cross between a homozygous red eyed female and a white eyed male. How many of the offspring are white eyed?

5. In humans, hemophilia is a sex-linked trait. Females can be normal, carriers or have the disease. Males will either have the disease or not – they will never be carriers. Draw a Punnett square to show a cross between a man who has hemophilia with a woman who is a carrier, but phenotypically normal. What is the probability that their children will have the disease?

6. A woman who is a carrier for hemophilia marries a man who does not have the disease. Draw a Punnett square to show the probability that their children will have hemophilia. What is the sex of children with the disease?

7. A woman with hemophilia marries a man without the disease. How many of their children will have hemophilia and what is the sex of affected children?

Exercise 4: ABO Blood Groups and Codominance

Although the basic composition and function of blood in each individual is the same, there are different human blood types. People can have one of four types of blood: A, B, AB, or O. A specific blood type is based on the presence of enzymes that determine the type of carbohydrates (A, B) or absence of these carbohydrates (O) on the surface of the red blood cells. Because there are two types of carbohydrates involved and both can be expressed on the same cell (**codominant inheritance**) there are four possible combinations or blood types (ABO groups), as shown in Figure 1. These carbohydrates can stimulate an immune response, so they are often referred to as antigens.

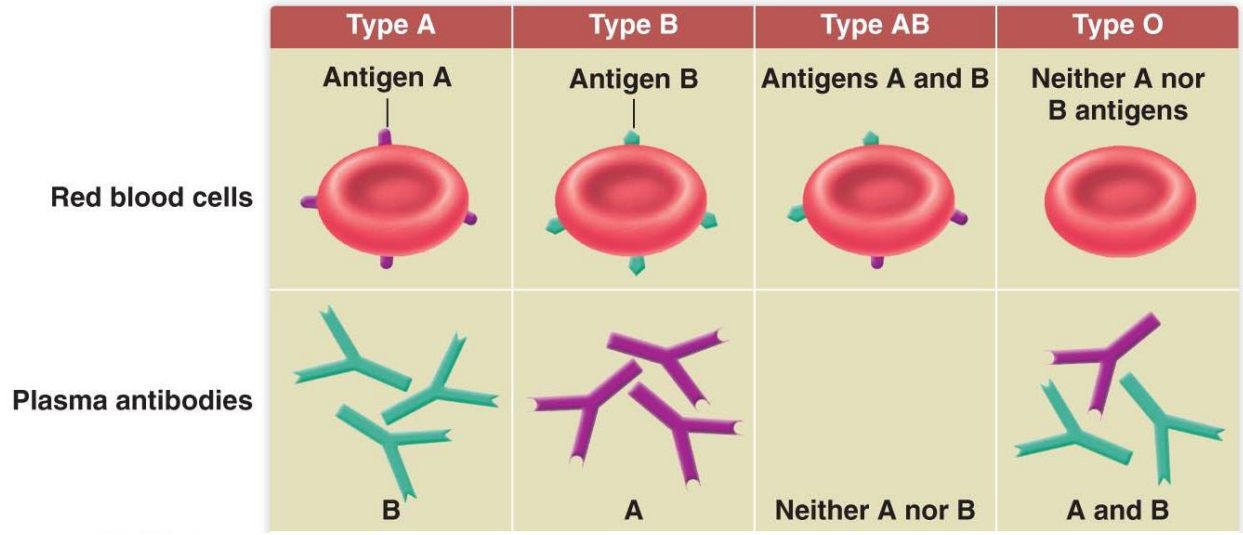


Figure 1. Four ABO blood types with their corresponding antigens and antibodies. This work has been released to the public domain by its authors Mukhopadhyay S, Davis C, and Wilson C .

The molecules (carbohydrates and proteins) that are expressed on your red blood cells allow your cells to make **antibodies** to protect against foreign cells. Your cells make antibodies against the proteins NOT expressed on your red blood cell surface (Table 1, below). If two different blood types are mixed during a transfusion, the blood cells may begin to clump together (**agglutinate**) in the blood vessels, possibly causing death. This clumping is due to the antibodies in your body binding to the foreign molecules (antigens) on the surface of the foreign red blood cells. Therefore, it is important that blood types be matched before blood transfusions take place.

Type O blood is referred to as the universal donor as it has neither A or B antigens on the red blood cells. However, Type O individuals cannot receive blood from any donors other than O as they have antibodies for A and B in the blood plasma. Type AB+ is the universal acceptor because they have no antibodies in the blood serum to cause an immune reaction. The + sign for this blood type comes from the presence of the rhesus (Rh) antigen or Rh factor on the red blood cells. The positive phenotype is dominant to the negative phenotype. You can use antibodies to test for this antigen similarly to how you test for the A and B antigens on the red blood cells.

Table 1: Relationships of the ABO Blood Types to the presence of antigens and antibodies in the blood.

Blood group	Antigen present on cells	Antibody in blood plasma
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither
O	neither	Anti-A and Anti-B

Rh factor	Antigen present on cells	Antibody in blood plasma
positive (+)	Rh antigen	None
negative (-)	no Rh antigen	Anti-Rh

With the information above and materials given to you by your instructor, you will determine the blood type of four different synthetic blood samples using antisera to the A, B, and Rh antigens that exist on human red blood cells. The procedure is the same as would be used for a real blood test, but for convenience and safety, the samples are synthetic and do not contain any biological materials.

Each team of four students will need:

- One vial each of synthetic anti-A, anti-B serum, and anti-Rh serum
- One vial each for synthetic blood samples 1-4
- Four blood typing trays
- Mixing sticks
- Paper towels

Procedure:

1. Using the dropper vial, place a drop of the first synthetic blood sample in each well of the blood typing slide. Replace the cap on the dropper vial. Always replace the cap on one vial before opening the next to prevent cross contamination.
2. Add a drop of synthetic Anti-A serum (blue) to the well labeled A. Replace the cap.
3. Add a drop of synthetic Anti-B serum (yellow) to the well labeled B. Replace the cap.
4. Add a drop of synthetic Anti-Rh serum (red) to the well labeled Rh. Replace the cap.
5. Using a different color mixing stick (blue for Anti-A, yellow for Anti-B, and red for Anti-Rh), gently stir the synthetic blood and anti-serum drops for 30 seconds. Remember to discard each mixing stick after a single use to avoid contamination.
6. Carefully examine the thin films of liquid mixture left behind. If a film remains uniform in appearance, there is no agglutination. If the sample appears granular or thick, agglutination has occurred.
7. Determine the blood type of the sample using the data table below. Answer yes or no as to whether agglutination occurred in each sample. A positive agglutination reaction indicates the blood type.

8. Record the results for the first blood sample in the data table.
9. Repeat steps 1-7 for synthetic blood samples 2, 3 and 4.

Table 2: Collected Results for Blood Typing Simulation

	Sample 1	Sample 2	Sample 3	Sample 4
Anti-A				
Anti-B				
Anti-Rh				
Blood type				

Clean-up: Wash all the blood sample trays with soap and water. If there is any stain, wipe with isopropyl alcohol. Throw all the sticks and paper towels in the trash. Wash your hands properly.

Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Name the antigens and antibodies present in Type A blood.

2. Name the antigens and antibodies present in Type O blood.

3. What does clumping mean in a blood typing test?

4. Which individuals are considered universal donors, and why?

5. Which individuals are considered universal acceptors, and why?

6. What blood types could an individual with blood Type A positive receive?

7. If two individuals with Type AB blood marry, what is the probability that they will have children with AB blood? Draw a Punnett square to illustrate your answer.

8. In a paternity dispute, a mother with Type A blood has a child with Type O blood. The man she claims is the father has Type B blood. Could this man be the father? Draw a Punnett square to illustrate your answer.

References

Portions of this lab exercise were modified from OERs:

Burran S, DesRochers D. 2015. Principles of Biology I Lab Manual. Biological Sciences Open Textbooks. Book 3. http://oer.galileo.usg.edu/biology-textbooks/3. CC BY-SA 4.0.

Mukhopadhyay S, Davis C, and Wilson C 2020. Fundamentals of Biology Lab Manual. Biological Sciences Open ALG Textbooks. https://alg.manifoldapp.org/projects/fundamentals-of-biology-lab-manual. CC BY-SA 4.0.