

# **Laboratory Manual**

*for*

## **BIOL-3: Introduction to Life Science**

*at*

### **Clovis Community College**

v1.0, Fall 2019

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Cover photo: Lesser sandhill cranes taking flight at Merced National Wildlife Refuge, November 25, 2018.  
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# Lab Safety and Course Instructions

## Course and book introduction

This lab course is designed to follow along with the lectures for BIOL-3, although different lecture instructors teach subjects in different orders.

In this lab book, questions that you need to answer are indicated by an arrow as follows:

→ Is there a study guide for the lab exams? Circle one: YES | NO

(Incidentally, the answer is “yes” -- the study guide is your lab manual, so it’ll be exactly as good as you make it.)

## Student responsibilities

You will be expected to do the following every week:

- Read over the lab assignment before class.
- Come to the lab, on time, wearing appropriate gear.
- Stay in class for the entire lab period.
- Do all the parts of the lab procedure.
- Contribute to your group equally and engage your brain!
- Turn in the assignment at the end of class.
- Clean up your lab station and common areas.
  - Observe special cleanup instructions each week
  - Make sure microscopes are at starting position and covered up
  - Use cleaner to wipe down your bench
- Complete the post-lab activities your instructor assigns.

Since you will be doing your assignments in groups, the normal criteria for plagiarism don’t really fit. **However**, this is not an excuse to turn your brain off! Mindless copying of answers (e.g. everyone in a group answers with the same really bad answer, or with identical spelling/grammatical errors that someone should have caught) will be graded harshly. If the group decides to “divide and conquer”, make sure you plan for debriefing time so that everyone ends up understanding the entire lab exercise.

## Lab safety instructions

Students must **read and sign the lab safety sheets** before we can proceed! When you have read the instructions below, sign the page that asks for a signature, tear it out, and give it to the instructor.

Safety is everyone’s business. Conduct yourself in a responsible and mature manner at all times in the lab. Preparation is key to safety: come prepared for lab. The following rules of lab safety and using common sense throughout the course will enhance your learning experience by increasing your confidence in your ability to safely use chemicals and equipment.

# Lab Safety and Course Instructions

## ATTIRE:

- Wear safety glasses or goggles during exercises in which glassware and solutions are heated, or when dangerous fumes may be present. DO NOT WEAR CONTACT LENSES. It is at the discretion of the lab instructor when safety glasses will be worn.
- You may be required to wear a lab coat when experiments are conducted, at the instructor's discretion. If lab coats are not required, wear clothing that, if damaged, would not be a serious loss, since chemicals may damage fabrics.
- **Your feet must be completely covered:** shoes with open toes and/or uncovered heels are not permitted in the lab. No exceptions!!! Shoes protect you against broken glass or spillage that may not have been adequately cleaned up.
- If you have long hair, please make sure to tie it back when working with chemicals or Bunsen burners.
- You should not wear dangling jewelry or any loose, baggy garments or accessories to avoid any unplanned contact with any chemicals.

## CONDUCT

- There is absolutely **no eating, drinking, or chewing in lab**. Even water! Toxic material may be present. You may leave your food and drink on the wire shelves near the doors and step outside to eat or drink.
- Clean your hands and the desk area, including desk top and edge, before and after each experiment, using the cleaning solutions provided.
- Note the location of emergency equipment, such as a first aid kit, fire extinguisher, fire blanket, ceiling showers, eyewash station and telephone (911).
- Keep aisles clear. Push chairs under desk when not in use. Access to exits and emergency equipment must be unobstructed.
- Keep your work area neat, clean, and organized. Before beginning lab, remove everything from your work area except the lab manual, pen and equipment used for the experiment. Place personal items in the laboratory drawers or cubicles at the end of the laboratory tables or in the back of the lab. You may keep your phone or personal computer out on the desk for lab unless you're told to put it away. Be sure to take all your personal items with you when you leave the laboratory.
- Please arrive on time, since laboratory directions and procedures are given at the beginning of the laboratory period. If you must arrive late, please enter and take your seat as quietly as possible to minimize your interruption of the class, and ask the instructor to explain the procedures before you begin.
- Read all experiments before you come to class to be sure that you understand all the procedures and safety precautions. Pay particular attention to oral and written safety instructions given by the instructor.
  - If you do not understand a procedure, ask the instructor, rather than a fellow student, for clarification. Confusion is dangerous!
  - Completely follow the procedure set forth by the instructor. Do not improvise any procedure and do not perform any unauthorized experiments without first getting the instructor's approval.
- Assume that all reagents are poisonous and act accordingly. Read the labels on chemical bottles for safety precautions and know the nature of the chemical you are using. Stopper all reagent bottles when not in use. If chemicals come into contact with your skin, wash immediately with water.
- DO NOT ingest any reagents, carry reagent bottles around the room, pipette anything by mouth, or pour chemicals back into containers.
- Dispose of chemicals, biological materials, used apparatus, and waste materials according to the directions of your instructor. Put all trash into the proper receptacles (for example: paper into trash cans, disposable glassware into recycling containers, used blood lancets into Sharps containers, etc.). Please do not put trash of any kind into the sinks, lab drawers or lab bench shelf spaces.
- DO NOT operate any equipment until you are instructed in its use.

## Lab Safety and Course Instructions

- When handling hot glassware use a test tube clamp or tongs. Use caution when using heat, especially when heating chemicals. DO NOT leave a flame unattended; DO NOT light a Bunsen burner near a gas tank or cylinder; DO NOT move a lit Bunsen burner.
- Make certain the gas jets are off when the Bunsen burner is not in use. Use proper ventilation and hoods when instructed.
- Use clean glassware at the beginning of each exercise, and wash glassware at the end of each exercise or before leaving the laboratory.
- Use extra care and wear disposable gloves when working with glass tubing and when using dissection equipment (scalpels, knives, or razor blades).
- Report all accidents to the instructor immediately, and ask your instructor for assistance in cleaning up broken glassware and spills.
- Report to the instructor any condition that appears unsafe or hazardous.
- Use caution during any outdoor activities. Watch for snakes, poisonous insects or spiders, stinging insects, poison oak, poison ivy, etc. Be careful near water.

### SPECIAL NOTES

- Some exercises in the laboratory manual are designed to induce some degree of cardiovascular stress. Students should not participate in these exercises if they are pregnant, have hypertension or any other known or suspected condition that might compromise health.
  - Students having any medical condition that may cause sudden loss of consciousness should consult their physician, and if accommodations are needed, they should provide the instructor with written directions from their physician.
  - Some of the chemical agents used in the labs may have unknown effects on human pregnancy. Pregnant students should consult their physician, and if any accommodations are needed then they should provide the instructor with written direction from their physician.
- 

### CUT THIS HALF PAGE OUT AND TURN IT IN

I have read and I understand the safety rules as presented in the book above. I agree to follow them and all other instructions given by the instructor and lab managers.

Laboratory Class:

Instructor Name and Class Time:

Name (Print):

Date:

Name (Signature):

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# Lab 1: The Scientific Method

## Learning Goals

1. Use the steps in the scientific method to answer a scientific question.
2. Describe the “real” scientific process.
3. Design a hypothesis and use a hypothesis to formulate a prediction.
4. Design an experiment with appropriate controls and run it.
5. Interpret and report your data.

## Agenda

1. Safety briefing & course introduction
2. Scientific investigations lab: pillbug behavior and preferences
  - a. Observe and sketch pillbugs
  - b. First behavioral experiment: design, execute, analyze
  - c. Second behavioral experiment: design, execute, analyze
  - d. Present results of your experiment to the class (3-5 minutes)

## Introduction to experimental science

The **scientific method**, to which you have had an introduction already in lecture, is the method by which science is done. An **experiment** is one way to implement the scientific method, in which a procedure is set up to test the validity of a hypothesis under specific controlled conditions. The key things that distinguish an experiment from other methods of scientific study, such as observational study, are that the procedure is repeatable and that the conditions are under the experimenter’s control as far as possible. For an experiment to be scientifically valid, the experimenter must pick one variable to manipulate and keep the others constant.

The goal of this lab is for you to design, run, interpret, and report an entire scientifically valid experiment on the behavior of pillbugs (*Armadillidium vulgare*).

## Observation and scientific drawing

Before you can ask a good question and formulate a hypothesis, you need to be familiar with your study system. In this part of the lab, you will do the following:

1. Observe your pillbugs closely, noting their behavior, preferences, and way of moving.
2. Make a scientific sketch of one of them.

Pillbugs are not really insects; they’re actually small terrestrial crustaceans (related to lobsters and shrimps) of the family Armadillidae. The ones you are looking at today belong to the species *Armadillidium vulgare*. They are native to Europe but have naturalized to North America, and are distinguished by a defensive behavior in which they roll up into a ball. They are decomposers, eating primarily decaying plant matter, and like to live under leaf litter. The females carry their eggs in a pocket full of water on their underside until they hatch.

# Lab 1: The Scientific Method

## Behavioral observation

*Materials: petri dish, filter paper, spray bottle, pillbugs, ruler or graduated cylinder, dissecting microscope or big lens, (optional) experimental materials*

One person from each group should go to the back and get a petri dish. Add a piece of dampened filter paper and carefully pick up 10 bugs and place them in the dish on top of the paper, then put the lid on the dish.

Observe the pillbugs' behavior in the dish for a little while. You may also use some of the experimental materials provided to do some preliminary tests of their preferences. For example, you could try to answer the following questions:

- What makes them roll up or relax?
- When you tilt the dish, what do they do?
- When they get flipped over, how do they right themselves?
- Where do they try to go? What kind of conditions do they seem to like?

## The dissecting microscope

In order to make a detailed drawing of the pillbug's external anatomy, you will need to use the dissecting microscope. The purpose of a dissecting microscope is to see a higher level of detail on things that are big enough to see with your naked eye, so its level of magnification is like that of a hand lens or jeweller's loupe.

→ Using the list of terms below, label the parts of the dissecting microscope on the image. Next to each name in the list, briefly describe the part and its function. (e.g. "Stage: Where you put the specimen").

Eyepiece/Ocular lens	
Arm	
Zoom (coarse focus) knob	
Fine focus knob	
Nose piece/Objective lens	
Stage	
Lighting switches: master, top light, bottom light	

# Lab 1: The Scientific Method

## Pillbug (*Armadillidium vulgare*)

### Scientific drawing

Place your box of pillbugs on the stage of the dissecting microscope. Turn on the overhead light, focus the objective lens, and look at their anatomy. Every person in the group should look at the pillbugs under the microscope; there are details that are hard to see any other way.

→ Make a scientific drawing of the pillbug in the space provided above.

You're going to make quite a few scientific drawings during this lab class. If you haven't done any scientific drawings before, you should know that there are some major differences from artistic drawing:

- The purpose of a scientific drawing is to communicate information. It's not important for it to be pretty, so don't worry about your artistic skills.
- Draw it big enough to fill up the box so that you have space for details.
- Draw what you see, not the picture in your head!
  - Look for details: How many segments does it have? How many legs? How do its antennae bend? Can you see the differences in how the plates are arranged in the three zones of its body (head, thorax, abdomen)? Make sure all these details show up in your drawing.
  - Draw multiple views: top and underside, walking and curled up.
- Label the animal's body parts in the drawing -- but don't label anything that you haven't actually drawn.

## Lab 1: The Scientific Method

# Designing and running experiments

In this section, we will first discuss experimental design with the whole class, then design and run behavioral experiments. You will do the following **with your group**:

1. Design a simple one-variable experiment to test the pillbugs' environmental or food preferences.
  - a. Run the experiment.
  - b. Interpret your data.
2. Based on the results of the first experiment, design a second experiment.
  - a. Run the experiment.
  - b. Interpret your data.
3. Report all your results to the class in a short presentation.

## Experimental design

In lecture, you have probably already learned about the basic steps in the scientific method:

1. Observation (I noticed that ...)
2. Question (I wonder if ... ?)
3. Hypothesis (I think ... is the most likely answer to that question, because ...)
4. Prediction (If I'm right, I expect that ...)
5. Experiment (Testing *only* and *exactly* for whether the hypothesis is correct.)
6. Data/results (What actually happened?)
7. Interpretation/conclusion (Did the experiment produce a reliable result? If so, does it support the hypothesis or not? What does this tell me about the reasoning that led me to that hypothesis?)
8. Communication (How do my experimental results compare to those of all the other people who are testing similar or related hypotheses using slightly different methods?)

By the time you get to step 5 and start designing an experiment, you should have a clear idea of the different possible outcomes that you're looking for, and of what you expect each of those possible outcomes to mean if you find it.

Some definitions of relevant terms follow:

- **Variable:** an aspect of the experiment that might affect the outcome in some way
  - **Experimental variable:** the variable you're deliberately changing to address the question (if there's more than one of these, you need more than one experimental group)
  - **Controlled variable:** any other variable that you are keeping the same throughout the experiment so that it can't affect the outcome
  - **External variable:** a variable that you can't (or didn't) control. Beware: these might invalidate your results!
- **Control group:** group of test subjects where you aren't introducing any changes in the experimental variable, for comparison to the experimental group
  - **Negative control:** a control group in which ordinary behavior is expected
  - **Positive control:** a control group in which a particular response is expected
- **Experimental group:** group of test subjects where you're changing the experimental variable to see what happens
- **Experimental error:** any way that your experiment doesn't reflect the real answers

An experiment should be designed to answer the question as cleanly as possible, minimizing the possible complications that arise from external variables. That means that, as far as possible, the *only* difference between the groups should be the experimental variable, and the other conditions should be uniform and optimal.

# Lab 1: The Scientific Method

## Experiment 1

### Designing the experiment

For the first experiment, you will need to **pick only one question**, based on the resources available to you. Choose a single question that compares two conditions.

Some possible questions you could ask:

- Do pillbugs prefer dry or moist environments?
  - What about very wet environments?
- Which of the available foods do the pillbugs prefer?
- Do pillbugs prefer to walk on sand or woodchips?
  - What about paper? What about bare plastic?
- Do pillbugs prefer light or dark environments?

Write your experimental plan out in the spaces given here.

→ Which **question** will you be investigating?

→ Once you have picked a question, think about the **observations** you have made. What have you observed about the pillbugs that might be relevant to your question?

→ What is your **hypothesis** (what answer to your question do you expect to find), and why?

→ What is your **prediction** (exactly what do you expect to happen during the experiment)?

### Running your experiment

When you're done setting up your experiment, you can run it. We will all follow approximately the same experimental procedure for the first experiment.

# Lab 1: The Scientific Method

Materials: 10 pillbugs, box with light/dark choice chambers and connecting piece, clear choice chamber, any other experimental materials specified in your experimental design above

1. Set up the two choice chambers with the two experimental conditions, keeping them as similar as possible in all ways other than the experimental variable. For example, if you are testing wet/dry preference, put the same amount of the same substrate (filter paper, woodchips, sand...) in two light or two dark chambers, then wet one of them with the spray bottle.
2. Join the chambers together using the junction piece from the black box, but leave the barriers in the junction piece.
3. Put 5 bugs in each chamber.
4. Pull the barriers out of the choice chambers and let them roam around so they can choose the condition they prefer. Start your timer.
5. Count how many bugs are in each chamber at 1-minute intervals for 10 minutes. Record your data in the table below. (Leave out any that are in the junction piece when you count.) Then proceed to Part C, Interpretation.

→ Record your data in this table.

Minutes	0	1	2	3	4	5	6	7	8	9	10
Condition 1:											
Condition 2:	5										

## Interpretation

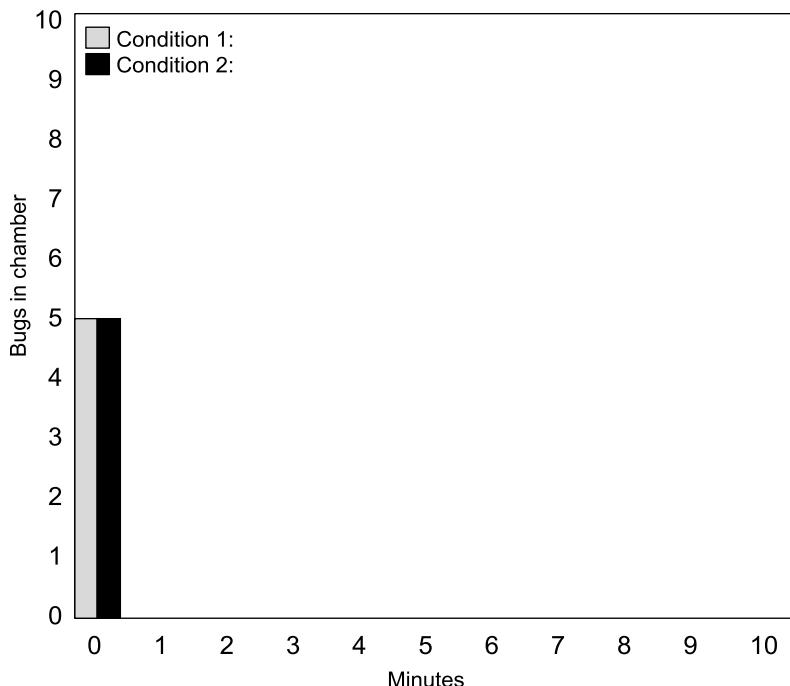
When you're done collecting your data, you need to interpret them. This step helps you to understand how the experiment went, what extraneous variables might have affected your results, and what the results mean for your hypothesis.

→ Graph your data on the plot at right.

First, write in the experimental conditions of the two chambers for Side 1 and Side 2 at the top. Then plot the number of bugs in each chamber with bars of the appropriate height. The starting condition has been plotted for you as an example. (Everyone should have started at the same state, with 5 bugs on each side.)

Questions:

→ What did the bugs prefer, or did they have any preference at all? Does the result support your hypothesis? Why do you think that might be?



## Lab 1: The Scientific Method

- What didn't go as expected -- were there any external variables that you hadn't accounted for, and how would you adjust your experiment to account for them?
  
- What do you know now that you didn't know before you did this experiment?

## Experiment 2

Once you've finished interpreting your data from the first experiment, do the second one. If you want to change anything about your experimental setup from the first experiment, go ahead and change it and then do the experiment again. If you're happy with your results on the first experiment, you can instead set up a new related experiment and test that. (For instance, if you tested dry/moist environments the first time, you can add a third chamber and try dry/moist/wet for comparison -- in this case you should add either 2 or 5 extra bugs, so that you can put the same number in each chamber.)

- Write out your new experimental plan below, and draw your chamber setup. Discuss it with the instructor before running it so they can help you eliminate external variables. A space has been provided for you to record your data.

### Question:

**Observations:** (This should include an explanation of the reason why you wanted to try this, especially if it's related to your first experiment.)

# Lab 1: The Scientific Method

**Hypothesis:**

**Prediction:**

**Methods:** Write out and/or draw your experimental setup and procedure for the second experiment in this space.

**Data:** Put your data table from the second experiment here.

Bugs in chamber

Minutes

- Plot the data for your second experiment at right. The axis labels are the same, but the details have been removed to allow you more freedom in the design of the second experiment than you had in the first one, so you will need to fill them in yourself.

## Lab 1: The Scientific Method

**Conclusions:** Describe and interpret the results of your second experiment, as you did for the first experiment, and compare them to the earlier data.

- What did the pillbugs do? Did it agree with your prediction?
- Did the results support your hypothesis? Why or why not?
- Did anything unexpected happen, and if so, did it expose hidden external variables that invalidated your experimental approach?
- What did you learn from this experiment?

## Presentations

Each group will present their data and interpretation to the whole class, using the whiteboard. You should plan on taking no more than 3 to 5 minutes for your presentation. We'll start the presentations 45 minutes before the end of class. Your presentation should address the following questions:

1. Which question did you test in your first experiment? (experimental variable)
2. What condition did you think the pillbugs/mealworms will prefer, and why do you think that? (hypothesis and prediction)
3. What actually happened? What did the bugs do? (data)
4. Did the data support your hypothesis? Was there anything unexpected about your experimental results? What did you learn? (interpretation)
5. For your second experiment, why did you change the things you changed? what were your hypothesis and prediction, and what were the results? Did they support your hypothesis from your first experiment?
6. What did you learn about pillbug behavior from your two experiments? (conclusions)

## Lab 1: The Scientific Method

→ Write your answers out and plan your presentation on this page before you present to the class.

## Cleanup

Clean up your station before you leave, according to the following instructions:

1. When you're done, put the bugs back in the container you got them from.
  - a. **DO NOT throw away your bugs**, or you will have to pick through the trash can to rescue them! There are more lab sections that will need to use the same bugs after you, and we maintain a breeding colony that needs them.
  - b. If the bugs won't leave the paper, you may put it in the pillbug tank with them.
2. Put the experimental materials in your chamber back in the container or throw it away, as appropriate.
  - a. Throw away pieces of food and paper.
  - b. Replace sand or woodchips in its container.
3. Disassemble the choice chambers, clean them out thoroughly, dry them, and put them back into the containers.
4. Wipe the eyepieces, objective lens, and stage of the dissecting microscope with a microscope wipe. Turn off and cover your dissecting microscope.
5. Spray your lab bench with cleaner and wipe it down.

# Lab 2 - Microscopy and Cells

## Learning Goals

1. Become comfortable with basic methods of physical measurement.
2. Understand the size of a cell relative to other objects.
3. Get familiar with microscopes.
  - a. Know the difference between a dissecting microscope and a compound microscope, and what you use them for.
  - b. Identify important parts of the machines.
  - c. Calculate total magnification.
  - d. Learn to properly focus a slide on the compound microscope.
4. Observe the differences between different eukaryotic cells.

## Agenda

1. Measurement, units, and scales
  - a. Metric units
  - b. Measurements
    - i. Block: length, area, volume, density
    - ii. Pebble: volume, density
2. Microscopy
  - a. Identify parts of compound microscope
  - b. Examine slides under lens
    - i. Printed letter "e"
    - ii. Red, yellow, and blue colored threads
    - iii. Cheek cell stained with methylene blue
    - iv. Plant cell (*Elodea*)

## Measurement, units, and scales

In this section, you will:

1. Review the principles of the metric system
2. Measure lengths on a wooden cube using a ruler
3. Calculate the cube's volume and surface area from length
4. Measure the cube's mass using a mass balance
5. Measure volume of a pebble by displacement of water

## Metric and imperial units

The metric system is the system of measurement used universally in the sciences, as well as for everyday household purposes in most of the world. Its main advantage over the US customary system, with which you are probably more familiar, is that it's entirely based on multiples of ten. For example, instead of the US customary system where 1 mile = 5280 feet and 1 foot = 12 inches, length in the metric system is expressed using the base unit of a meter and multiples of ten: 1 kilometer = 1000 meters and 1 meter = 100 centimeters. This makes calculations much simpler, because multiplying by 10 is the same as moving the decimal point in our decimal numeric system:  $4 \times 10 = 40$ ,  $4 / 10 = 0.4$ , and so on.

## Lab 2 - Microscopy and Cells

Each kind of measurement has a basic unit (length = meter, mass = gram, etc.). The basic units in the metric system are based on specific values in the natural world, chosen for convenience. Thus, the metric temperature unit is the degree Celsius ( $^{\circ}\text{C}$ ), which is defined such that water freezes at  $0^{\circ}\text{C}$  and boils at  $100^{\circ}\text{C}$  at sea level on Earth. Similar standard references exist for each base unit.

When this base unit is multiplied by some power of 10, its name can be adjusted using a system of prefixes. For example, if the length of an object is 2500 meters (abbreviated "m"), its length can also be written as 2.5 kilometers (abbreviated "km"), because the prefix "kilo-" means "1000 times". All conversions in the metric system are accomplished simply by multiplying or dividing by 10 some number of times; the number of times you multiply or divide by 10 is called the power of 10. Positive powers of ten indicate that you're multiplying; negative powers of ten indicate that you're dividing.

A more compact way of writing such numbers, called scientific notation or exponential notation, lets you skip writing down all the zeroes and instead just write the power of 10. For example, the value 65,000,000 in scientific notation would be written as  $6.5 \times 10^6$  (usually pronounced "six point five times ten to the sixth"), which you can think of as "6.5 and then move the decimal point six spaces to the right". Similarly, 0.0000065 could be written as  $6.5 \times 10^{-6}$  -- the negative power of ten means that the base number is divided by 106.

The first chart on the next page gives some of the metric prefixes and the power of 10 that they indicate the base number is being multiplied by, written both with zeroes and with scientific notation. The commonly used system of measurement in the U.S. is called the imperial or English system. If that's what you're used to, the table of conversions between English and metric systems on the opposite page may help you.

Official metric prefixes exist all the way out to  $10^{24}$  (yotta) and  $10^{-24}$  (yocta), but the ones further out on the ends of the scale don't get used very often in real life. However, they're not useless: the observable universe is about 880 yottameters from one side to the other, and the mass of one proton is about 1.66 yoctograms.

Answer the following questions using the prefixes chart on the facing page:

- How many millimeters make one meter? \_\_\_\_\_
- How many micrometers make one millimeter? \_\_\_\_\_
- How many centimeters make one kilometer? \_\_\_\_\_
- How many kilobytes will fill up a 1-gigabyte drive? \_\_\_\_\_
- The most abundant single species on Earth, by mass, may be the Antarctic krill *Euphausia superba* (pictured at right). It is estimated that the total global biomass of these tiny shrimplike ocean animals is about 500 megagrams. A single krill is as long as your pinky finger and weighs about 1 gram.
- Approximately how many Antarctic krill are there on Earth? \_\_\_\_\_



## Measurement

In this section we will practice measuring and converting various different kinds of quantitative measurements, including length, area, volume, mass, and density. It's important to make sure that everyone remembers how to take measurements and convert between different metric units, because you'll need to do it in many future labs in this course. (This is probably as much math as you'll need for the lab, though.)

Materials: small clear ruler with cm and mm, pebble, wooden block, water, graduated cylinder, mass balance, class laptop

## Lab 2 - Microscopy and Cells

Prefix	Symbol	Exponential notation	Written out
peta	P	$10^{15}$	1 000 000 000 000 000
tera	T	$10^{12}$	1 000 000 000 000
giga	G	$10^9$	1 000 000 000
mega	M	$10^6$	1 000 000
kilo	k	$10^3$	1 000
hecto	h	$10^2$	100
deca	da	10	10
---	---	1	1
deci	d	$10^{-1}$	0.1
centi	c	$10^{-2}$	0.01
milli	m	$10^{-3}$	0.001
micro	$\mu$	$10^{-6}$	0.000 001
nano	n	$10^{-9}$	0.000 000 001
pico	p	$10^{-12}$	0.000 000 000 001
femto	f	$10^{-15}$	0.000 000 000 000 001

Above: table of metric prefixes and their numeric equivalents.

Below: table of conversions between Metric and English (Imperial) measurement systems.

English to Metric				Metric to English			
inches (ins)	X 25.4	=	millimetres (mm)	mm	X 0.04	=	ins
feet (ft)	X 0.3	=	metres (m)	m	X 3.3	=	ft
yards (yds)	X 0.9	=	metres (m)	m	X 1.1	=	yds
miles (mi)	X 1.6	=	kilometres (km)	km	X 0.6	=	mi
sq inch (in <sup>2</sup> )	X 6.5	=	sq centimetre (cm <sup>2</sup> )	cm <sup>2</sup>	X 0.16	=	in <sup>2</sup>
sq feet (ft <sup>2</sup> )	X 0.09	=	sq metres (m <sup>2</sup> )	m <sup>2</sup>	X 11	=	ft <sup>2</sup>
sq yard (yd <sup>2</sup> )	X 0.8	=	sq metres (m <sup>2</sup> )	m <sup>2</sup>	X 1.2	=	yd <sup>2</sup>
cu. in (in <sup>3</sup> )	X 16	=	cu.centimetres	cm <sup>3</sup>	X 0.06	=	in <sup>3</sup>
cu. ft (ft <sup>3</sup> )	X 0.03	=	cu.metres (m <sup>3</sup> )	m <sup>3</sup>	X 35	=	ft <sup>3</sup>
cu. yd (yd <sup>3</sup> )	X 0.8	=	cu.metres (m <sup>3</sup> )	m <sup>3</sup>	X 1.3	=	yd <sup>3</sup>
(liq) quart (qt)	X 0.9	=	litre (l)	l	X 1.05	=	qt
gallon (gal)	X 0.004	=	cu.metres (m <sup>3</sup> )	m <sup>3</sup>	X 264.2	=	gal
(advp) ounce (oz)	X 28.3	=	grams (g)	g	X 0.035	=	oz
(advp) pound (lb)	X 0.45	=	kilogram (kg)	kW	X 1.34	=	hp
horsepower (hp)	X 0.75	=	kilowatt (kW)	kg	X 2.2	=	lb
ft per second (ft/s)	X 0.304	=	met. Per second (m/s)	m/s	X 3.28	=	ft/s
ounce-force (ozf)	X 0.278	=	newtons (N)	N	X 3.597	=	ozf
pound-force (lbf)	X 4.448	=	newtons (N)	N	X 0.224	=	lbf
foot pounds (ft.lb)	X 1.355	=	newtons-metres (N.m)	N.m	X 0.737	=	ft.lb
foot pounds (ft.lb)	X 1.355	=	joules (j)	j	X 0.737	=	ft.lb
in. pounds (in.lb)	X 0.112	=	newtons-metres (N.m)	N.m	X 8.85	=	in.lb
lb per foot (lb/ft)	X 14.59	=	newtons-metres (N.m)	N.m	X 0.068	=	lb/ft
cycles per sec (cps)	X 1	=	hertz (Hz)	Hz	X 1	=	cps
Brit therm unit (Btu)	X 1055	=	joules (j)	j	X 0.0009	=	Btu

## Lab 2 - Microscopy and Cells

### Linear measurements

- In the box at right, draw the first two centimeters of your ruler, from 0 to 2 cm. Label the longer lines that indicate centimeters “cm” and the shorter lines that indicate millimeters “mm” on your drawing.

If you’re not used to metric units, you need to tune your brain to it using real objects.

- With your group, estimate the length (in metric units -- m, cm or mm) of the following items. Then go measure them with a ruler or meter stick, write down the actual measurement as well, and convert it into the English system using the conversions above.

Item	Estimate	Measurement	Converted to feet & inches
Distance from your table to classroom door			
Width of classroom doorway			
Laptop screen			
“Q” key on laptop keyboard			

### Butterfly photo: measuring length

On the right is a printed image of a swallowtail butterfly, *Papilio alexenor*, at about life size.

- Measure the length of the butterfly’s body (from head to tail) and the maximum width of its wingspan (from the tip of the left wing to the tip of the right wing), record them below, and do the following calculations.



“Papilio alexenor” is licensed under CC BY SA 4.0.  
A derivative from the original work: “Southern Swallowtail (Papilio alexenor orientalis)”, by Bernard Dupont licensed under CC BY SA 4.0.

Body length: \_\_\_\_\_ mm = \_\_\_\_\_ cm = \_\_\_\_\_  $\mu$ m

Wingspan: \_\_\_\_\_ mm

Ratio of body length to wingspan: \_\_\_\_\_

(Note: When you divide a length by a length, you should get an answer without any units.)

### Wooden block: length, area, and volume

- Measure the length of an edge of your wooden block in cm. The block is a cube, so all its edges are the same length.

Edge length: \_\_\_\_\_ cm

- Use this measurement to calculate its volume and surface area.

$$\text{Volume} = \text{length} \times \text{width} \times \text{depth} = \text{length}^3 = \text{_____ cm}^3$$

$$\text{Surface area} = \text{side area} \times 6 = \text{length}^2 \times 6 = \text{_____ cm}^2$$

## Lab 2 - Microscopy and Cells

### Pebble: measuring volume by displacement

Measure the volume of your pebble by displacement, using the following procedure:

1. Fill your graduated cylinder with about 100 mL of water at the tap.
2. Record the exact initial water volume from the cylinder.
3. GENTLY drop the rock into the water. (Don't splash!)

→ Read the volume again. Subtract the final volume measurement from the initial volume measurement to find the volume of the pebble. ( $1 \text{ mL} = 1 \text{ cm}^3$ )

$$\text{Volume} = \underline{\hspace{2cm}} \text{ mL} = \underline{\hspace{2cm}} \text{ cm}^3$$

→ Would you be able to measure the volume of the pebble with a ruler, the way you measured the wooden block? What makes the displacement method more suitable for the pebble?

### Pebble and wooden block: Mass and density

Density is an important attribute of a material that indicates how much matter is in a given volume of that material. Take the pebble and the block to the mass balance and measure their masses in grams. Then, using the measurements you have just taken, you can now calculate the density of stone and of wood. Notice that the units of density are grams per cubic centimeter: this value would apply to any piece of this material, regardless of its size and shape.

	<i>Pebble</i>	<i>Wooden block</i>
Mass	g	g
Volume (copied from above):	$\text{cm}^3$	$\text{cm}^3$
Density = mass/volume	$\text{g/cm}^3$	$\text{g/cm}^3$
	<i>Density of stone</i>	<i>Density of wood</i>

## Microscopy

In this section, you will do the following:

1. Identify the parts of a compound microscope
2. Calculate the total magnification for each objective lens
3. Use the compound microscope to examine and draw several objects:
  - a. A printed letter "e"
  - b. Colored threads
  - c. Animal cells
  - d. Plant cells
  - e. Organisms in a drop of pond water

*Materials: small clear ruler, mounted specimen of a small organism, letter "e" slide, colored thread slide, Elodea leaf, pond water, toothpick, blank slides, cover slips, methylene blue solution, dissecting microscope, compound microscope*

## Lab 2 - Microscopy and Cells

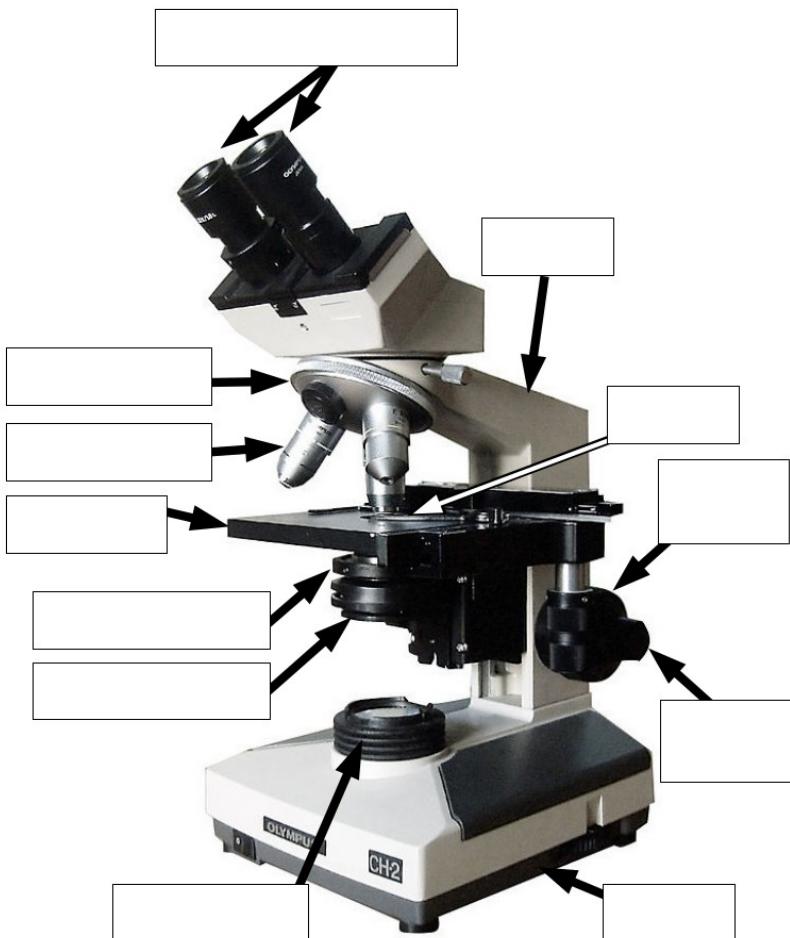
### The compound microscope

The **compound microscope** is the one with the rotating arrangement of several different objective lenses above the stage. The compound microscope is the tool you will use to look at things smaller than you can see with the naked eye. At its highest magnification (4000x), you can see individual bacterial cells.

#### Parts of the compound microscope

→ Label the parts of the compound microscope using the list of terms given here:

Eyepiece	Diaphragm	Slide holder clip
Nosepiece	Condenser	Coarse focus knob
Base	Stage	Fine focus knob
Light source	Arm	Objective lenses



#### Learning to use the compound microscope

You're going to be using the compound microscope in every lab for the next several weeks, so now is your time to figure out how to use it. For the rest of the lab session, you will be practicing focusing on slides of different materials. All the people in your lab group should take turns using the microscope.

The guide on the next page explains the basics of how to use the compound microscope. You may want to bookmark this page for later reference.

The **field of view** is the part of the stage that you can see magnified through the lens. The size of the field of view depends on the total magnification of the lens combination you use.

For each objective lens, multiply its magnification by the ocular lens magnification (10x) to get the total magnification. Then focus your ruler under the compound microscope and figure out how many millimeter marks are visible on the screen. Use that to estimate the width of the field of view at each magnification.

→ Compound scope magnification and field of view with different objective lenses:

Objective lens	Total magnification	Field of view (mm), based on ruler
4x		
10x		
40x		

## Lab 2 - Microscopy and Cells

### ----- Compound microscope procedures -----

#### Focusing a slide:

1. Pick up the slide by the edges or by the label. Don't put your finger over the subject -- your fingertips will leave a mark on it.
2. Press the lever on the top of the stage to open the slide holder clip, then slot the slide into it.
3. Turn the lenses counterclockwise until the lowest objective lens (4x) is in front.
4. Looking through the eyepieces, turn the coarse focus knob until the slide comes into focus. Rotate the knobs on the back of the stage until the subject is in your field of view, then tweak the fine focus knob until you can see it well.
5. If you need to zoom in to a higher magnification:
  - a. Turn the stage control knobs until the part you want to zoom in on is in the center of your field of view.
  - b. Rotate the lenses clockwise to get to the next-lowest lens, without changing the focus. Then refocus on the subject using the **fine focus knob only**.

#### Displaying the microscope image on your lab computer screens:

1. Start the Motic Image Plus application from your lab laptop (lower left corner of desktop).
2. Put it in the live microscope view mode (choose File -> Capture or click the "play" button in the left toolbar).
3. Make sure the following conditions are set:
  - i. Video Device (top right) set to "**Motic 3.0MP**"
  - ii. Light switched **on** (right side of microscope)
  - iii. Plunger on right side of scope (next to red "Motic" label) pulled **out**

#### Notes and tips for microscope use:

- If you take out the slide or change the stage height, you need to start the focusing process again from the 4x lens!
- Use the coarse focus knob ONLY when you're on the lowest objective lens, not when you're at a higher magnification!
- Finding a good subject and getting a good focus on it can take several minutes. Don't get impatient, just keep working on it.
- Looking through the eyepieces takes some practice. Notice that you can adjust their distance to fit the distance between your own eyes. Try with glasses on and off. You can also try looking through one eye and closing the other, or even looking only at the computer screen. Find a method that works for you.
- If you have a lighting problem, raise your hand and the instructor will help you adjust the lighting system as necessary.

Your instructor may also ask you to take pictures of slides at different magnifications; if so, use the instructions given in the "Compound microscope procedures" box above to display the field of view on the lab computer, take screenshots using the Motic Image Plus application, and submit them for credit as your instructor directs.

#### Looking at prepared slides: "e" and threads

In this section, you will look at prepared slides of a printed letter "e" and a stack of colored threads, and use these to explore the optical properties of the compound microscope.

First, look at your letter "e" slide with your naked eye (no microscope).

## Lab 2 - Microscopy and Cells

- In the left space below, draw the “e” as it appears on the slide to your naked eye. Make sure to draw it in the orientation that you see it.

1. “e” slide viewed with naked eye	2. “e” slide viewed at 40x magnification

Now focus it under the **low power objective lens** (4x) and display it on your lab computer screens, using the above procedure.

- In space 2 above, draw the “e” as it appears under the microscope.  
→ What’s the difference between the two drawings?

Finally, use the stage control knobs to move the slide to the right on the stage.

- Did the image that appears in the microscope move **left or right?** (circle one)

Next, focus the slide with colored threads under the low power lens. Notice how you can adjust the focus so that either the top or the bottom of the thread is in focus, but you can’t get all the threads in focus at once. This is because the *focal plane* (the range of distances in which a lens gives a sharp image) is shallower than the thickness of the stacked threads.

Adjust the height of the condenser using the knob on the left side of the stage.

- What does this do to the location of the focal plane?

### Wet mounts of living animal and plant cells

In this section, you will prepare and examine slides of living cells from two sources, an *Elodea* leaf and epithelial cells from your own cheek. Fresh specimens are prepared as **wet mount** slides: that is, a drop of liquid specimen or a dry specimen with a drop of liquid (water or stain) is placed on the slide, then covered with a cover slip. The flatter the wet mount is, the easier it is to focus under the microscope.

#### Plant cells: Elodea leaf

*Elodea* is a genus of aquatic plants collectively referred to as “waterweed”. They are fast-growing and reproduce by fragmentation, which makes them an important part of an ecosystem in their native range and an invasive weed elsewhere. Since they are so easy to keep, they are also commonly used in aquaria and as a model organism in biology labs.

## Lab 2 - Microscopy and Cells

Prepare the *Elodea* leaf wet mount:

1. Take a clean glass slide and place a single drop of distilled water in the center of it.
2. Using the tweezers, pluck one leaf from the *Elodea* sprig and place it on the water drop.
3. Take a glass coverslip, remove the paper, and place it on top of the leaf, then press down lightly to flatten the leaf.  
(The flatter you make it, the easier it will be to look at.)
4. Finally, focus the leaf under the microscope as specified before.

→ In the boxes below, draw the plant cells as they appear under the low, medium, and high power objective lens. Remember the rules of scientific drawing that we learned last week.

40x	100x	400x

### Animal cells: human oral mucosa

Your own body constantly produces and sheds cells. **Epithelial** cells make up the tissues on the surfaces of your body, and are particularly fast-growing and quick to slough off. The inside of your mouth is a tissue called the oral mucosa; it's lined with epithelial cells that form a tissue called stratified squamous epithelium, which forms a protective and fast-healing layer. We will sample these cells today and look at them under the microscope.

Because squamous epithelial cells are colorless, we will be dyeing them with a stain called **methylene blue**, which binds to cell membranes and DNA and makes them more visible.

Prepare the cheek cell wet mount:

1. If you like, go to the water fountain outside and rinse your mouth with water.
2. Take a clean glass slide and a toothpick.
3. Unwrap the toothpick and gently scrape it against the inside of your cheek to pick up some cells.
4. Then roll it across the middle of the slide to smear the cells off onto it.
  - If you see a little bit of grainy colorless material on the slide, congratulations, you've got it.
5. Put one drop of methylene blue on top of the cell smear.
6. Top it with a cover slip, laying it down carefully at an angle to minimize bubbles.
  - If necessary, use a cloth to blot away excess methylene blue at the edge of the cover slip.

Focus these cells under the microscope. The instructor will show you an example on the screen at the front of the room.

→ As with the *Elodea* cells, draw them at low, medium, and high magnification in the boxes.

## Lab 2 - Microscopy and Cells

40x	100x	400x

### E. Pond water

Once you are done with the preceding parts, the instructor will help you prepare a drop of pond water in a well slide.

- How many different kinds of things can you spot in the water? Which ones are living organisms? Can you find some that are moving around?
  - Try different objective lenses. What level of magnification gives you the best view of each organism?
- In the boxes below, draw at least three different organisms from the pond water sample. Next to each drawing, write the magnification that you used to get that image. If you're having trouble finding three different organisms, ask the instructor for help.

Magnification:	Magnification:	Magnification:

## Cleanup

Clean up your station before you leave.

1. Return your "e" and thread slides to the box. Discard the onion and cheek slides.
2. Put as much pond water as possible back in the pond aquarium. Wash and dry your deep well slides carefully and put them back in the box. Discard the cover slip.
3. Reset the microscopes:
  - a. Turn the light off and close the Motic Image Plus software.
  - b. Put the compound scope on its lowest (shortest) objective lens and lower the stage to its lowest position.
  - c. Get an alcohol wipe and clean the glass parts of the eyepieces and objective lenses.
  - d. Put the covers back on both scopes.
4. Spray your lab bench with cleaner and wipe it down.

# Lab 3: Diffusion and Osmosis

## Learning Goals

1. Identify cell structures.
2. List the differences between prokaryotes and eukaryotes.
3. List the differences between plant and animal cells.
4. Define diffusion and osmosis.
5. Define hypertonic, hypotonic and isotonic solutions.
6. Describe the importance of the plasma membrane.

## Agenda

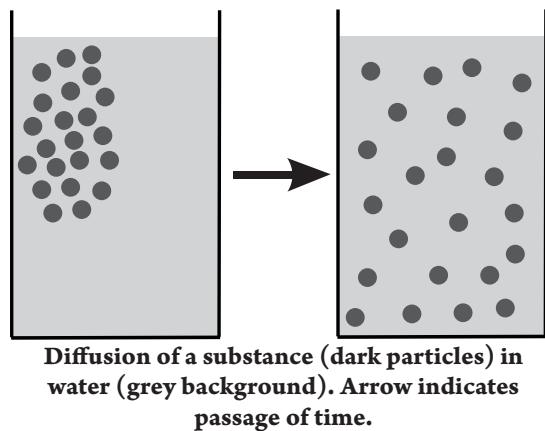
1. Roll call; read Introduction; pre-lab briefing
2. Diffusion and osmosis experiments
  - a. Observing diffusion under the microscope
  - b. Comparing diffusion rates in agar and water
  - c. Diffusion through dialysis tubing (“plasma membrane” simulation)
  - d. Osmosis in potatoes
3. Observe osmosis in plant and animal cells
  - a. Effects of solutions on plant cells (*Elodea*)
  - b. Effects of solutions on animal cells (red blood cells)
4. Plant and animal cell models
5. Clean up

## Introduction

In any substance, molecules constantly move around and bump into each other at random in a process called Brownian motion. The higher the temperature, the more they move. The distance they can move is controlled by the state of matter that they’re in: molecules in a solid can only move a small distance, while those in a liquid can wander around more freely, and the molecules in a gas can move quite a long way. This random movement and collision means that, over time, molecules tend to get spread out evenly as far as they can go. For example, if a small amount of a particular molecule in liquid form is dropped into a large amount of a different liquid, the random motion of both kinds of molecule will eventually result in them being evenly mixed throughout the entire volume.

The result of this is that molecules tend to move away from areas where they are more concentrated and toward areas where they are less concentrated. A difference in concentration between two adjacent areas is called a concentration gradient. The molecules are said to move “down” the concentration gradient (from areas of high concentration to areas of low concentration) in a process called **diffusion**, illustrated at right. When diffusion has gone on for long enough, the system will be evenly mixed and no concentration gradient will exist; at this point, although molecules keep moving randomly, there will be no dominant direction of movement overall.

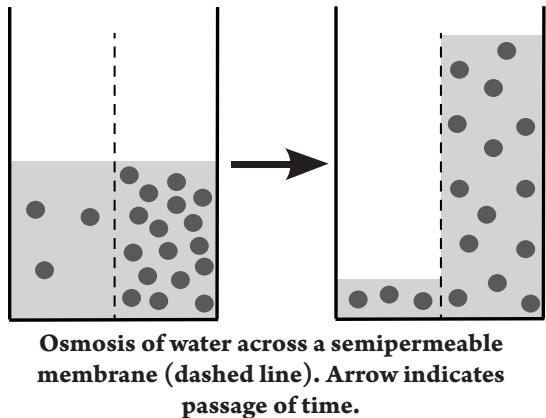
If a diffusion gradient is interrupted by a membrane whose pores are large enough for water molecules to pass through it, but not large enough for biological molecules, called a semipermeable membrane, the result is that only the water diffuses across the membrane. The water goes from the side with a higher water concentration to the side with the lower water concen-



## Lab 3: Diffusion and Osmosis

tration. This diffusion of water through a selectively permeable membrane is called **osmosis**, and is illustrated at right.

A concept called **tonicity** describes the relationship between the inside and the outside of a cell, or indeed any other bag made of a semipermeable membrane. If the solution outside the bag is lower in solutes (higher water concentration) than the solution inside the bag, it's described as being in a **hypotonic** solution, and water will flow through the membrane into the bag. If the solution outside the bag is higher in solutes, it's a **hypertonic** solution, and in that case water will flow out of the bag. If the two sides of the membrane have the same proportion of solutes to water and therefore the same tonicity, the solution is described as **isotonic**.



## Diffusion and osmosis

### Diffusion through liquid and solid matter

In this part, you will observe diffusion of a dye through different substances. The dyes we will use are potassium permanganate ( $\text{KMnO}_4$ ) and methylene blue. First, you will watch the process of diffusion and compare the diffusion speed of two different dyes in water and agar. Agar is a clear polysaccharide jelly derived from seaweed, widely used in both biology labs and food production.

*Materials: agar plate, empty plate lid, straw, water, potassium permanganate solution, potassium permanganate crystals, methylene blue, white paper, ruler*

→ Set up diffusion in solid agar jelly and water.

1. Use the straw to put two holes in the agar in the agar plate. Set the plate down on a white piece of paper.
2. Fill the empty plate with enough water to cover its bottom completely. Set it down on the white paper as well, and wait until it stops sloshing.
3. Your instructor will come to each table and add the dyes: a very small pinch (5-10 crystals) of the dry potassium permanganate crystals into the center of the water in the plate, a drop of potassium permanganate solution in one hole in the agar, and a drop of methylene blue solution in the other hole. When they add the crystals to the water plate, start your timer.
4. Measure and record the diameter of the dye spot in the water at the times given in the table below. Stop recording once the dye has filled the whole plate.
5. Measure and record the diameter of the dye spot in the agar at the times given in the table below.

→ Record your results in the table.

Time (min)	Methylene blue in agar (cm)	KMnO <sub>4</sub> in agar (cm)	KMnO <sub>4</sub> in water (cm)
0			
5			
15			
30			
60			

## Lab 3: Diffusion and Osmosis

Calculate the speed of each diffusion experiment.

Speed of methylene blue diffusion in agar (mm/min): \_\_\_\_\_

Speed of  $\text{KMnO}_4$  diffusion in agar (mm/min): \_\_\_\_\_

Speed of  $\text{KMnO}_4$  diffusion in water (mm/min): \_\_\_\_\_

- Compare the three different speeds of diffusion. Why is there a difference? (Express your answer in terms of molecule size and how molecules move in solids and liquids.)

### Diffusion across a semipermeable membrane

In this part you will see the effects of osmosis and diffusion across a semipermeable membrane. We will use an artificial semipermeable membrane called dialysis tubing, which is essentially a sheet of cellophane with very small holes in it. The holes in this dialysis tubing allow small molecules to pass through, but not large ones. The substances you will add to the system are water ( $\text{H}_2\text{O}$ ), glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ), starch ( $(\text{C}_6\text{H}_{12}\text{O}_5)_n$  -- that is, many glucose molecules bonded together), and Lugol's iodine, the last being a mixture of potassium iodide ( $\text{KI}_3$ ) with elemental iodine (I) that changes from orange-brown to dark blue in the presence of starch. The results of this procedure will show you which of these molecules are small enough to pass through the pores in the dialysis membrane and which are too large.

*Materials: precut presoaked dialysis tubing, thread or clips, beaker, water, starch/glucose solution in squeeze bottle, iodine solution, sugar test strip*

- Set up a semipermeable membrane separating solutions of glucose, starch, and iodine in water to observe the process of osmosis and see which molecules can pass through its pores.

1. Take a segment of dialysis tubing and fold it over about 1 cm from one end, then clip or tie it in the middle of the folded section to seal the end tightly.
2. Open the other end of the tube and insert the nozzle of the squeeze bottle of starch/glucose solution. Squeeze the bottle to fill your tube about two-thirds full.
3. Fold and either clip or tie the top end of the tube as you did the bottom, forming a "sausage" filled with starch/glucose solution. Rinse it under the tap to get rid of any solution that got on the outside.
4. Weigh the tube and record the weight here: \_\_\_\_\_ g
5. Put the tube in the beaker, add enough water to cover it completely, and add 2-3 drops of iodine solution. Start a timer for 45 minutes. (Do part III while you wait.)
6. When the timer finishes, weigh the tube again and record the weight: \_\_\_\_\_ g
7. Using a glucose test strip, test the water in the beaker.

Questions:

- How much water did the tube gain or lose by osmosis? \_\_\_\_\_ g (\_\_\_\_\_ mL)

- Fill in the following results table. To test for glucose, compare the color of your glucose test strip to the key on the package. To test for iodine and starch, look for the characteristic blue color that results from the reaction between iodine and starch.

## Lab 3: Diffusion and Osmosis

Molecule:	Water	Glucose	Starch	Iodine
Present in beaker at end?	-----			
Present in tube at end?	-----			
Passes through the dialysis membrane?				

### Tissue in hypertonic and hypotonic solution

**Tonicity** is the relative concentration (amount of solutes) of two different solutions. Most often, one solution's tonicity is described in relation to another solution: a **hypertonic** solution has a higher concentration than the other solution you're comparing it to, while a **hypotonic** solution has a lower concentration than the one you're comparing it to. When they're the same, you call it **isotonic**. In the context of living cells, the one we are describing as hypertonic or hypotonic is usually the solution outside the cell, and we are comparing it to the inside of the cell. In this part, you will compare the effects on living plant tissue (a piece of potato tuber) of being immersed for one hour in a hypertonic, an isotonic, and a hypotonic solution.

→ Set up an experiment to measure the effects of tonicity on potato tissue.

1. Weigh three potato pieces and record their weights in the table below.
2. Immerse one in DI water (hypotonic solution) and the other in the saline solution provided (hypertonic solution).
3. Wait 1 hour. (Work on sections B and C during this time.)
4. Weigh them again at the end of the hour and record the new weight in the table.

→ Complete the following data table.

Solution	Initial weight	Final weight	Difference
Deionized water (hypotonic)			
0.9% saline (isotonic)			
10% saline (hypertonic)			

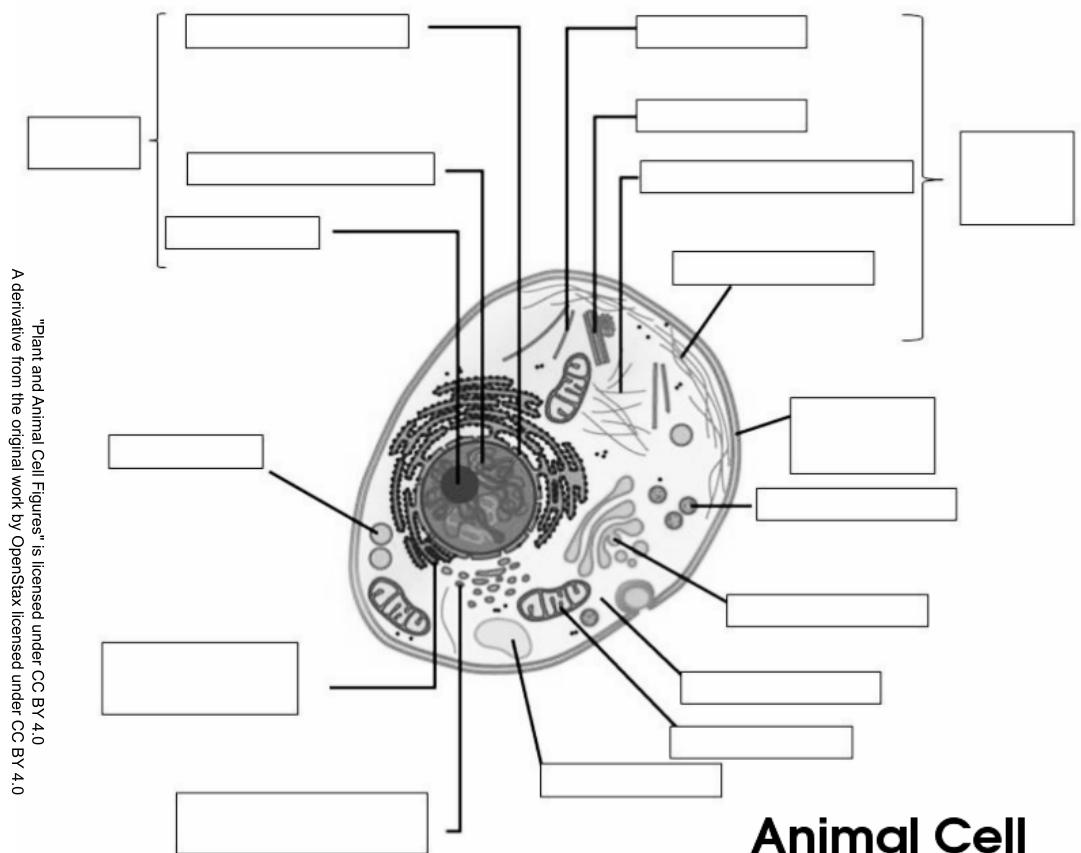
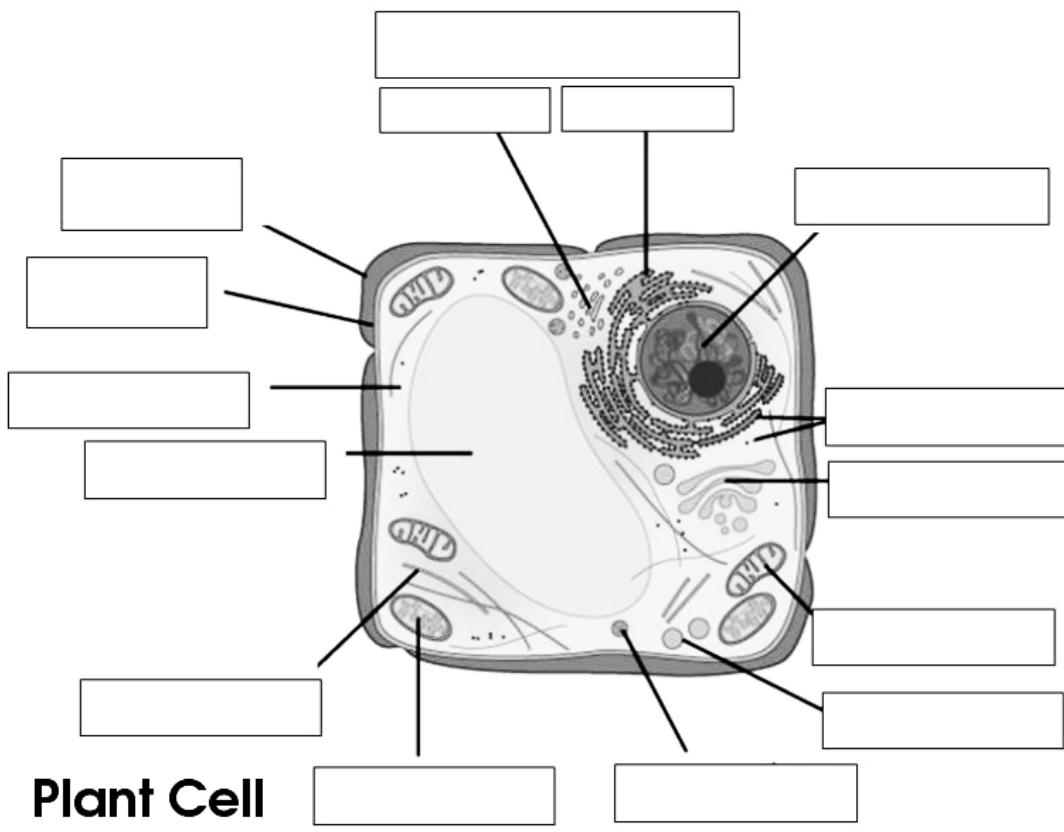
→ What accounts for the difference in weight between each potato piece at the beginning and at the end?

### Plant and animal cells

#### Cell models

Complete and label the animal and plant cell drawings on the following pages. Then use the colored tape (a color will be assigned to your group) to label the 3D animal and plant cell models with the cell parts named on the list posted next to them. Make sure there's a label with your group number and section number in the picture. The instructor will come check your answers when you're done labeling the models. Once you're done taking your pictures, peel your tape pieces off so the next group can do it.

## Lab 3: Diffusion and Osmosis



## Lab 3: Diffusion and Osmosis

→ Label the following parts on the plant and animal cell models.

Mitochondrion	Golgi apparatus	Bound ribosome	Free ribosome
Nucleus	Endoplasmic reticulum	Lysosome	Cell membrane
Cytoplasm	Chloroplast (plant only)	Nucleolus	Spindle (animal only)
Cytoskeleton	Central vacuole (plant only)	Cell wall (plant only)	Plastid (plant only)

## Relative sizes of different cells

Use the laptop on your lab table to go to this website: <https://learn.genetics.utah.edu/content/cells/scale/>

→ Run the application. Use it to discuss and answer the following questions:

How many times larger is a skin cell than a red blood cell? \_\_\_\_\_ times

How many times longer is the smallest cell shown in this chart (*E. coli* bacterium) than the largest virus (measles virus)?

\_\_\_\_\_ times

If you wanted to look at a paramecium under the microscope so that it filled the microscope's field of view completely, how wide would the field of view need to be? Give your answer in **millimeters**.

\_\_\_\_\_ mm

## Observing osmosis in living cells

In this section, you will demonstrate the effects of hypertonic and hypotonic solutions on living cells. **Before you start this section**, set up your microscope to project to the computer screen, as instructed in last week's lab. This should make the current microscope image display on your screen. If it doesn't, ask your instructor for help.

### Plant cells (*Elodea*)

You will first test the effects of different osmotic pressures on plant cells.

→ Prepare a wet mount of *Elodea* in deionized (DI) water, a hypotonic solution.

1. Place one drop of DI water on a slide.
2. Remove an *Elodea* leaf from the plant and place it flat on the slide.
3. Put a cover slip on top of the leaf and press it down gently to eliminate bubbles.

Examine the leaf with the compound microscope under 400× magnification (i.e. the 40× objective). (Reminder: start at 4×, focus with the coarse focus knob, and center the leaf; then switch to 10×, focus with the fine focus knob, and center on an interesting spot; then switch to 40× and focus with the fine focus knob one more time.) This is what *Elodea* cells look like in the **hypotonic** water where they live.

Next, you will examine the cells' reaction in two unknown solutions A and B. One of these is hypertonic and the other is hypotonic; you will determine which is which.

## Lab 3: Diffusion and Osmosis

- Prepare wet mounts of *Elodea* in the two different unknown solutions.
4. Label two slides “A” and “B”.
  5. Prepare these slides with a single drop of liquid as before, but instead of DI water, use a drop of solution A on the first slide and a drop of solution B on the second slide.
  6. Put cover slips on the slides and gently press down to eliminate bubbles.

Examine both slides under the 40x objective and make a drawing of each. Discuss your observations with your group.

Solution A	Solution B
Tonicity:	Tonicity:

Questions:

- Which solution is hypotonic and which is hypertonic? Label the drawings accordingly in the bottom row where it says “Tonicity”, and indicate the features that led you to this conclusion.
- What effects did the two solutions have on the cells and why? Answer in terms of osmotic pressure and the movement of water in and out of the cell.

## Animal cells (red blood cells)

Next you will observe the effects of hypotonic and hypertonic solutions on animal cells, specifically red blood cells. Your instructor will prepare three blood slides and show them on the screen at the front of the class. One will be prepared with a hypotonic solution, another with a hypertonic solution, and the third in isotonic solution.

## Lab 3: Diffusion and Osmosis

→ Observe the red blood cells in each solution and draw them in the boxes.

Solution A	Solution B	Solution C
Tonicity:	Tonicity:	Tonicity:

Questions:

- What effects did each solution have on the red blood cells and why? Answer in terms of osmotic pressure and the movement of water in and out of the cell.
- What factor makes plant cells' reactions to hypertonic and hypotonic solutions different from those of animal cells?

## Cleanup

Clean and replace all the equipment you used.

1. Throw away your potato pieces and dialysis tubing, scrape the agar out of the dish and discard it, and pour the water down the sink.
2. Wash and dry all your glassware and return it to where you picked it up.
3. Clean up your *Elodea* slides.
  - a. Peel the leaf off and throw it away.
  - b. Rinse off the slide, dry it, and put it in the box.
4. Reset your microscope.
  - a. Return it to beginning position: stage down, objective lens to 4×, lights off, Motic application closed.
  - b. Wipe the eyepieces and lenses well with a dry wipe. If that doesn't get all the smudges off, repeat the procedure with an alcohol wipe.
  - c. Put the cover back on.

# Lab 4: Microbiology

## Learning Goals

1. Understand the diversity of microorganisms.
2. Identify the three bacterial shapes and a variety of protists observed.
3. Identify the common features of fungi and the structures of a typical fungus.
4. Make and view a stained bacterial smear slide.
5. Formulate and test hypotheses about environmental microbiology.

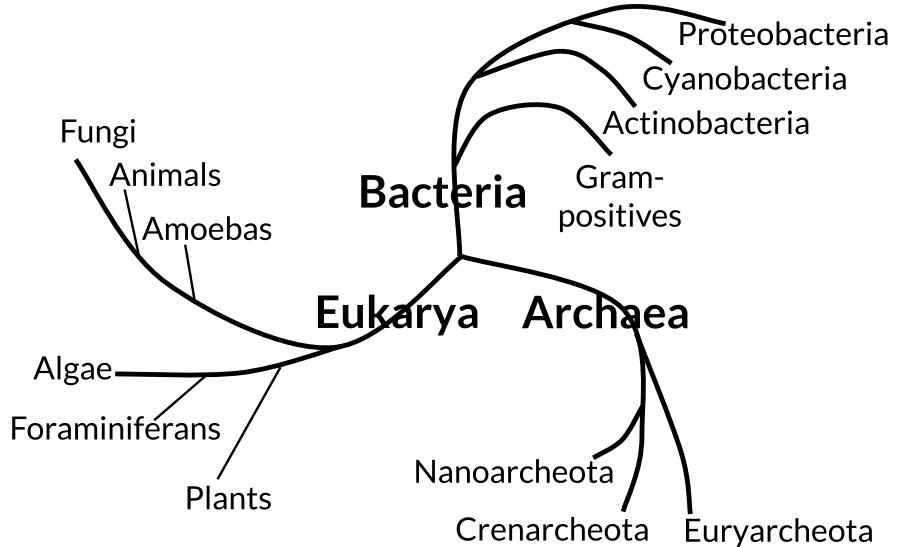
## Agenda

1. Microbial diversity
  - a. Drawings from fixed slides
  - b. Smear/stain slides
2. Set up environmental microorganism experiment
3. Handwashing with luminol

## Microbial diversity

Microbiology is the study of microorganisms, which are also called microbes. It therefore encompasses all organisms whose mature forms are too small to see without a microscope, including almost all unicellular organisms and those made up of only a few hundred cells.

The phylogenetic diversity of this group is much, much larger than that of macroscopic organisms: while almost all macroscopic organisms belong to the domain Eukarya, there are numerous species of microorganisms in each of the three domains, including all Bacteria and Archaea as well as members of every kingdom of Eukarya. (see figure at right). Microbes therefore account for the majority of biodiversity on our planet. For most of Earth's history, they were the only life forms; from the origin of life about 3.8 billion years ago up to some time in the last billion years when multicellularity became common, it appears all or almost all organisms were unicellular. Even today, the majority of biomass on Earth probably consists of microbes.



Phylogenetic tree of all three domains of life. Note the three-way split at the base of the tree and branching relationships between all the members of each branch.

Bacteria and Archaea have a metabolic flexibility that eukaryotes lack, and as a result have adapted to live in nearly every environment on Earth and eat almost everything, including substances toxic to eukaryotic life such as acids, metals, petroleum and natural gas. Bacteria, especially, have an amazing variety of metabolic pathways; some of them don't even perform normal cellular respiration, instead using other reactions to produce the energy they need to live. The field of microbiology has only begun to explore the diversity of microbial life, and by some estimates only about 1% of microbial species have been identified by scientists.

## Lab 4: Microbiology

# Microbe drawings

View each of the slides of microbes. For each one, make a drawing of it in the space indicated.

Slides: bacterial shapes (bacillus, coccus, and spirillum), cyanobacteria (Oscillatoria and Anabaena), algae (Spirogyra, Volvox, and diatoms), protozoans (amoeba and Paramecium), and fungi (Penicillium and Coprinus).

Slide name	Organism classification	Drawing
Bacteria cell types	Domain: Bacteria	(Draw a small group of each shape.)  Bacillus (rod):    Coccus (sphere):    Spirillum (spiral):
<i>Oscillatoria</i>	Domain: Bacteria  Kingdom: [not defined]  Phylum: Cyanobacteria  Class: Cyanophyceae  Order: Oscillatoriales  Family: Oscillatoriaceae  Genus: <i>Oscillatoria</i>	(Draw several colonies.)

## Lab 4: Microbiology

Anabaena	Domain: Bacteria Kingdom: [not defined] Phylum: Cyanobacteria Class: Hormogoneae Order: Nostocales Family: Nostocaceae Genus: <i>Anabaena</i>	(Draw several colonies.)
Spirogyra	Domain: Eukarya Kingdom: Plantae Phylum: Charophyta Class: Zygnematophyceae Order: Zygnematales Family: Zygnemataceae Genus: <i>Spirogyra</i>	(Draw at high enough magnification to see the cells' internal structure.)
Volvox	Domain: Eukarya Kingdom: Plantae Phylum: Chlorophyta Class: Chlorophyceae Order: Chlamydomonadales Family: Volvocaceae Genus: <i>Volvox</i>	(Draw one colony. Adjust condenser height to increase depth of field.)
Diatoms	Domain: Eukarya Supergroup: SAR Kingdom: Heterokonta Phylum: Ochrophyta Class: Bacillariophyceae	(Draw several different shaped diatoms.)

## Lab 4: Microbiology

Amoeba	Domain: Eukarya Kingdom: Unikonta Phylum: Amoebozoa Class: Tubulinea Order: Tubulinida Family: Amoebidae Genus: <i>Amoeba</i> Species: <i>Amoeba proteus</i>	(Draw one amoeba in detail. Note its shape and internal features.)
Paramecium	Domain: Eukarya Supergroup: SAR Kingdom: Alveolata Phylum: Ciliophora Class: Oligohymenophorea Order: Penicula Family: Parameciidae Genus: <i>Paramecium</i>	(Draw one paramecium in detail. Note its shape and the small hairs on the outside.)
<i>Saccharomyces</i> (bread/beer yeast)	Domain: Eukarya Kingdom: Fungi Phylum: Ascomycota Class: Saccharomycetes Order: Saccharomycetales Family: Saccharomycetaceae Genus: <i>Saccharomyces</i> Species: <i>Saccharomyces cerevisiae</i>	(Draw several cells. If possible, draw one that is in the process of budding.)

## Lab 4: Microbiology

<i>Penicillium</i>  (bread mold responsible for penicillin)	Domain: Eukarya  Kingdom: Fungi  Phylum: Ascomycota  Class: Eurotiomycetes  Order: Eurotiales  Family: Trichocomaceae  Genus: <i>Penicillium</i>	(Draw the edge of the clump. Note the brush-shaped fruiting structures.)
<i>Coprinus</i> l.s.  (a mushroom, the fruiting body of a fungus. Not really a microbe!)	Domain: Eukarya  Kingdom: Fungi  Phylum: Basidiomycota  Class: Agaricomycetes  Order: Agaricales  Family: Agaricaceae  Genus: <i>Coprinus</i>	(Draw the gills and spores.)

**Questions:**

- Based on the phylogenetic tree on page 1, which of these organisms are most closely related to each of the following? (There may be more or less than one answer.)
- Land plants (Eukarya > Plantae > Chlorophyta > Embryophyta)?
  - Kelp (Eukarya > SAR > Heterokonta > Phaeophyceae > Laminariales)?
  - *E. coli* (Bacteria > Proteobacteria > Gammaproteobacteria > Enterobacteriales > Enterobacteriaceae > *Escherischia coli*)?
  - Volcanic hot spring microbes (Archaea > Crenarcheota > Thermoprotei > Sulfolobales)?
  - You (Eukarya > Animalia > Chordata > Mammalia > Primates > Hominidae > *Homo sapiens*)?

## Lab 4: Microbiology

# Studying microorganisms

Microbes are everywhere: billions of them live on every surface and in every environment in the Earth, from the surface of your own skin to tiny cracks deep in Earth's crust. However, although people had suspected the existence of tiny invisible beings since ancient times, the technology to actually see them was not developed until the 1700s. The discovery, in the 1800s, that some microbes cause diseases (these are called **pathogens**) prompted scientists to pay them more attention. Researchers developed techniques to isolate, grow, and examine microbes in the laboratory.

The traditional techniques of microbiology include microscopy and culture, both of which you will get to try during this lab. These techniques provide enough information about differences between microbes that scientists were able to begin describing and categorizing them, based on only a few traits:

- their cell shape
- the appearance of cultured colonies
- what food sources they eat
- what waste products they produce

However, this limited scientists' ability to find different microbes and tell them apart from each other. Many microbes can't be grown outside their natural environments, and they can be very difficult to tell apart under a microscope. Those that couldn't be grown in the lab, and those which couldn't be told apart based on their appearance, food, or waste products, were basically impossible to study until DNA sequencing was invented.

Modern DNA sequencing allows us to get the complete genome of a single cell taken directly from the wild, or to mix together the genetic information of a whole sample from a particular environment to see what genes are most common in that environment. From this, we have learned that many microbes live in diverse interdependent communities and have complex ecological interactions, and also that the phylogenetic diversity of microbes is far vaster than we had ever expected. This is an active area of research, with new discoveries made every day.

## Mouth cell smear

You can see the shapes of microbes by simply making a slide. In this section, you will make a smear of human and bacterial cells from your own mouth.

So far this term, you have been preparing slides using the *wet mount* technique, in which the subject is suspended in a drop of liquid between the slide and the cover slip. In this section, you will prepare slides of bacterial cells using a different technique. A *smear* is a method of slide preparation in which the subject is spread onto the surface of the slide and allowed to dry. No cover slip is used.

### Instructions:

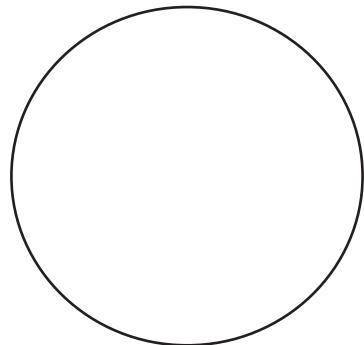
1. Run a toothpick around your gumline to collect a very small sample of cells. (If there's a visible glob of white material on the toothpick, that's more than enough.)
2. Put a single drop of water on a slide, put the toothpick in it, and mix well to disperse the sample into the water.
3. Add a single drop of methylene blue and mix again.
4. Set the slide aside (without a cover slip!) to let it dry. This will take at least 5 minutes.

Once it's dry, examine it under the microscope. You will see your own epithelial cells, whose appearance will be familiar from the cheek cells you sampled for lab 2 (like little fried eggs), as well as some shapeless dark lumps of cellular debris. On the epithelial cells and throughout the material you will see small dark flecks; these are bacteria.

## Lab 4: Microbiology

### Questions:

- Find an epithelial cell with bacteria on it. Note the size of the bacterial cells relative to the human cells. Draw a picture of it at right, focusing on illustrating the contrast in shape and size between the two cell types. (It may take you a while to find a good place to look on the slide, so keep looking around until you find a clear example.)
- How much larger are the eukaryotic (epithelial) cells, compared to the bacterial cells? Give an approximate number.



Human mouth epithelial and bacterial cells, smear , 400x magnification

## Environmental microbes

There are microbes all around you and inside you, wherever you are, and they are so small they move from one place to another with almost no effort. When you touch something, when you flush the toilet, even when dust just settles on a surface, environmental microbes are transferred. Most of the time this is no problem, because only a small proportion of microbes are **pathogenic** (cause illness), and most of those need to get into a cut or land on your mucous membranes to get started. However, in environments where contamination can cause major problems, such as in an operating room or a microbiology lab, avoiding the transfer of microbes is a constant effort.

## Environmental microbiology experiment

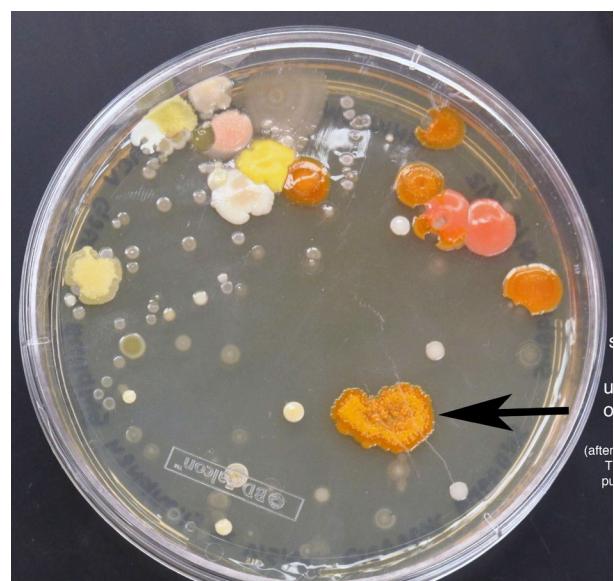
In this section, you will design and execute an experiment to test an environmental microbiology hypothesis of your own devising. Your samples need to incubate, so you will prepare them this week, set them in the incubator, and next week see what you grew.

- With your group, come up with a question regarding presence of more/fewer microbes (5-second rule, water temperature, surface touching, metal disinfection, wet vs dry surface...) and formulate your hypothesis.

### Question:

### Hypothesis:

- With your group, design an experiment to test your hypothesis. Make sure your experiment has the appropriate control. Use the space provided to outline and/or draw out your experiment. Have your instructor approve your experimental design and then begin collecting your samples.



Results of growing an environmental microbe sample on agar. You will make something like this. (Credit: Jnims on Wikimedia Commons, under CC-BY 3.0)

## Lab 4: Microbiology

### **Experimental design:**

### **Prediction:**

### **Sampling Directions:**

1. On the bottom of your group's agar plate, draw lines to cut it in four equal sections. On the top, write your group's name, number, or some other identifying information so you can find your plate next week.
2. Take a sterile swab and open it up carefully.
3. Dip the cotton end of the swab into the tube of water, squishing and rolling it against the bottom of the tube to get the air out of the cotton. Once you stop seeing bubbles, withdraw the swab to above the water line, roll it around the inside of the tube again to squeeze the excess water out.
4. Roll the swab back and forth on your chosen surface five to ten times so that the damp swab picks up anything that's living on it.
5. Open up your agar plate and roll the swab back and forth in the sector of the plate that you labeled. This will transfer the microbes you picked up onto the growth medium and let them start growing.
6. Close it back up and repeat until all four sectors are filled.
7. Tape the plate shut on both sides and put it in the incubator. Next week, you will see what you grew...

## Lab 4: Microbiology

### Spreading germs

Washing your hands is widely regarded as one of the most effective ways to stop the spread of pathogenic microorganisms, which are often colloquially called “germs”. However, it matters a lot how you do it. In this section, you will use a substance called “Glo-Germ” to simulate getting germs all over your hands. Glo-Germ glows under ultraviolet (UV) light, and is designed for teaching medical professionals good handwashing technique.

#### Instructions:

1. Take a small blob of Glo-Germ oil, about the size of a quarter, in the palm of your hand. Spread it over both your hands, as if you were applying lotion. Make sure to cover your hands completely, front and back, and get it between your fingers and in and around your fingernails. Wipe off the excess with a paper towel.
  - a. One person in your group should be excused from this. They will run the stopwatch during the handwashing part.
2. Place your hands under the UV light to see how many “germs” are on your hands before you wash them.
3. Now wash your hands. The person without Glo-Germ on their hands should time everyone’s handwashing.
  - a. At least one group member should wash their hands very thoroughly as though for a medical procedure.
  - b. Another should do it quickly, as if they don’t think they’re particularly dirty.
4. Put your washed hands back under the UV lamp to see how good of a job each of you did at getting rid of the “germs” on your hands.

#### Questions:

- What handwashing technique worked the best to remove the Glo-Germ? Discuss what each of you did differently.
- What other factors affected how clean you could get your hands? (Cracked or chapped hands? Calluses? Fingernail length? Artificial fingernails?)

Last, you will use Glo-Germ powder to demonstrate how well you have been cleaning your bench this term.

#### Instructions:

1. Lightly dust an area of your bench with a small amount of Glo-Germ powder. Use the brush to distribute it evenly.
2. Clean it with lysol spray and a paper towel, as you usually do. Be honest.
3. Use the UV light to look for any remaining “germs”.

#### Questions:

- Have you been doing a good job cleaning your bench (and other germy surfaces in your life), or is there something you need to do differently?

## Lab 4: Microbiology

### Cleanup

Clean up the slides, sampling equipment, and Glo-Germ.

1. Return all the slides to their correct locations in the trays. Be careful not to mix them up or drop them.
2. Discard the wet mount and smear slides.
3. Reset the microscope as usual.
  - a. Turn off the light
  - b. Wipe the stage, objective lenses, and condenser to get rid of any remaining methylene blue
  - c. Use the coarse focus knob to move the stage all the way down
  - d. Turn the objective lens to 4x (scanning power)
  - e. Put the cover on the microscope
4. Discard all disposable equipment used to make slides and plates, including loops, toothpicks, and swabs. The water tubes used for sampling do not need to be discarded.
5. Make sure your group's agar plate is labeled, taped shut, and placed in the incubator.
6. Clean up any spilled Glo-Germ liquid or powder, and wipe your bench with lysol solution.

# Lab 5: Respiration and Photosynthesis

## Learning Goals

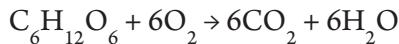
1. Name the reactants and products of both photosynthesis and cellular respiration in plants and animals.
2. Identify the function of cellular respiration and photosynthesis.
3. Identify components of the leaf important for photosynthesis
4. Identify the anatomical structures of a leaf, including the cuticle, epidermis, palisade mesophyll, spongy mesophyll and stomata.
5. Describe what happens in the light and dark reactions of photosynthesis.
6. Explain the role of the mitochondria and oxygen in cellular respiration.

## Agenda

1. Lab Introduction
2. Cellular respiration in yeast
3. Chemical products of photosynthesis
4. Microscopic examination of cells and tissues
5. Plant pigment chromatography
6. Cleanup

## 1. Cellular respiration in yeast

All living cells fuel themselves by a chemical reaction called **respiration**: they consume glucose and oxygen to produce energy, emitting water and carbon dioxide as byproducts.



Yeasts are eukaryotic cells, specifically single-celled fungi. All fungi are **heterotrophs** (hetero = other, troph = feed); that is, they cannot produce their own food as plants do, but instead must digest external food sources for energy. Yeasts are specialists in the direct digestion of sugars. The type of yeast most widely used by people is *Saccharomyces cerevisiae*, which is involved in the production of bread, wine, beer, and a wide variety of other foods. Yeast cells are about 10 micrometers across, and one gram of instant yeast holds about 25 billion of them.

In this section, you will set up flasks with yeast and different concentrations of yeast food (sugar), then observe the amounts of carbon dioxide produced by the yeast cells' respiration and fermentation over the next hour.

### Procedure

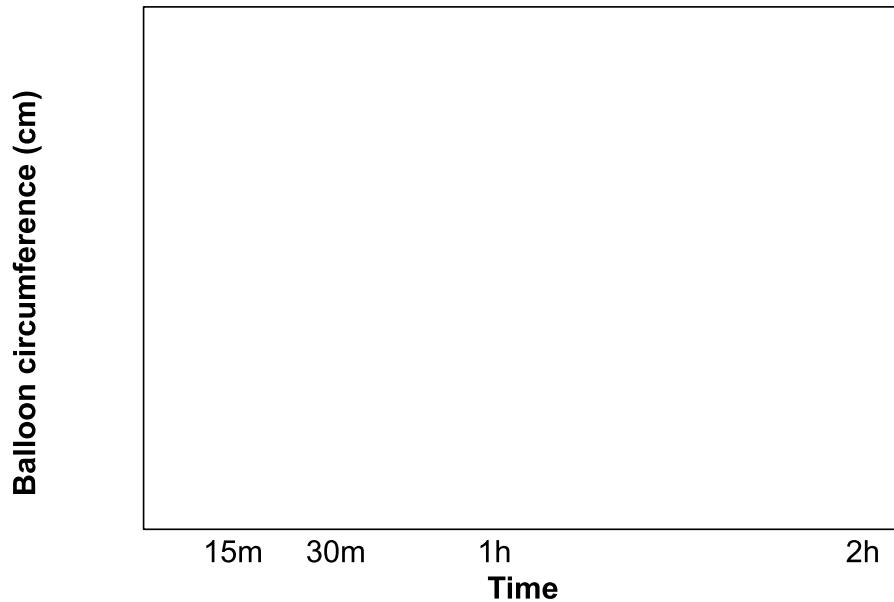
1. Prepare four flasks labeled "0%", "10%", "50%", and "Sat."
2. Add 10 mL of the prepared yeast solution and 30 mL of the appropriate sucrose solution to each one.
3. Prepare a yeast slide by placing 1 drop of mixed yeast and 10% sucrose solution from the 10% flask on a slide and cover with a cover slip. Put it aside to examine later in the lab (in part 3).
4. Cover each flask with a balloon, secure the balloon with a rubber band, and set them all on your lab bench. Set a timer.
5. At the indicated times, observe the amount of CO<sub>2</sub> in the four balloons. Measure the circumferences of the four balloons with the flexible measuring tape and record your measurements in the table. Between observations, work on the other parts of the lab.

## Lab 5: Respiration and Photosynthesis

→ Record your measurements of the balloon circumferences in the table below.

	Circumference of balloon at each sucrose concentration			
Time	0%	10%	50%	saturated
15 minutes				
30 minutes				
1 hour				
2 hours				

Graph your results. Fill in the numbers you need for balloon circumference on the y-axis. Use four different lines to show the results for the four different bottles.



Questions:

- How long did it take for the yeast to get started on its cellular respiration process?
- Did the yeast perform cellular respiration when you didn't provide it with any sugar? (i.e. does it contain or produce its own sugar?)
- Were any of the sugar solutions in the experiment too concentrated for the yeast to use?
- What are the reactants and the products in cellular respiration?

**Reactants:**

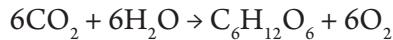
**Products:**

## Lab 5: Respiration and Photosynthesis

→ If you kept running this experiment for several more hours, what reactant do you think would get used up first?

## 2. Respiration and photosynthesis in *Elodea*

Plants are **autotrophs** (auto = self, troph = feeding): they consume glucose in respiration to fuel their cells, just like any other organism, but unlike heterotrophs they produce their glucose themselves. The chemical reaction that captures light energy and stores it as glucose is called **photosynthesis**. They start with water and carbon dioxide, capture light to synthesize the hydrogen from water and carbon from CO<sub>2</sub> into glucose, and release oxygen as a byproduct.



Notice that this is exactly the opposite of respiration! The laws of physics dictate that if a reaction releases energy, as respiration does, its opposite must consume energy. The energy consumed in photosynthesis comes from light harvested by the green pigment molecule **chlorophyll**, which contains at its center a magnesium atom that captures photons and stores the energy in a molecule called **ATP**. This is the first step of photosynthesis, called the **light-dependent reactions** (or light reactions): chlorophyll consumes light and water to produce ATP and release oxygen. In the second step, called the **light-independent reactions** (or dark reactions or Calvin cycle), a protein called **rubisco** uses the energy in the ATP to take the carbon atoms from carbon dioxide and reassemble them into the sugar **glucose**.

In this experiment, you will see plants engage in both photosynthesis and respiration. To monitor the progress of these reactions, you will take advantage of one of the properties of carbon dioxide, which is that when it dissolves in water it makes the water more acidic. (This is also the chemistry behind ocean acidification.) By adding a pH-sensitive dye to the water, you will therefore see when the plant has released or consumed CO<sub>2</sub>, because the color will change.

### Procedure:

1. Label eight test tubes, with a wax marker, 1A-4A and 1B-4B.
2. Put 150 mL of DI water in a beaker and add 3 mL of phenol red. Stir to mix. It will be bright pink, indicating a pH of 7 (neutral).
3. Use a drinking straw to blow bubbles into the beaker. This will dissolve CO<sub>2</sub> from your breath in the water, acidifying it. Blow bubbles for 30 seconds. It should still be pink.
4. Fill the test tubes marked A each about two-thirds full with the pink water. This should use up half of the 150 mL, leaving you with about 75 mL in the beaker.
5. Go back to blowing bubbles into the beaker. This time, keep going just until you see the color change to yellow, indicating that the water has become acidic (pH < 7).
6. Fill the test tubes marked B each about two-thirds full with the yellow water. This will use up the remaining water.
7. Using tweezers, take four large pieces of *Elodea* (each 8-12 cm long) from the tub (DO NOT break them up!) and insert them into tubes 1A, 1B, 3A, and 3B. Make sure they are pushed all the way to the bottom of the tube and submerged in the water.
8. Place tubes 1A, 2A, 1B, and 2B in one container and 3A, 3B, 4A, and 4B in the other. Take a piece of white paper and put it underneath your desk lamp, then put the 1 & 2 test tubes on the paper so that it gets as much light as possible. Put the 3 & 4 container in the dark cabinet underneath your lab bench.
9. Start a two hour timer.
10. Also prepare a slide of an *Elodea* leaf as you did in lab 3: pluck a single leaf from one of the *Elodea* sprigs, place it on a slide with a drop of DI water, and cover it with a cover slip, pressing down to flatten it and exclude bubbles. Put it aside to examine in part 3.

## Lab 5: Respiration and Photosynthesis

Once you've started your timer, discuss the structure and purpose of the experiment with your group and answer the following questions about it.

→ What questions are being asked in these experiments? (Hint: there are two questions.)

1:

2:

→ For each of the questions, what is your hypothesis, and why?

1:

2:

→ What are the experimental variables? Which tubes are the controls for each of the experimental variables? (Review the idea of "controls" from lab 1 if necessary.)

Experimental variable 1:

Control tube number:

Experimental variable 2:

Control tube number:

Then proceed to the next part of the lab while the plants do their work.

→ When your timer ends, examine the color of the water in each test tube and document it in the table. In the last column, note whether it changed between the start and end of the experiment.

Tube # and corresponding treatment	Water color at start (time=0)		Water color at end (time=2h)		Did the color change? Y/N
	A	B	A	B	
1: <i>Elodea</i> , light	Pink	Yellow			
2: No <i>Elodea</i> , light	Pink	Yellow			
3: <i>Elodea</i> , dark	Pink	Yellow			
4: No <i>Elodea</i> , dark	Pink	Yellow			

Questions:

→ What did the *Elodea* plant do in the water with CO<sub>2</sub>? Did it require light?

→ What did the *Elodea* do in the water with no CO<sub>2</sub>? Did it do it more in the light environment or in the dark environment?

## Lab 5: Respiration and Photosynthesis

- How does the presence of plants affect CO<sub>2</sub> concentrations?

When cleaning up, rinse your *Elodea* fragments gently with water and place them back in the tub. **DO NOT THROW THEM AWAY.**

## Environmental microbiology follow-up

After setting both of your 2-hour experiment timers, you can go get the agar plate you made last week with the environmental samples of microorganisms from different surfaces and determine the results of your experiment.

**Questions** to ask as you examine the agar plates:

- Describe the different appearances of the colonies you found from each sample. How many different types of microorganisms can you identify on your group's plate by colony appearance (color, shape, surface appearance)?
- Which place sampled by your group had the largest apparent microbial diversity? Was it the same one that had the highest microbial abundance?
- Do your results support or reject your original hypothesis?
- What conclusions can you draw based on your experiment?

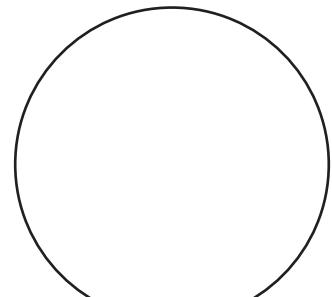
## Lab 5: Respiration and Photosynthesis

### Microscopy

#### Living yeast cells

Examine the yeast slide that you prepared in part 1. These tiny unicellular fungi are alive, because you took them from the live culture in your experiment, so they'll be respiring and growing on the slide.

- Draw a picture of a few yeast cells as they appear at 400x in the space at right.
  - See if you can find one that's dividing.
  - Are there bubbles? If so, make sure they're accurately depicted in your drawing.



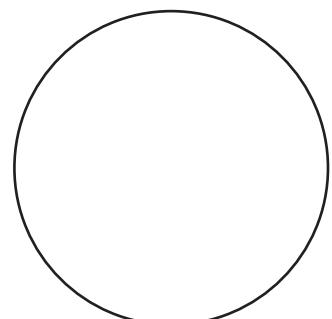
*Saccharomyces cerevisiae, wet mount, 400x magnification*

#### Living Elodea cells

**Chloroplasts** are the organelles in a plant cell in which photosynthesis takes place. Just like mitochondria, chloroplasts have their own DNA and reproduce themselves within their host cell, and their presence inside plant cells (actually the entire group Archaeplastida, which includes both red and green algae and land plants) is thought to be the result of an event where a photosynthetic bacterium, called a **cyanobacterium**, took up residence inside a eukaryotic cell. Inside a chloroplast, there are membrane-bound compartments called **thylakoids**, in which the actual chlorophyll is kept and where the light reactions of photosynthesis occur. The dark reactions take place in the space between the thylakoids.

Examine the Elodea slide that you prepared in part 2. This was picked recently enough that it's still acting like it's alive. Notice the chloroplasts moving around inside the cells. This is called "cytoplasmic streaming": the cytoplasm is moving around in a stream inside the cell, carrying the chloroplasts with it. It's thought to expose the chloroplasts more evenly to light, improving their efficiency at performing photosynthesis.

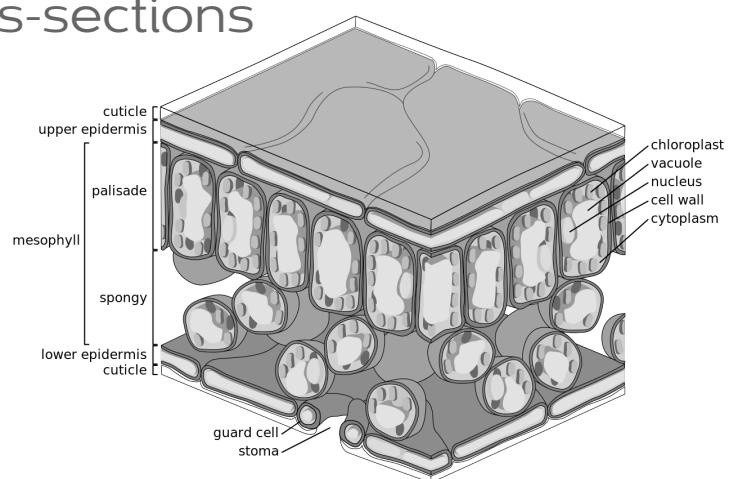
- Draw a few of the Elodea cells as they appear at 400x. Focus on depicting the size and location of the chloroplasts accurately.
- Leave the slide on the microscope for a few minutes without moving it. Does the cytoplasmic streaming speed up or slow down in the cells exposed to the microscope's light source, compared to the cells not in the light?



*Elodea chloroplasts, wet mount, 400x magnification*

#### Prepared slides of leaf cross-sections

The leaves of most land plants have multiple layers, much like our skin, to protect their insides from drying out. (*Elodea* and other water plants, which have no such problems, have a simpler leaf structure.) Most of the photosynthesis takes place in a layer called the **palisade parenchyma**, which contains neatly stacked cells full of chloroplasts. The palisade parenchyma, along with a gappy layer of irregular cells called the **spongy mesophyll** that contains the **veins** used for transport of substances around the plant, is sandwiched between two layers of flat cells called the upper and lower **epidermis** which keep water in.



## Lab 5: Respiration and Photosynthesis

In some plants, a waxy **cuticle** coats the epidermis for more effective protection against dehydration.

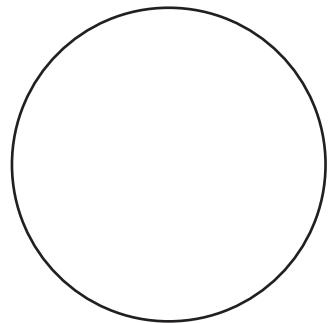
Examine one of the pre-prepared leaf structure slides. The cells have been stained to make them easier to find.

- Draw the cross section of the leaf as it appears under the microscope at 400x. Label the different structures and cell layers (upper and lower epidermis, palisade parenchyma, spongy mesophyll, vein) described above.

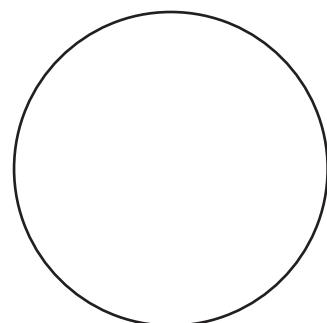
Although preventing dehydration is vital, the leaf also needs to exchange gases with its environment to function. Little holes in the leaf, called **stomata** (singular **stoma**), allow the passage of water, carbon dioxide, and oxygen in and out of the leaf. A pair of cells called **guard cells** open and close the stomata.

Prepare a wet mount of two small pieces of a leaf from a geranium plant (*Pelargonium peltatum*). Mount one piece with the top side of the leaf up and the other with the underside up. Again, these are so recently picked they still think they're alive, so the stomata will still be trying to control the leaf's water balance. Examine the underside first.

- Draw a few of the stomata from the underside of the leaf as they appear at 400x.  
→ Are there stomata on the top face or only on the bottom face? Why might that be?



Cross-section of leaf,  
400x magnification



Stomata on *Pelargonium* leaf,  
400x magnification

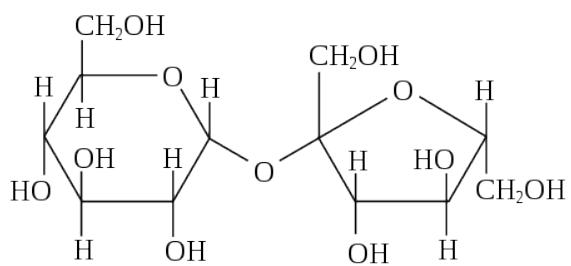
## Cleanup

1. Part 1:
  - a. Empty the vials of yeast down the drain.
  - b. Rinse out the balloons and return them and the rubber bands to the box.
  - c. Wash out the flasks and put them in the racks upside-down.
2. Part 2:
  - a. Rinse the *Elodea* pieces gently with water and return them to the container. Do not touch them with your hands.
  - b. Pour the colored water down the drain.
  - c. Rinse out the tubes and put them in the racks upside-down.
  - d. Dry the beakers and hang them on the rack above the sink.
3. Part 3:
  - a. Discard the *Pelargonium*, *Elodea*, and yeast slides.
  - b. Replace the leaf cross-section slides in their holder.
4. As always, clean and reset your microscope, wipe your lab bench, and close your computer programs but don't log out of the computer.

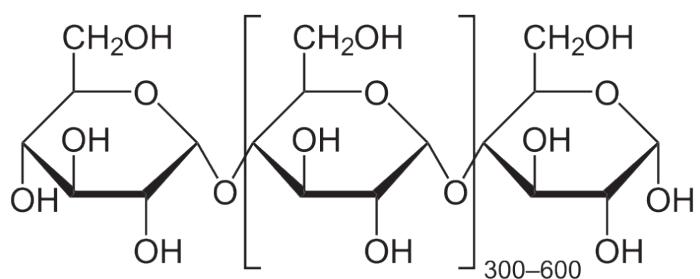
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## Lab 5: Respiration and Photosynthesis



a. A sugar molecule (sucrose)



b. A starch molecule (amylose)

# Lab 6: Dietary Macromolecules

## Learning Goals

1. Understand the uses of dietary macromolecules in the human body.
2. Become familiar with laboratory tests for sugars, carbohydrates, fats, and proteins.
3. Identify the presence of sugars, carbohydrates, fats, and proteins in foods and drinks.

## Agenda

1. Lab Introduction
2. Run the tests for macromolecules on positive and negative controls
  - a. Biuret test for protein
  - b. Iodine test for starch
  - c. Benedict's test for sugar
  - d. Alcohol wash for fats
3. Identify whether each type of macromolecules is present in three different drinks
4. Present your results to the class
5. Clean up

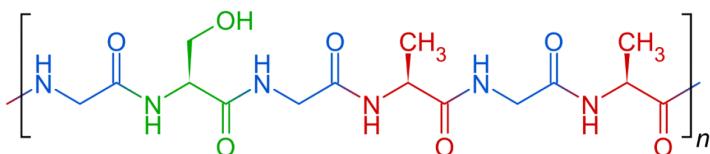
## Introduction

The food that humans and other animals eat is manufactured by other organisms for their own use. It supplies us with both energy (stored in its chemical bonds) for our activities and raw materials for the maintenance of our bodies.

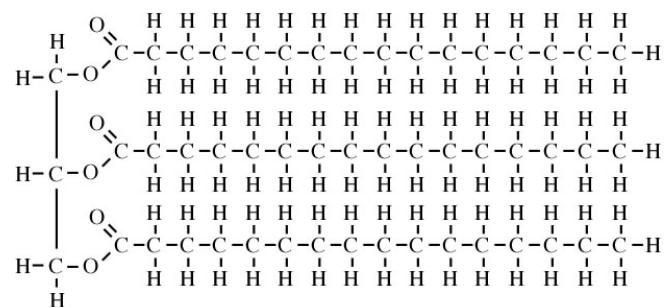
**Macronutrients** are the molecules that make up the vast majority of our food, and which we digest for food and energy. (The others, called micronutrients, will not be addressed in this lab.) There are three main classes of macronutrients:

- Carbohydrates: made up of sugar molecules, used by organisms for energy storage (p. 46, at left)
  - Sugars (“simple carbohydrates”): only one or two sugar molecules
  - Starches (“complex carbohydrates”): long chains of sugar molecules bonded together
- Proteins: long chains of amino acids, used by organisms for structural and functional purposes (below)
  - There are 20 different amino acids used in the body; these are combined in different ways to produce all the proteins.
  - Some amino acids can be manufactured, while others must be supplied in food
- Fats (lipids): long chains of carbon atoms, used in cell membranes and for energy storage; oily/greasy texture indicates that they are hydrophobic (repelled by water) (below)
  - Most dietary fats are *triglycerides*, made up of three carbon chains attached to a molecule of glycerol

These are also referred to as **dietary macromolecules**, where “dietary” means they can be digested and used for energy and “macromolecules” refers to their large molecular size.



- c. (above) A protein molecule (fibroin)  
d. (right) A triglyceride fat molecule (palmitin)



## Lab 6: Dietary Macromolecules

# Tests for dietary macromolecules

Laboratories have developed tests specific for the presence of a variety of molecules. The tests we will use today are **colorimetric** tests; that is, when the substance it tests for is present in the sample, the **reagent** will change color. The word “reagent” is related to the word “react”: a reagent is a substance that reacts with something. Each of the four types of dietary macromolecule reacts with only one of the reagents we will use, which allows you to test for the presence of each of the four types separately. One of your group members should be in charge of each of the four tests.

In this section, you will try each reagent on a **positive control** (definitely contains the substance you’re testing for), a **negative control** (definitely doesn’t contain the substance), and one or two experimental solutions (which may or may not contain the substance).

When conducting colorimetric tests, it is important to consider the color *change*, not the final resulting color. Be careful not to mistake a simple color mixture for a reaction!

### Testing for proteins: biuret test

The biuret test quickly detects the presence of protein in a sample. This reagent is a combination of two chemicals, copper sulfate ( $\text{CuSO}_4$ ) and either sodium or potassium hydroxide ( $\text{NaOH}$  or  $\text{KOH}$ ). **The biuret reagent is corrosive and could harm you! You need to wear gloves, be careful not to spill it, and if you get any on your skin, wash it off immediately.**

The biuret reagent is a clear blue color; in the presence of protein, it turns bright purple. When reading the result, keep in mind the color of the sample you’re mixing it with.

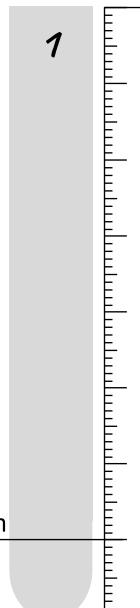
Questions:

- What color does the biuret reagent give for a *negative* result (no protein present)?
- What color does it give for a *positive* result (protein present)?

Materials: two clean test tubes, wax pencil, mm/cm ruler, egg white solution, distilled water, dropper bottle of biuret reagent

Procedure:

1. With the wax pencil, mark each tube at 1 cm from the bottom (as shown in the picture at right) and number them 1 and 2.
2. Fill each tube up to the 1 cm mark with the appropriate solution (see table below).
3. Add to each tube 5 drops of biuret reagent. Tap or swish gently to mix. Observe the color of each and record your data in the table below.



- Complete the data table.

Tube #	Contents	Color	Protein? (Y/N)
1	Egg white solution		
2	Distilled water		

Questions:

- Which tube is the positive control? (shows what a positive result looks like)

## Lab 6: Dietary Macromolecules

- Which tube is the negative control? (shows what a negative result looks like)
- If you got a positive result from the negative control, or vice versa, what might have happened to cause that result?

### Testing for sugars: Benedict's reagent

- What's the difference between a sugar and a starch? (The answer is in the introduction section above.)

We will use a test called Benedict's test to test for the presence of sugar. Benedict's reagent is a clear blue solution composed of copper sulfate, sodium citrate, and sodium carbonate. When heated to boiling, it reacts with most monosaccharides and some disaccharides (but not polysaccharides), changing its color. The amount of color change in the reagent indicates approximately the concentration of sugars in the sample, ranging from its original blue (no sugar) through green and yellow to reddish-orange (high sugar concentration).

**Note: This procedure is more dangerous than the other four. It involves not only a corrosive reagent like the previous test, but also a boiling hot water bath (100° C/212° F). Again, wear gloves, and be careful when executing it!**

- What color does Benedict's reagent give for a *negative* result (no sugar present)?
- What color does it give for a *strong positive* result (lots of sugar present)?

*Materials: four clean test tubes, wax pencil, cm/mm ruler, glucose solution, distilled water, two other sample solutions to be tested, Benedict's reagent, boiling water bath*

Procedure:

1. With the wax pencil, mark each tube at 1 cm from the bottom and number them 1-4.
2. Fill each tube with the appropriate solution up to the 1 cm mark. Add 8 drops of Benedict's solution to each tube.
3. Incubate all the tubes in the hot water bath for 4 minutes.
4. Carefully remove the test tubes from the hot water bath and put them back in your test tube rack. Observe the color of each and record it in the table.

- Complete the data table.

Tube #	Contents	Prediction	Color	Sugar concentration (zero, low, high)
1	Glucose solution			
2	Distilled water			
3	Vegetable oil			
4	Onion juice			

Questions:

- Which tube is the positive control? (shows you what a positive result looks like)
- Which tube is the negative control? (shows you what a negative result looks like)

## Lab 6: Dietary Macromolecules

→ Which of the experimental tubes contained sugar? Was either result surprising?

### Testing for carbohydrates: Lugol's iodine

Carbohydrates are made up of long chains of sugar molecules. The reagent we use as an indicator for their presence is a solution called Lugol's iodine, which contains a form of iodine called the triiodide ion ( $I_3^-$ ). On its own, this solution is orange-brown, but when it touches starch, the triiodide ion becomes entangled in the long polysaccharide chain, forming a complex with a dark blue color. This reaction is very sensitive; it changes color visibly in the presence of even very small amounts of starch. **Note: Lugol's iodine stains skin and clothes.**

- What color does the iodine reagent give for a *negative* result (no starch present)?
- What color does it give for a *positive* result (starch present)?

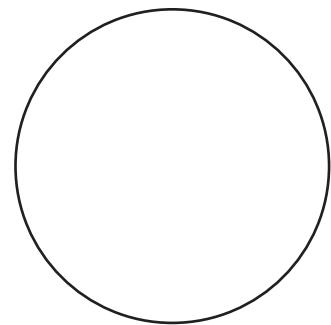
A clear demonstration of this reaction is given by staining a slice of potato and looking at it under the microscope. Potatoes are the potato plant's starch-storage organs; each cell contains many granules of starch, which makes the tuber a valuable food source to the plant and to us.

#### A. Examining starch granules in potato tissue

Materials: potato, razor blade, dropper bottle of iodine solution, slide, cover slip

1. Use the razor blade to shave off the thinnest possible slice of potato, as thin as a piece of paper. Rinse the potato slice in water, then place it on a microscope slide.
2. On one side, add a small drop of iodine, then place the cover slip on top.
3. Examine the slide on your compound microscope. Beginning at low power, find a spot where you can clearly see through the potato. Look for the small oval starch granules, which will be white on the unstained side and blue-black on the iodine stained side.

- Draw a few of the potato cells with stained starch granules (as they appear at 400x total magnification) in the space at right.



Potato stained with iodine,  
400x magnification

#### B. Testing for starch in food samples

Next, you will test several solutions for the presence of starch using the same reagent.

Materials: four clean test tubes, wax pencil, cm/mm ruler, starch solution, distilled water, experimental solutions A and B

Procedure:

1. With the wax pencil, mark each tube at 1 cm from the bottom and number them 1-4.
2. Fill each tube up to the 1 cm mark with the appropriate solution. Record the identities of the two experimental solutions in the table.
3. Add 5 drops of iodine to each tube and swirl to mix.

- Complete the data table.

## Lab 6: Dietary Macromolecules

Tube #	Contents	Color (brown or blue)	Starch present?
1	starch solution		
2	distilled water		
3	glucose solution		
4	onion juice		

Questions:

- Which tube is the positive control? (shows you what a positive result looks like)
- Which tube is the negative control? (shows you what a negative result looks like)
- Which of the experimental tubes contained sugar? Was either result surprising?

## Testing for fats: alcohol wash

Fats are nonpolar molecules and therefore don't dissolve in water. However, they will dissolve in alcohol, which none of the other macronutrients will do. The alcohol wash test takes advantage of that fact. The test substance is mixed thoroughly with isopropyl alcohol and then allowed to settle. If any fats are present, they will dissolve in the alcohol, leaving it cloudy. If not, the alcohol will settle clear.

Materials: three clean test tubes, wax pencil, cm/mm ruler, test tube corks or Parafilm, vegetable oil, distilled water, starch solution

Procedure:

1. With the wax pencil, mark each tube at 1 cm and 5 cm from the bottom and number them 1-3.
2. Fill each test tube to the 1 cm mark with the appropriate solution.
3. Fill all tubes to the 5 cm mark with the 90% isopropyl alcohol. Either cork them or cover them tightly with Parafilm, as your instructor specifies.
4. Shake each one vigorously for 15 seconds (that's longer than you think it is!), then uncork it and replace it in the test tube rack.
5. Wait 3 minutes. While you're waiting, wash the corks with soap.
6. Observe the top layer of liquid in each test tube, which contains the isopropanol (it floats on top of both water and oil). Record whether it's clear or cloudy.
  - If you can't decide, hold the tube up to a piece of printed paper or a computer screen with text on it. If you can see the letters through it, record it as being clear. If you can't see them, record it as cloudy.

→ Complete the data table.

Tube #	Contents	Clear or cloudy?	Lipids present?
1	vegetable oil		
2	distilled water		
3	starch solution		

## Lab 6: Dietary Macromolecules

- Which tube is the positive control? (shows you what a positive result looks like)
- Which tube is the negative control? (shows you what a negative result looks like)
- Were you surprised by the results from the experimental tube? If so, why?

## Testing food samples

You have just demonstrated how to perform and read tests for the four main classes of dietary macromolecules. Next, you will use these tests on real samples. All the unknown samples provided are “real” foods or drinks -- i.e. things you can get at the grocery store, rather than lab supplies. You will analyze them using the four macronutrient assays you just learned, present them, and compare your results with the rest of the class. We have provided some unknown liquids, labeled “A”, “B”, etc. These are your test subjects.

Procedure:

1. Choose any three of the unknown liquids provided. For each one, label four test tubes with the letter of that unknown and the nutrient you’re testing for (e.g. “B Starch”). Write the letter ID of the unknown in the “ID” column of the table, next to the number.
2. For each unknown, add a sample of about 1 mL to each of the four test tubes labeled with its letter. (1 mL is about the same as 1 cm high in the test tube.) When you’re done, your test tube rack should contain three sets of four identical samples.
3. Look, swirl, sniff, and otherwise observe each of your unknown liquids to form a hypothesis about what macronutrients are likely to be present in them. Record your hypotheses in the “Hypothesis” column in the table below (you can just write “yes” or “no” for each nutrient).
4. You will use all four of the dietary macromolecule tests on each unknown to test your hypotheses. In the “Reagent” column, write the name of the reagent that you will use to test the unknown for that nutrient.
5. Run the tests exactly the same way you did in the first half of the lab.
  - a. If you can’t see the color of the biuret or Benedict’s reagents (this often happens in opaque or colored samples), add five more drops and swirl it again.
  - b. If you think you made an error in doing one of your tests, you can get more of the unknown substance and test it again.
6. Record what you see in each tube in the “Test color” column of the table.
7. In the “Result” column, record whether or not your test showed that macronutrient to be present in the sample.
8. In the “What is it?” column, make your best guess as to what each of the unknown drinks was.
9. Proceed to cleanup and organizing your presentations, below.

- Record your data in the following table.

## Lab 6: Dietary Macromolecules

ID	Nutrient	Reagent	Test color	Result	What is it?
#1:	starch				
#1:	sugar				
#1:	fat				
#1:	protein				
#2:	starch				
#2:	sugar				
#2:	fat				
#2:	protein				
#3:	starch				
#3:	sugar				
#3:	fat				
#3:	protein				

## Presentation of results

When you're done, inform the instructor of your results. They will put everyone's results up on the board and reveal the actual identities of all the unknown drinks.

- What foods did you test?
- What were your hypotheses about the macronutrients they would contain?
- Did your results support your hypotheses?
  - If any of your results were surprising, mention which ones.

## 3. Cleanup

1. Wash your test tubes using liquid dish soap (not hand soap) and bottle brushes. Rinse them out, then set them upside down in the large test tube rack in the sink. Place the test tube racks next to them on the counter.
2. Discard the potato slide.
3. Reset the microscope to beginning position (stage down, lights off, 4x objective lens in front). Wipe the eyepieces, objective lenses, condenser, and light source with a dry lens cloth.
4. Close applications on the laptop, but do not log out.
5. Spray and wipe your lab station.

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# Lab 7: Enzymes

## Learning Goals

1. Understand the idea of a catalyzed reaction and the roles of catalyst and substrate.
2. Explain the role of enzymes in cell homeostasis and digestion.
3. Explore the effects of temperature, pH, and enzyme concentration on enzymatic activity in different enzymes.

## Agenda

1. Catalase experiments
  - a. Catalase reaction
  - b. Effect of enzyme concentration on reaction
  - c. Effect of temperature on reaction
  - d. Effect of pH on reaction
2. Amylase experiment

## Introduction

A chemical reaction, as you have learned already, starts with a set of **reactants**. The chemical bonds in the reactants are consumed during the reaction, and their atoms are rearranged into the **products**. In some reactions, a third role is that of **catalyst**. A catalyst is a molecule that is involved in a reaction but is not produced or consumed. Instead, its presence makes the reaction happen when it otherwise would not, or allows it to happen faster than it would without the catalyst. The reactant on which a catalyst acts is called the catalyst's **substrate**.



**Enzymes** are a class of biological molecules, mostly proteins, which act as catalysts for biological chemical reactions. Organisms produce thousands of different enzymes to catalyze all the different chemical reactions that allow them to live. Most of these reactions do not occur on their own, or occur only very slowly; the presence of the right enzyme allows the reactions to proceed fast enough for the cell to maintain its internal conditions and perform its biological functions.

Today we will experiment with two different enzymes, **catalase** and **amylase**. (The “-ase” suffix is common in enzyme names.) Catalase is a cleanup enzyme that detoxifies a poison inside the cell, while amylase is a digestive enzyme that breaks down food molecules.

## Catalase experiments

Catalase is an enzyme produced by organisms that breaks down the toxic molecule hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) into water and oxygen. Hydrogen peroxide is a toxic byproduct of some cellular reactions involving oxygen, and it causes cell damage, so catalase is produced by all oxygen-breathing organisms. The catalase you will use in this lab is extracted from cow liver.

Hydrogen peroxide breaks down slowly by itself, but the reaction occurs much faster in the presence of catalase. The catalase reaction is as follows:



## Lab 7: Enzymes

→ What molecule plays each role in this reaction?

**Reactants:**

**Products:**

**Catalyst:**

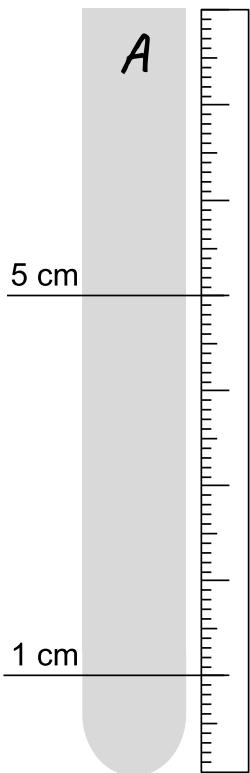
**Substrate:**

### Catalase activity

Your first task is to see what the catalase reaction looks like. Just as you did in Lab 5 for the macronutrient tests, you will test a positive control and two negative controls. The bubbles of oxygen produced by the reaction will serve as an indication of whether the reaction happened.

Procedure:

1. With a wax marker, label 3 clean test tubes at 1 cm and 5 cm from the bottom. (The drawing at right shows you how to do this.)
2. Mark one of the test tubes "A". Fill tube A to the 1 cm mark with catalase solution, then up to 5 cm with hydrogen peroxide. Swirl gently to mix and wait about 20 seconds, then watch what happens. Record whether the reaction occurs (i.e. whether oxygen gas was produced in the test tube), then set it down in the test tube rack.
3. Mark the next tube "B". Fill tube B to 1 cm with water and then to 5 cm with hydrogen peroxide. Again, swirl gently to mix, wait about 20 seconds, record whether the reaction occurs, then set the tube back down in the test tube rack.
4. Mark the last tube "C". Fill tube C to 1 cm with catalase solution and then to 5 cm with sucrose solution. Again, swirl gently to mix, wait about 20 seconds, record whether the reaction occurs, then set the tube back down in the test tube rack.



→ Record your results in the table.

Tube	Contents (solution 1 + solution 2)	Reaction occurred?	Positive or negative?	Missing reactant? (If so, which one?)
A	+			
B	+			
C	+			

→ What substances remain in tube A after the reaction ends (i.e. stops bubbling)?

Hydrogen peroxide

Water

Oxygen

Catalase (Circle all that apply)

Touch the test tube containing the positive control and feel that it is warm. Some chemical reactions are **endergonic** (absorb energy), while others are **exergonic** (release energy). The energy often comes in the form of heat.

→ Which type of reaction is this: endergonic or exergonic ? (Circle one)

# Lab 7: Enzymes

## Effects of conditions on catalase enzyme activity

Next, you will examine how the catalase reaction is affected by the conditions under which the reaction takes place. You will test it at different concentrations, temperatures, and pH.

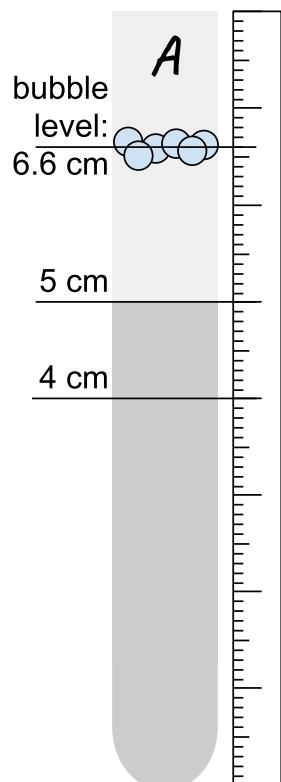
### A. Catalase concentration

The reaction occurs every time a molecule of catalase and a molecule of  $\text{H}_2\text{O}_2$  bump into each other. The catalase molecule can break 40 million  $\text{H}_2\text{O}_2$  molecules per second (an unusually high speed for an enzymatic reaction). In this section, you will determine whether the amount of catalase affects how fast the  $\text{H}_2\text{O}_2$  is consumed.

Procedure:

1. Label 3 clean test tubes "A", "B", and "C".
2. Mark tube A at 4 cm and 5 cm, tube B at 4 and 6 cm, and tube C at 4 and 7 cm.
3. For each tube, do the following steps:
  - a. Fill to the first mark with hydrogen peroxide.
  - b. Fill to the second mark with catalase.
  - c. Swirl to mix, wait 20 seconds, and make a third mark with the wax marker at the maximum height to which the bubbles rise (see picture at right, above).
  - d. Measure how high the bubbles rose above the surface of the liquid (the distance between the second mark and the third mark) and record it in the table.
4. Set the three test tubes down in your test tube rack and set a timer for 5 minutes. When it ends, look at all three again and determine whether any bubbles are still rising in each one. If so, mark and record the bubble height again; if not, write "none".

→ Record your results in the table.



Tube	Proportions	Bubble height after 20 sec	Bubble height after 5 min
A	4 cm $\text{H}_2\text{O}_2$ : 1 cm catalase		
B	4 cm $\text{H}_2\text{O}_2$ : 2 cm catalase		
C	4 cm $\text{H}_2\text{O}_2$ : 3 cm catalase		

Questions:

→ Did increasing the proportion of enzyme in the mixture make the reaction go faster?  
If so, why?

→ Did the proportion of enzyme in the mixture affect how long the reaction took to go to completion? If so, why?

## Lab 7: Enzymes

→ Would you expect the same effect from increasing the concentration of substrate? Why or why not?

### Temperature

Heat is the random movement of molecules. The higher the temperature of a substance, the more random movement is happening in it. This is true regardless of the state of matter. Since reactions occur when the reactant and catalyst molecules bump into each other, you might expect more heat to increase reaction speed, and in general it does. However, high heat can also damage biological molecules or affect their function; proteins are particularly temperature-sensitive, and most enzymes are proteins. As a result, most enzymes have an optimum operating temperature, below which they work slower because of the low rate of random molecule movement, and above which they become damaged (**denatured**) and stop working at all. In this section, you will run the catalase reaction at different temperatures to try to find the temperature at which catalase works best.

Procedure:

1. Label 3 clean test tubes “A”, “B”, and “C”. Mark each at 1 and 5 cm.
2. Fill each tube up to the 1 cm mark with catalase at the appropriate temperature, according to the table below (A: boiling; B: body temperature; C: ice).
3. To tube A, add hydrogen peroxide up to the 5 cm mark, then swirl to mix, wait 20 seconds, and record how high the bubbles rise above the liquid surface, just as before. Repeat for tubes B and C.
4. Set the three test tubes down in your test tube rack and set a timer for 5 minutes. When it ends, look at all three again and determine whether any bubbles are still rising in each one. If so, mark and record the bubble height again; if not, write “none”.

Tube	Temperature	Bubble height after 20 sec	Bubble height after 5 min
A	Boiling (100°C)		
B	Body temp (37°C)		
C	Ice (0°C)		

### pH

pH is a measure of how acidic or basic a solution is. The more H<sup>+</sup> ions are dissolved in a solution, the higher the acidity and the lower the pH; the more OH<sup>-</sup> ions, the more basic the solution, and the higher the pH. In a neutral solution (pH 7), the concentrations of H<sup>+</sup> and OH<sup>-</sup> ions are balanced; this is how deionized water is. The presence of these ions can **denature** a protein and affect its functioning, either by chemically interacting with its side groups and changing the shape of the protein, or by binding to the part that would normally grab onto the substrate molecule (the active site). In this section, you will run the catalase reaction at three different pH levels to find its optimum pH.

Procedure:

1. Label 3 clean test tubes “A”, “B”, and “C”. Mark each at 1, 3, and 7 cm.
2. Fill all three tubes with catalase up to 1 cm. Make sure you are using the *non-buffered* catalase solution that will be at this station -- the catalase from the other stations is buffered and will not work correctly!

## Lab 7: Enzymes

3. Fill each tube up to the 3 cm line with water at the appropriate pH, as indicated in the table below (A: acidic; B: neutral; C: basic).
4. To each tube, add hydrogen peroxide up to the 7 cm line. Once again, swirl to mix, wait 20 seconds, and record how high the bubbles rise above the surface of the liquid.
5. Set the three test tubes down in your test tube rack and set a timer for 5 minutes. When it ends, look at all three again and determine whether any bubbles are still rising in each one. If so, mark and record the bubble height again; if not, write "none".

Tube	pH	Bubble height after 20 sec	Bubble height after 5 min
A	1 (acidic)		
B	7 (neutral)		
C	10 (basic)		

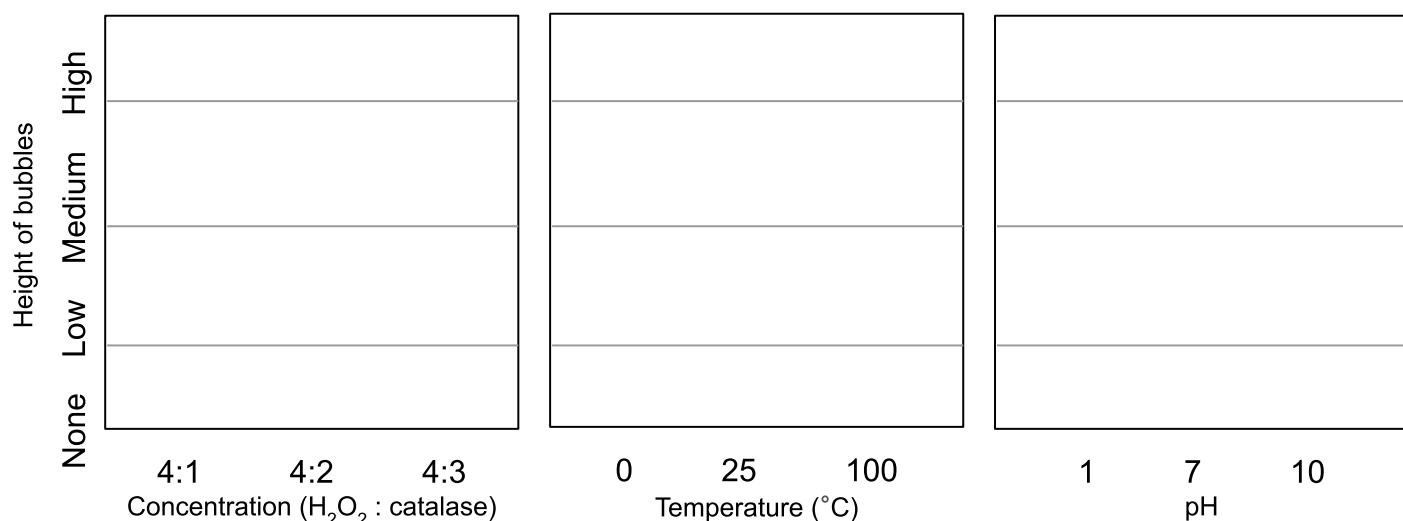
Questions:

- Does adding more catalase make the reaction go faster? What about more  $\text{H}_2\text{O}_2$ ?
- Does adding more catalase or more  $\text{H}_2\text{O}_2$  change how long it takes for the reaction to go to completion? If so, how?

## Comparing your data

- Plot the data recorded in all three tables on the following graphs. Draw and connect the data points for the 20-second data and the 5-min data in two different colors.

When you are done with all three of the catalase experiments, your group will draw their graphs on the board in the location indicated by your instructor.



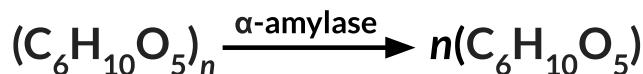
## Lab 7: Enzymes

Questions:

- What are the optimum conditions of temperature and pH for the functioning of catalase?
- What conditions of temperature and pH denatured the catalase?
- Do these results make sense given what you know about the biological function of catalase and where this catalase comes from?

## 2. Amylase experiments

You have just done several experiments with the enzyme catalase, which converts hydrogen peroxide into water and oxygen. In this section, you will experiment with a different enzyme, **amylase**, which breaks down complex carbohydrates (starches), turning them into maltose, a sugar which consists of two glucose molecules bonded together.



In the human body, amylase is produced in the saliva; chewing your food, therefore, starts the process of digestion by breaking the starches down into maltose, which is then broken down further into glucose by another enzyme, maltase, in the small intestine. The optimal temperature for amylase is about body temperature. In the following exercises, you will experiment with the digestion of starches by amylase in your own saliva.

The colorimetric tests you will use are familiar to you from the previous lab. To test for the presence of starch, we will use the iodine stain, which changes from reddish-brown to dark purplish-blue when it contacts starch, but which does not react with sugar. To test for the presence of sugar, we will use Benedict's reagent, which must be heated to 80°C for 2-4 minutes to react, and which indicates the presence of sugars with warmer colors (i.e. blue = no sugars, green = trace sugars, and so on to red = large amount of sugars). In this section, you will investigate the effects of saliva amylase on a starch solution.

*Materials: six 16mm test tubes, wax marker, ruler, DI water in a squeeze bottle, vinegar in a dropper bottle, starch solution in a squeeze bottle, 80C water bath, Benedict's solution in a dropper bottle, Lugol's iodine solution in a dropper bottle*

Procedure:

1. Prepare six test tubes. Label two "A", two "B", and two "C".
2. Take one test tube from each labeled pair to prepare your solutions. Mark each of these three tubes at 1 cm, 2 cm, and 5 cm. Set the other tube of each pair aside to be used in step 10 (dividing the sample in half).
3. Fill the marked tube A with DI water up to the 2 cm mark.
4. Spit enough saliva into marked tubes B and C to fill them up to the 1 cm mark. (This will take a few minutes.)
5. Fill tube B with DI water up to the 2 cm mark.
6. Fill tube C with vinegar up to the 2 cm mark.
7. Tap each tube against the palm of your hand until the saliva and water or vinegar are well mixed.
8. Fill all three marked tubes up to the 5cm mark with starch solution. Swirl vigorously until the starch solution is well mixed into the contents of the tube.
9. Let all the solutions stand for 15 minutes while the amylase digests the starch.

## Lab 7: Enzymes

10. Now you will divide each sample in half for testing.
  - a. Using a clean plastic pipette, transfer half of the mixture in the full tube "A" into the empty tube "A". Then put the pipette in the disposal container.
  - b. Repeat for "B" and "C".
11. Test one of each pair of tubes with Benedict's test for sugar, as you did with the food samples last week (add 5 drops of Benedict's reagent, swirl to mix well, 80 C water bath for 10 minutes, read color). Record your results in the table.
12. Test the other one of each pair with the iodine test for starch (add 1 drop Lugol's iodine solution, swirl to mix, read color). Record your results.

→ Complete the results table.

Mixture	Reactants	Sugar present?	Starch present?
A	Water + starch		
B	Water + saliva + starch		
C	Vinegar + saliva + starch		

Questions:

- Which mixture was the negative control? **A      B      C** (Circle one)
- Did the tube with the active amylase completely digest all the starch into sugar, or was there some starch left in the tube after the digestion?
- Which experimental treatment denatured the amylase?
- Amylase is produced and mixed with food in the mouth. It then travels into the rest of the digestive tract. Where in the digestive tract do you think it gets denatured and stops digesting starch into sugar? Compare this to any advice you may have heard on how long to chew your food.

## Lab 7: Enzymes

### 3. Enzyme videos

Using any of the materials available in lab, create a stop motion movie illustrating the enzymatic reaction assigned to your lab group in the table below. You can use a free app like Stop Motion Studio. It is a good idea to demonstrate your reaction once for your instructor before you begin filming. One of your groups members must upload the film by next week's lab. Make sure everyone's name appears in the first shot of your film, along with which enzyme your film is about. The grading rubric is as follows:

- Correctly labels and portrays the enzyme, substrate and product (3 pts)
- The reaction is accurately portrayed (1 pt)
- Creativity and effort (1 pt)

Table #	Enzyme	Table #	Enzyme	Table #	Enzyme	Table #	Enzyme
1	Lipase	3	Pepsin	5	Catalase	7	Maltase
2	Lactase	4	Trypsin	6	Amylase	8	Sucrase

### 4. Cleanup

1. Just like last week, wash all the test tubes.
  - a. Put solid matter into the garbage can and liquids down the sink.
  - b. Then wash out the tubes with the blue soap in the large pump bottles and a bottle brush.
  - c. Leave them upside-down in the metal rack to dry.
2. Put all the materials back from your enzyme videos and wipe the table.

# Lab 8: Organ Systems

## Learning Goals

1. Identify the main functions of each organ system in the human body.
2. Define anatomy and physiology.
3. Define homeostasis and provide relevant examples.
4. Explain the relationship between structure and function in the human body.

## Agenda

1. Visit each of the stations set up in the classroom. At each one complete the activities.

## Introduction

Anatomy is the scientific study of the body's structures. Some of these structures are very small and can only be observed and analyzed with the assistance of a microscope. Other larger structures can readily be seen, manipulated, measured, and weighed. The word "anatomy" comes from a Greek root that means "to cut apart."

Whereas anatomy is about structure, physiology is about function. **Physiology** is the scientific study of the chemistry and physics of the structures of the body and the ways in which they work together to support the functions of life. Much of the study of physiology centers on the body's ways of maintaining chemical and physical homeostasis. Homeostasis is the state of steady internal conditions maintained by living things. A steady internal state, including all the chemical and physical conditions that affect functioning, is necessary for their continued survival. The study of physiology certainly includes observation, both with the naked eye and with microscopes, as well as manipulations and measurements. However, current advances in physiology usually depend on carefully designed laboratory experiments that reveal the functions of the structures and chemical compounds that make up the body.

In this lab, we will explore the structure and function of the major organ systems in the human body. Each of the stations set up in the classroom represents one of the organ systems. As you go through the different stations, pay attention to the relationship between the structure (shape) of each tissue, organ, and organ system and its function. Also note if the organ system works to maintain the homeostasis of the body and think about the ways that the different organ systems interact with each other. You can record this information in the table on page 18 to study later.

## Station 1: The Muscular and Skeletal System

### The Skeletal System

The skeletal system includes all the parts of the body composed of bones and cartilage. **Bone** is a hard, dense connective tissue that forms most of the adult skeleton, the support structure of the body. The skeletal system performs the following functions:

- supports the body
- facilitates movement
- protects internal organs
- produces blood cells
- stores and releases minerals and fat

## Lab 8: Organ Systems

The skeleton is subdivided into two major divisions, axial and appendicular. The **axial** skeleton forms the vertical, central axis of the body and includes all bones of the head, neck, chest, and back. It serves to protect the brain, spinal cord, heart, and lungs. It also serves as the attachment site for muscles that move the head, neck, and back, and for muscles that act across the shoulder and hip joints to move their corresponding limbs. The **appendicular** skeleton includes all bones of the upper and lower limbs, plus the bones that attach each limb to the axial skeleton.

## The Muscular System

All muscle cells have the distinctive abilities to contract (change their own shape), and to transmit an electrical signal from one cell to the next one. Skeletal muscle is present throughout the body and is responsible for movement. It has a striated (streaky) appearance under the microscope because its contracting cells are organized into bundles called fibers that all point in the same direction (like the fibers in a rope). The skeletal muscles act together with the skeleton to move the body.

Muscle cells require a lot of energy to move the body. During strenuous activities, energy stores are depleted, and oxygen is consumed faster than it can be resupplied. Luckily, muscle cells can continue to produce ATP through lactic acid fermentation, even when your circulatory system is unable to keep up with their oxygen requirements. As the lactic acid builds up, though, this becomes less and less effective. As strenuous activity continues, muscles become fatigued and exhibit a decline in force. This buildup of lactic acid is also responsible for the muscle swelling that bodybuilders describe as “pump”, as blood comes in to flush out the lactic acid and bring oxygen and nutrients to repair damaged cells.

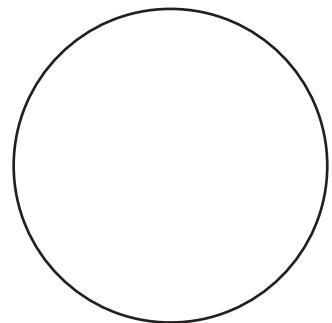
### Activities:

1. Observe the slide of skeletal muscle tissue under the microscope. Identify the striations. Draw your observations in the space at right.
2. On the skeleton below, color the axial and appendicular skeleton different colors.
3. Complete the Muscle Fatigue activity. Record your data in the table below.

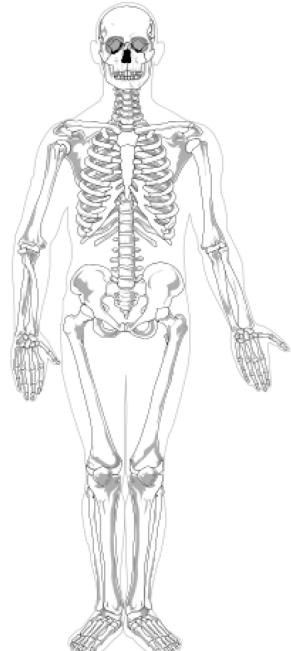
Biceps measurement before lifting:	cm (relaxed):	cm (flexed):
Number of reps in first set:		
Biceps measurement after lifting:	cm (relaxed):	cm (flexed):
Number of reps in second set:		

### Questions:

- Define the appendicular and axial skeleton. What are the function(s) of each?
- Provide one example of how the structure of the axial function fits its function.
- After exercising, which nutrients will need to be replenished? What is the name for this return to a steady state?



Skeletal muscle tissue slide,  
400x magnification



The human skeleton, front view. Label the axial and appendicular skeleton. Credit: Mariana Ruiz Villarreal via Wikimedia Commons.

# Lab 8: Organ Systems

## Station II: The Nervous System

Motion, sensation, and thought, as well as many other less obvious functions, are all controlled by the nervous system. The Nervous System can be divided into two major regions: the central and peripheral nervous systems. The central nervous system (CNS) is the brain and spinal cord, and the peripheral nervous system (PNS) is everything else.

The brain is contained within the cranial cavity of the skull, and the spinal cord is contained within the vertebral cavity of the vertebral column. The peripheral nervous system is so named because it is on the periphery—meaning beyond the brain and spinal cord. Depending on different aspects of the nervous system, the dividing line between central and peripheral is not necessarily universal.

The nervous system is made up of **neurons**, specialized cells that can receive and transmit chemical or electrical signals, and **glia**, cells that provide support functions for the neurons. There is great diversity in the types of neurons and glia that are present in different parts of the nervous system. Neurons are highly specialized to fit their function. Dendrites, which protrude from the cell body receive signals from the environment or other neurons and transmit signals through a longer protrusion called an **axon**. Axons can be very long, sending signals all the way from your spinal cord to your big toe.

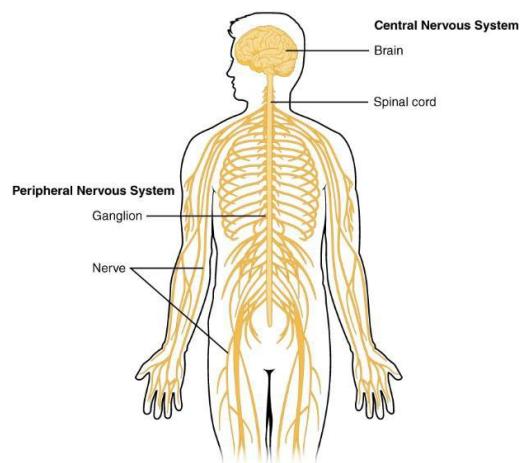
### Activities:

- Observe the slide of nervous tissue under the microscope. Identify a neuron and draw your observations in the space at right.
- Complete the Reaction Time activity and record your partner's results here.

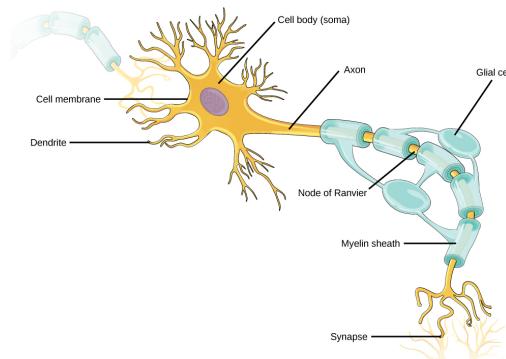
Stimulus	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Visual						cm
Auditory						cm
Tactile						cm

- Complete the Mapping Nerve Endings activity and record your partner's results here.

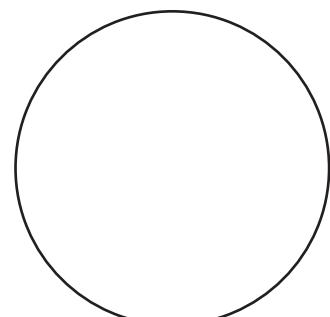
Skin area tested	Trial 1	Trial 2	Trial 3	Average
Right index finger				mm
Upper arm				mm
Back of neck				mm



The structures of the PNS, the ganglia and nerves, can be seen as distinct structures. The structures in the CNS are not obvious at this scale, but are visible in prepared tissue under the microscope. (Credit: OpenStax, licensed under CC BY 4.0.)



Neurons contain organelles common to other cells, such as a nucleus and mitochondria. They also have more specialized structures, including dendrites and axons. (Credit: OpenStax under CC-BY 4.0)



**Neural tissue, 400x magnification**

## Lab 8: Organ Systems

### Questions:

- What is the difference between the central and peripheral nervous system? Identify the function of each.
  
- Which part of the body were you able to differentiate the smallest distance in the Mapping Nerve Endings Activity? Given the function of the different body parts tested, does this make sense? Why or why not?
  
- Study the neuron model. What structure(s) allow the neuron to fulfill its function of receiving and sending signals?

## Station III: The Senses

Within the realm of physiology, senses can be classified as either general or specific. A **general sense** is one that is distributed throughout the body and has receptor cells within the structures of other organs. Mechanoreceptors in the skin, muscles, or the walls of blood vessels are examples of this type. A **special sense** is one that has a specific organ devoted to it, namely the eye, inner ear, tongue, or nose.

To test your vision, we will use the Snellen eye chart (located by the door). You may be familiar with the idea of 20/20 vision, which as a standard means that you can see from 20 feet away what a person with normal vision can see from 20 feet away. Compare this to someone who is **near-sighted** and may have 20/100 vision, meaning they have to be 20 feet away an object to see it as clearly as a person with normal vision could see from 120 feet away. Another important aspect of good vision is depth perception or the ability to perceive how far away an object is, which requires both eyes working together.

Our ears are complicated machines with many small, even microscopic parts. Together these parts allow us to hear sounds and determine where they came from. To localize sound, your brain compares the time it takes for the sound to get to your left and right ear. For example, if the sound comes from your left, then it will reach your left ear before it reaches your right. If sound is coming from somewhere along the midline of your head, that is to say it is an equal distance from both ears, then the pinna (outside of your ear) is perfectly shaped to funnel the sound to determine where it came from.

## Lab 8: Organ Systems

Activities:

1. Complete the activity “Testing for Hearing Localization”. Record your partner’s results.

Most accurate direction	
Least accurate direction	

2. Complete the activity “Testing Visual Acuity”. Record your and your partner’s results.
3. Complete the activity “Testing Binocular Vision and Depth Perception”. Record results.

	Visual acuity			Depth perception	
	Left eye	Right eye		Binocular (both eyes)	Monocular (one eye)
You	/	/		/10	/10
Partner	/	/		/10	/10

Questions:

→ What is the difference between a general and special sense?

→ Why do you think it is easier to localize sound that comes from in front of you? Think about the shape of your ears.

## Station IV: The Circulatory System

This system is responsible for moving blood, along with the substances it carries, around the body. It works with the respiratory system to exchange oxygen and carbon dioxide, with the digestive system to move nutrients, and with the urinary system to remove waste. The center of the cardiovascular system is the heart, the fist-sized muscle that generates the force to move blood through the vessels. The blood vessels are a branching system of smooth muscle tubes, about 60,000 miles long in the average adult human, that vary in diameter from nearly 3 cm (the aorta) to only a few micrometers (the capillaries).

The figure at right illustrates the flow of blood through the heart and the body. Blood circulates from the right side of the heart, through capillaries in the lungs (the **pulmonary** circuit), back into the left side of the heart, through the capillaries in the rest of the body (the **systemic** circuit), and then back into the right side of the heart to start again. Each blood cell makes this whole circuit about once per minute.

## Lab 8: Organ Systems

The most common measurement of cardiovascular health is **blood pressure**. When blood pressure is measured, it is recorded as a ratio of two numbers (e.g., 120/80 is a normal adult blood pressure), expressed as systolic pressure over diastolic pressure. The **systolic pressure** is the higher value (typically around 120 mm Hg) and reflects the arterial pressure resulting from the ejection of blood during ventricular contraction, or systole. The **diastolic pressure** is the lower value (usually about 80 mm Hg) and represents the arterial pressure of blood during ventricular relaxation, or diastole.

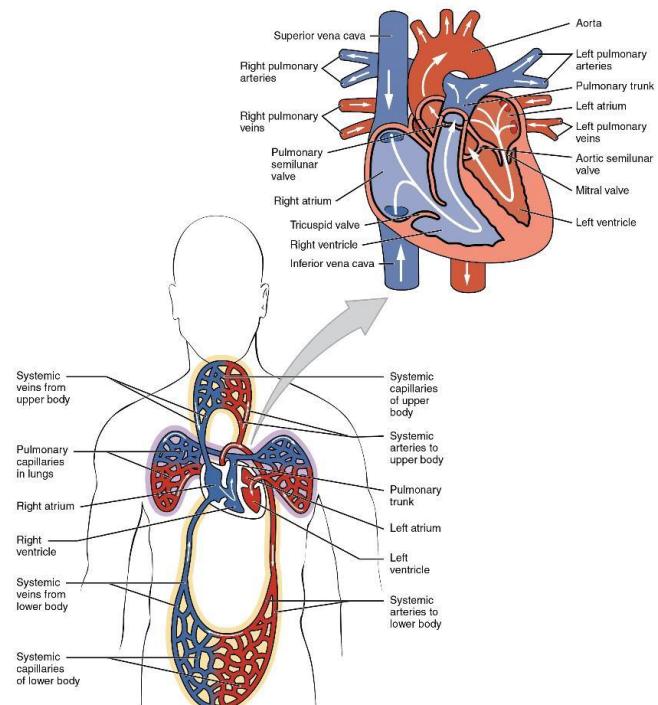
### Activities:

1. Observe the blood film slide under the microscope. Using the guide next to the microscope, identify examples of the different kinds of white blood cells. Draw your observations in the space provided.
2. On the heart diagram at right, indicate the flow of blood through the heart using arrows. Use a blue colored pencil to represent deoxygenated blood and red to represent oxygenated blood.
3. Complete the Pulse Rate & Blood Pressure Activity and record your results.

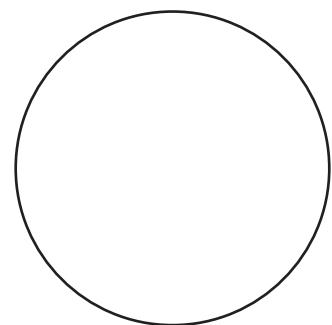
Your pulse:	beats/min
Your partner's pulse:	beats/min
Your blood pressure:	
Your partner's blood pressure:	

### Questions:

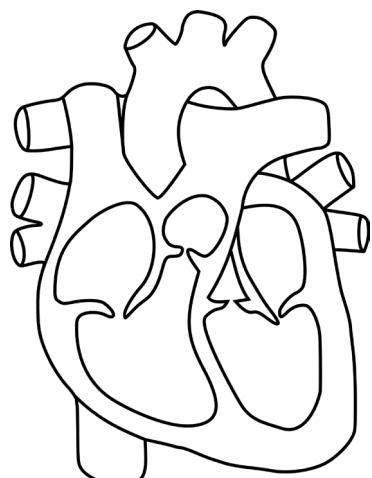
- What is the difference between the pulmonary and the systemic circuit of the circulatory system?
- Study the anatomy of the heart model and consider the flow of blood through the pulmonary (right) and systemic (left) circuits. List one observation of how the structure of the heart fits its function.



(Credit: OpenStax, licensed under [CCBY-4.0](#).)



Red and white blood cells,  
400x magnification



## Lab 8: Organ Systems

### Station V: The Respiratory System

Hold your breath. Really! See how long you can hold your breath as you continue reading...

How long can you do it? Chances are you are feeling uncomfortable already. A typical human cannot survive without breathing for more than 3 minutes, and even if you wanted to hold your breath longer, your nervous system would override your conscious instructions. This is because every cell in the body needs to run the oxidative stages of cellular respiration, the process by which energy is transferred from storage molecules to adenosine triphosphate (ATP). For ATP to be phosphorylated, oxygen is consumed as a reactant and carbon dioxide is released as a waste product. Carbon dioxide is exhaled and oxygen is inhaled through the respiratory system, which includes muscles to move air into and out of the lungs, passageways through which air moves, and microscopic gas exchange surfaces covered by capillaries (small blood vessels). The circulatory system transports gases from the lungs to tissues throughout the body and vice versa.

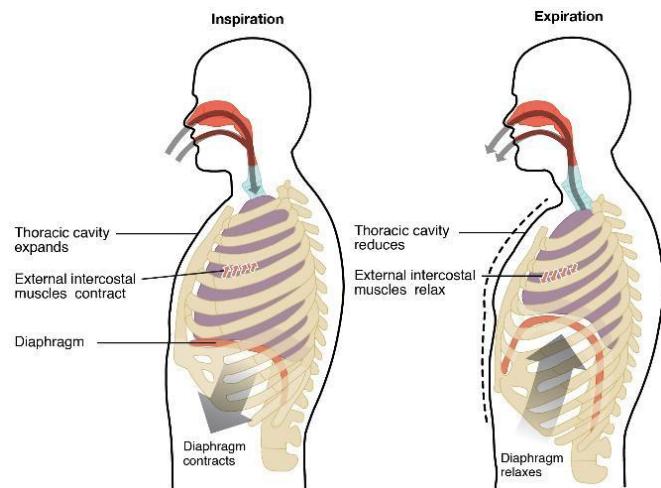
Breathing comprises two major steps: inspiration (inhalation) and expiration (exhalation) (see figure). Inspiration is the process that causes air to enter the lungs, and expiration is the process that causes air to leave the lungs. A respiratory cycle is one sequence of inspiration and expiration. During inspiration, the diaphragm and the other muscles contract. When the diaphragm contracts, it creates more space for the lungs. This increase in volume decreases the pressure in the lungs to below atmospheric pressure, which creates a pressure gradient that drives air into the lungs. The process of normal expiration is passive, meaning that energy is not required to push air out of the lungs. Instead, as the diaphragm relaxes, the thoracic cavity and lungs decrease in volume, pressing air out of the lungs. A variety of diseases can affect the respiratory system, such as asthma, emphysema, chronic obstruction pulmonary disorder (COPD), and lung cancer. All of these conditions affect the gas exchange process and result in labored breathing and other difficulties.

#### Activities:

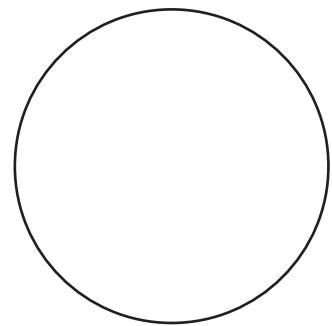
1. Observe the cross section of a lung slide under the microscope. Try to identify an alveolus, the microscopic sac that inflates with air as you inhale. The walls of the alveoli are only one cell thick. Draw your observations.
2. Using tape, label the model with: trachea, left lung, right lung, bronchi, bronchioles.
3. Complete the “Bell Jar Model for Breathing” activity.

→ What happened when you pulled the rubber sheet down?

→ What happened when you released it back to its original position?



Inspiration and expiration occur due to the expansion and contraction of the thoracic cavity, respectively. Credit: OpenStax, licensed under CC BY-4.0



Lung cells in alveolus,  
400x magnification

# Lab 8: Organ Systems

## Questions:

- Think back to previous labs. Why is it so important that our bodies have a constant supply of oxygen? What biochemical process is the oxygen used for?
- Why do the lungs inflate when the diaphragm contracts? (Think about the bell jar.)

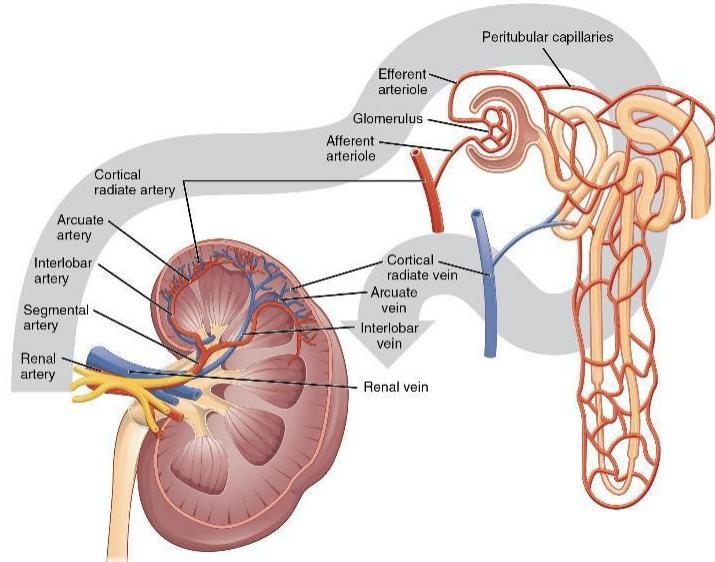
## Station VI: The Urinary System

The urinary system has roles you may be well aware of: cleansing the blood and ridding the body of wastes probably come to mind. The urinary system, controlled by the nervous system, also stores urine until a convenient time for disposal and then provides the anatomical structures to transport this waste liquid to the outside of the body. Failure of nervous control or the anatomical structures leading to a loss of control of urination results in a condition called incontinence.

However, the urinary system also has a number of less obvious functions. It regulates many of the chemical conditions in the body, including the concentration of solutes in the blood; body pH, a function shared with the lungs and the blood; and blood pressure, a role shared with the heart and blood vessels. The kidneys even control the concentration of red blood cells, producing the substance that causes red blood cell production, and perform the final synthesis step of vitamin D production. Each of these functions is vital to your well-being and survival. If the kidneys fail, these functions are compromised or lost altogether, with devastating effects on homeostasis. The affected individual might experience weakness, lethargy, shortness of breath, anemia, widespread edema (swelling), metabolic acidosis, rising potassium levels, heart arrhythmias, and more.

The kidney is made up of millions of microscopic “functional units” called **nephrons**, which cleanse the blood and balance the constituents of the circulation. A “tuft” of high-pressure capillaries, about  $200\text{ }\mu\text{m}$  in diameter, called the **glomerulus**, facilitates the movement of fluid from the blood into the tubules of the nephron to be filtered. The fluid that passes out of the tubules, which has a high concentration of wastes and other solutes, flows out of the nephron into the ureter and is collected in the bladder until it can be eliminated.

Since the kidneys are so vital to maintaining chemical homeostasis, **urinalysis** (a urine test) is a simple, non-invasive tool to diagnose a plethora of disorders. A urinalysis involves checking the appearance, concentration and chemical content of the urine. For example, proteins are very large molecules that are not filtered out of the blood. The presence of proteins indicates kidney damage that is allowing proteins to “leak” from the blood to the urine. The table below indicates a few conditions that can be implicated by a urinalysis, though many will require additional tests to confirm a diagnosis.



Macroscopic (left) and microscopic (right) structure of the kidneys, including how the urinary tubules interact with the capillaries in the glomerulus. Credit: OpenStax, licensed under CC-BY 4.0

## Lab 8: Organ Systems

Test	Abnormal Conditions Reported by Test
pH	Acidic urine (below 4.5)— diabetes, starvation, dehydration, respiratory acidosis Alkaline urine (above 7.5) - kidney disease, kidney failure, urinary tract infection, respiratory alkalosis
Glucose	Diabetes
Proteins	Kidney Disease
Ketones	Starvation, prolonged vomiting, diabetes, hyperthyroidism and other metabolic disorders

### Activities:

1. On the small body model, label the kidneys, the ureters and the bladder using tape. You may have to remove some other organs to find these.
2. Observe the kidney slide under the microscope. You will not be able to directly observe a nephron, but you should be able to find a glomerulus, which will appear as a distinct ball of capillaries. Draw your observations in the space at right.
3. Complete the Urinalysis Activity. Record your results in the table.

Sample	pH	Glucose	Protein	Ketones	Diagnosis
Normal					None (within normal limits)
Patient 1					
Patient 2					
Patient 3					
Patient 4					

### Questions:

- Why is urinalysis such an important diagnostic tool?
- Name two ways the kidneys works with other body systems to maintain homeostasis.

## Station VII: The Digestive System

The digestive system is continually at work, yet people seldom appreciate the complex tasks it performs. Consider what happens when you eat an apple. You enjoy the apple's taste as you chew it, but in the hours that follow, unless something goes amiss and you get a stomachache, you don't notice that your digestive system is working. You may be taking a walk or studying or sleeping, having forgotten all about the apple, but your stomach and intestines are busy digesting it and absorbing its vitamins and other nutrients. By the time any waste material is excreted, the body has extracted all it can use from the apple. In short, whether you pay attention or not, the organs of the digestive system perform their specific functions, allowing you to use the food you eat to keep you going.

## Lab 8: Organ Systems

The easiest way to understand the digestive system is to divide its organs into two main categories. The first group is the organs that make up the digestive tract. The second group is comprised of the accessory digestive organs, which mechanically or chemically break down of food and allow the assimilation of its nutrients into the body. Accessory digestive organs, despite their name, are critical to the function of the digestive system.

Also called the gastrointestinal (GI) tract or gut, the **digestive tract** is a one-way tube through the body. The main function of the organs of the digestive tract is to nourish the body. This tube begins at the **mouth** and terminates at the **anus**. Between those two points, the canal is modified into the **pharynx**, **esophagus**, **stomach**, **small and large intestines**, and **rectum** to fit the functional needs of the body. Both the mouth and anus are open to the external environment; thus, food and wastes within the digestive tract are technically considered to be outside the body. Only through the process of absorption do the nutrients in food enter into and nourish the body's "inner space."

Each **accessory digestive organ** aids in the breakdown of food. Within the mouth, the **teeth** and **tongue** begin mechanical digestion, whereas the **salivary glands** begin chemical digestion. Once food products enter the small intestine, the **gallbladder**, **liver**, and **pancreas** release secretions, such as bile and enzymes, essential for further digestion. Together, these are called accessory organs because they sprout from the lining cells of the developing gut (mucosa) and augment its function; indeed, you could not live without their vital contributions, and many significant diseases result from their malfunction. Even after development is complete, they maintain a connection to the gut by way of ducts.

### Activities:

1. Using tape, label the digestive system model with mouth, esophagus, stomach, small intestine, large intestine, rectum, pancreas, liver and gallbladder.
2. Measure the length of each organ of the digestive tract and record your results.

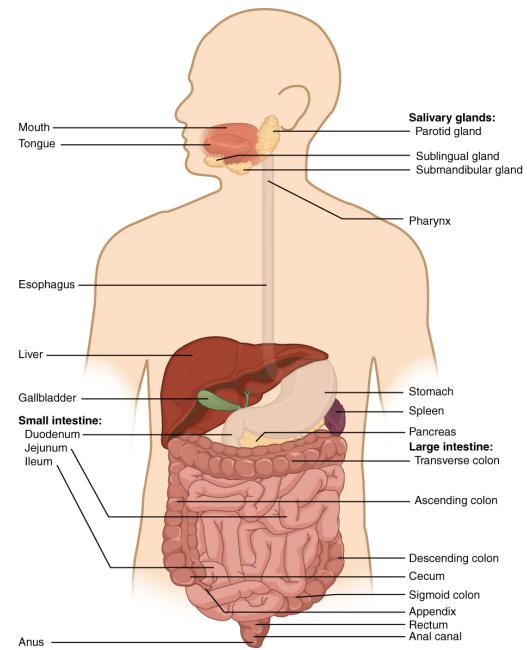
Mouth	Pharynx	Esophagus	Stomach	Small intestine	Large intestine	Rectum

3. Watch the video cued up on the laptop (<https://www.youtube.com/watch?v=5ufESc1bK78>) and answer the questions below:

→ What triggers the contractions of the esophagus?

→ What is secreted by the pancreas into the small intestine? Why is this important?

→ What is absorbed by the large intestine?



## Lab 8: Organ Systems

### Questions:

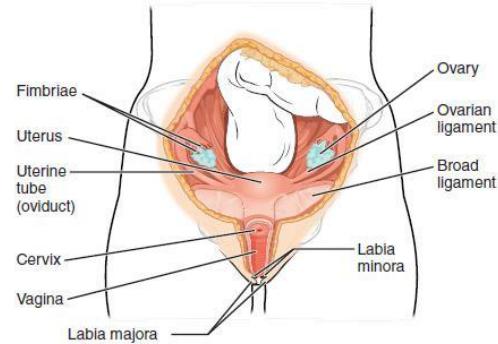
→ What is the difference between the organs of the digestive tract and the accessory organs?

→ Why do you think the digestive tract is so long?

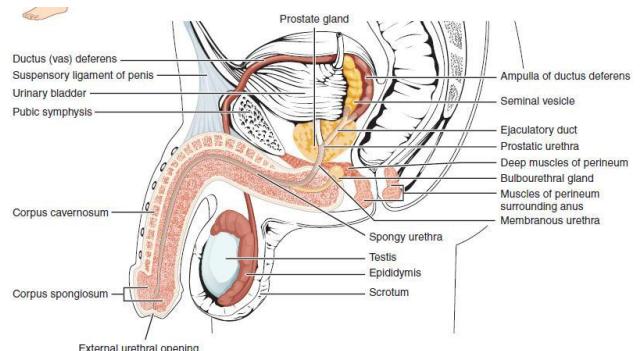
## Station VIII: The Reproductive System

The function of the reproductive system is to produce gametes (reproductive cells) and reproductive hormones, support the developing fetus, and deliver it to the outside world. The reproductive system is unique among the body systems in that it is not required for homeostasis or survival, only for the production of offspring. The female and male reproductive systems are built from the same structures in the developing embryo, but perform somewhat different functions in the adult: the female reproductive system contains the necessary structures to provide a full life support system for the developing fetus, while the male reproductive structure must excrete its gametes in order for them to participate in fertilization. Female gametes are large, while male gametes are small; in fact, in terms of sex cells, this is the definition of male and female. In monosexual organisms like mammals, this difference is often reflected in other aspects of the reproductive strategies of males and females: within a majority of species, females have fewer offspring and invest more resources in each one, while males invest less in a larger number of offspring.

The female reproductive system is located primarily inside the pelvic cavity. The ovaries, the female gonads, produce large gametes called **oocytes** (egg cells), as well as a variety of reproductive hormones that regulate oocyte production and other aspects of the reproductive cycle. A single oocyte is usually produced every few weeks. The oocyte migrates down the **fallopian tubes** to the **uterus**; if any viable sperm are present in the fallopian tube, the oocyte may fuse with (usually) one of them and become **fertilized** en route to the uterus. Fertilization occurs when a sperm and an oocyte combine and their nuclei fuse. Because each of these reproductive cells is a haploid cell containing half of the genetic material needed to form a human being, their combination forms a diploid cell. This new single cell, called a **zygote**, contains all of the genetic material needed to form a human—half from the mother and half from the father. If the reproductive hormones have prepared the uterus for **implantation**, the zygote can settle into the wall of the uterus. At this point, the reproductive system builds a structure to supply the zygote with blood (the **placenta**) and protect it from the intrauterine environment (the **amniotic sac**). Over the next nine months, the zygote develops into an embryo with distinct tissues, then into a fetus with its own distinct organ systems, and then into a fully formed infant, consisting of trillions of cells with myriad



Internal views of normal human female (top) and male (bottom) reproductive systems. Credit: OpenStax under CC-BY 4.0



## Lab 8: Organ Systems

specialized functions, and capable of life outside the womb. This development depends upon the appropriate synthesis of structural and functional proteins, which is governed mainly by the maternal reproductive system, but also influenced by the genetic material inherited from the parental egg and sperm, as well as environmental factors.

The function of the male reproductive system is to produce sperm and transfer them to the female reproductive tract. In males, the urethra is shared between the bladder and the urinary system. The paired testes produce both sperm and the hormones called androgens, which support reproductive physiology. (The most important androgens are testosterone and progesterone.) Several accessory organs and ducts aid the process of sperm maturation and transport the sperm and other seminal components to the penis, which delivers sperm to the female reproductive tract.

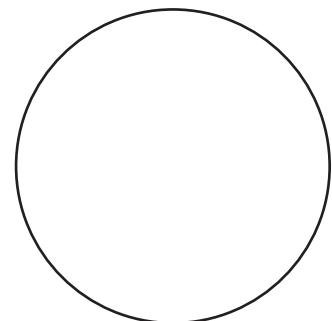
### Activities:

1. Observe the ovary slide under the microscope. Identify a Graafian follicle and draw your observations in the space at right. The Graafian follicle contains one oocyte and many follicle cells around it, separated by a large cell-free space.
2. Observe the sperm slide under the microscope and draw your observations in the space at right.
3. Complete the Condom Demonstration Activity and record the results below.

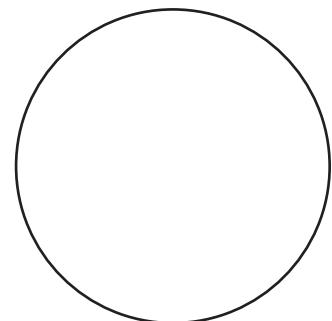
Material	Color of water
Latex	
Natural	
Latex with holes	

### Questions:

- What are two differences between the male and female reproductive strategies?
- A complete human genome is made up of 46 chromosomes. For a zygote after fertilization to also have 46 chromosomes, how many must be in an oocyte or sperm cell?
- Given that bacteria and viruses are very small, which condom would provide the best protection against sexually transmitted infections?



Graafian follicle & oocyte,  
400x magnification



Sperm cells, 400x  
magnification

## Lab 8: Organ Systems

**Organize your thoughts.**

Organ System	Main Functions	Main Organs	Example of Homeostasis or Structure and Function
Muscular and Skeletal System			
Nervous			
Senses			
Circulatory			

## Lab 8: Organ Systems

Respiratory			
Urinary			
Digestive			
Reproductive			

# Lab 9: DNA and Gene Expression

## Learning Goals

1. Extract and examine DNA from plant cells.
2. Identify the differences between DNA and RNA and their roles in gene expression.
3. Use the genetic code to transcribe and translate a gene.
4. Identify the roles of mRNA, rRNA, and tRNA in transcription and translation.

## Agenda

1. DNA extraction from living plant tissue
2. DNA models: replication, transcription, translation

## Strawberry DNA extraction

DNA molecules in plants and animals are billions of base pairs long, and they're usually unrolled inside the nucleus so that the cell can make mRNA transcripts -- they're only wound up tightly into chromosomes just before cell division. This means that they look like very long, thin threads: the DNA molecule is only 2 nanometers (0.000 000 002 m) wide, but if you laid all the DNA from a single human cell end to end, it would be about 2 meters long.

In this section, you will extract DNA from strawberry cells. The procedure for extracting DNA involves first breaking the cell walls with a blender, then dissolving the cell membrane and the nuclear membrane with a homogenizing medium made up of salt, sodium citrate, and EDTA. Strawberries are a good first DNA extraction to try, because they have an unusually large amount of DNA. While most cells in animals are either **haploid** sex cells (1 version of each chromosome) or **diploid** body cells (2 versions of each chromosome), hybridization in plants often results in their cells having more than two versions of each chromosome. The body cells of strawberry plants, including the fruit, are **octoploid**! Having more than two versions of each chromosome (**polyploidy**) is not uncommon in hybridized plants, but even for a plant, eight is an extremely large number.

### Procedure:

1. Place two or three frozen strawberries in the blender. Cover with 100 mL of cold homogenizing medium.
2. Blend for at least 1 minute, or until the mixture is smooth.
3. Pour the blended strawberries into a beaker and place the beaker in an ice bath. Wait 10 minutes for the chemicals to work on the cells in the mixture.
4. While you're waiting, prepare a filter by folding a piece of cheesecloth several layers thick, attaching it to a second beaker with a rubber band, and poking the middle down to form a basin. Place this beaker in the ice bath as well.
5. After 10 minutes, strain the strawberry mixture through the cheesecloth. You can stir it gently with a glass stirring rod to make it filter a little faster. When you're done, remove and discard the cheesecloth.
6. Pour a few milliliters of the filtered strawberry mixture into a test tube. Then, very gently and carefully, add one to two milliliters of cold ethanol by squirting it down the side of the tube. The goal is to float a layer of ethanol on top of the strawberry liquid.
7. Now hold it and wait as the DNA begins rising into the alcohol. It will look like a mass of slime or very fine silk thread with bubbles in it.
8. After a few minutes, there will be a lot of DNA in the ethanol. If you like, you can gently reel it up onto a glass stirring rod, or you can just look at it in the beaker.

## Lab 9: DNA and Gene Expression

Your instructor may ask you to take a photo of the DNA you extracted.

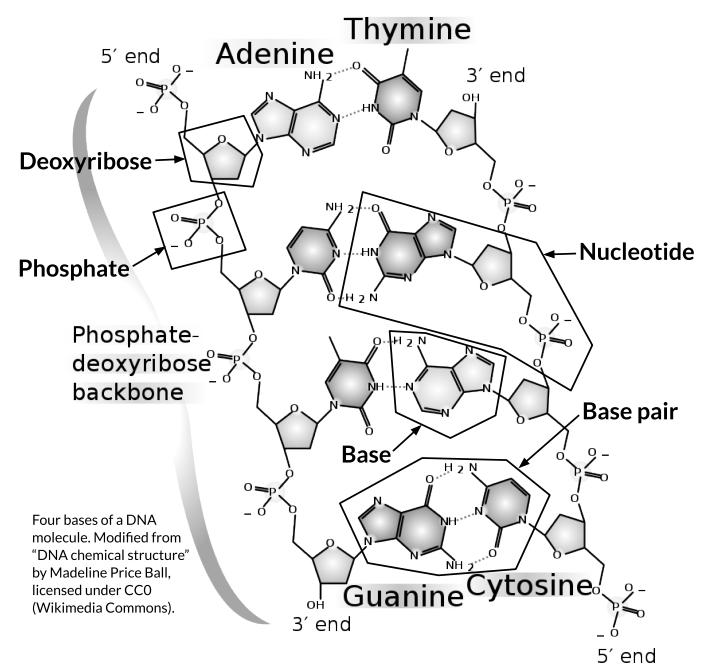
Questions:

- What was the purpose of each step in this extraction process? In particular, why did we need to use the blender and the homogenizing medium?
- If you put the wad of extracted DNA under a microscope, do you think you would be able to see the DNA molecules? Why or why not?
- Does the DNA look the way you previously imagined DNA to look? Explain.

## DNA structure, transcription, and translation

The nucleic acids, **ribonucleic acid (RNA)** and **deoxyribonucleic acid (DNA)**, are the molecules that all cells use to store and transmit their genetic information. DNA, which is a two-stranded nucleic acid, stays inside the nucleus except during cell duplication, is used for the long-term storage of the genome. RNA, which is single-stranded and is present everywhere in the cell in multiple forms, allows the information encoded in the DNA to be assembled into usable protein molecules outside the nucleus. We have only known about nucleic acids since the 1950s; before that, nobody knew how genetic information was stored. The discovery of DNA and RNA has produced a huge scientific boom: being able to read the genetic code means that we can now compare genomes, identify specific genes and determine their functions, and even insert new genes into cells' DNA.

Nucleic acid molecules are polymers, which means they are made up of a repeating string of smaller molecules. Each of these smaller molecules is called a nucleotide, and consists of three parts: a sugar (ribose or deoxyribose) and a phosphate,



## Lab 9: DNA and Gene Expression

which make up the “sides” or “backbone” of the molecule, and the nitrogenous base, which makes up the “rungs” of the molecule. There are five of these, adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U); the first three are present in both DNA and in RNA, while T is only in DNA and U is only in RNA. The information in a genetic sequence is stored as a series of nitrogenous bases.

Information transfer happens by the bonding of complementary bases: during DNA synthesis or RNA transcription, C and G always bond, and A and T/U always bond. Thus, each time a nucleic acid molecule is duplicated, it produces an inverse of the original: for example, a DNA polymerase making a new strand based on a strand with sequence AA-CATGATGC would produce a new strand with sequence TTGTACTACG. The end of a nucleotide with the phosphate group is referred to as the 5' (pronounced “five prime”) end, and the other end is called the 3' end. The enzymes that assemble nucleic acids always attach a 3' end to a 5' end.

Differences between DNA, RNA, and protein molecules				
Molecule	Subunits	Structure	Location	Function
DNA	Deoxyribose nucleotides	Double helix	Chromosomes inside nucleus	Long-term storage of genetic information
RNA	Ribose nucleotides	Single strand	Throughout cell	Turning genetic information into proteins
Protein	Amino acids	Folded single strand	Everywhere in the organism (inside & outside cells)	Many functions: structural, enzymatic, signaling, transport...

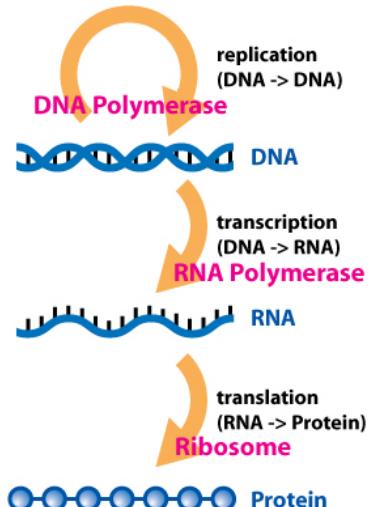
The figure at right summarizes how genetic information is transferred inside the cell. The arrows represent the actions of the enzymes that turn one kind of information-containing molecule into another. The transfer of information from DNA to RNA to protein, but not backwards, was one of the earliest important results to be established about the way genetic information is processed in the cell, and as a result is called the “central dogma” of molecular biochemistry.

In this section we will be using the DNA kits to make models of DNA and mRNA. You will assemble a model DNA molecule and model the process of transcription and translation to understand how replication of the DNA occurs. We will also use the DNA code of a particular gene to determine the amino acid sequence of the resulting protein and explore the effect of single-codon mutations.

### DNA

DNA is a two-stranded nucleic acid. The two strands are antiparallel -- that is, the nucleotides are oriented in opposite directions, with the 5' end of one strand and the 3' end of the other strand at the same end of the molecule. The molecule's flat ladder-like structure (as depicted in the figure on page 1) coils up into a right-handed double spiral, the familiar “double helix” shape that we recognize as a DNA molecule. This structure makes the molecule more stable.

The copying of a DNA molecule, which happens before mitosis or meiosis, is called replication. This process unzips the whole molecule and uses each half to assemble a new opposing strand. Thus, each of the two new DNA molecules contain one old strand from the original DNA molecule and one newly-assembled strand; DNA replication is therefore described as “semiconservative”.



In the cell, information flows from DNA to RNA to protein. (Credit: Daniel Horspool, Wikimedia Commons, CC BY-SA 3.0)

## Lab 9: DNA and Gene Expression

→ Use the DNA kit to assemble the model of a segment of a DNA molecule.

1. First look at the available pieces and figure out which pieces correspond to each subunit of the DNA molecule. Write the colors of the pieces that represent the four types of bases here so you can refer to it easily.

A color:

C color:

G color:

T color:

2. Then assemble the coding strand using the yellow “deoxyribose” pieces, based on the (very short) nucleotide sequence assigned to your lab group in the following table. The DNA molecule should be 15 base pairs long; do not use the sequence given in the instruction booklet, use the one given in the table below.
3. Finally, assemble the template strand using the grey “deoxyribose” pieces, one base at a time, starting at the 5' end. At each base in the strand, all A's should be facing T's and all C's should be facing G's. Follow the assembly instructions in the kit, but **do not** assemble the stand as described in the instruction booklet.
4. Label the **coding strand** (your assigned sequence as printed in the table above) and the **template strand** (the opposing sequence) using tape.
5. Show your DNA molecule to your instructor and answer the questions below before proceeding.

Group	Sequence (coding strand)	Group	Sequence (coding strand)
1	ATGATAACCTCGATGA	5	ATGCGATGAGCGTAG
2	ATGTTAACGGGTTAG	6	ATGGCCTGTCAATAA
3	ATGGCCACCAGATGA	7	ATGCTCTTACATTAG
4	ATGAGATTGTCGTA	8	ATGTCGAAGTCTTGA

Questions:

→ Write out the nucleotide sequence of your group's template strand:

→ Your assigned gene is 18 bases long. How many bases long are the shortest real genes? How about the longest real genes?

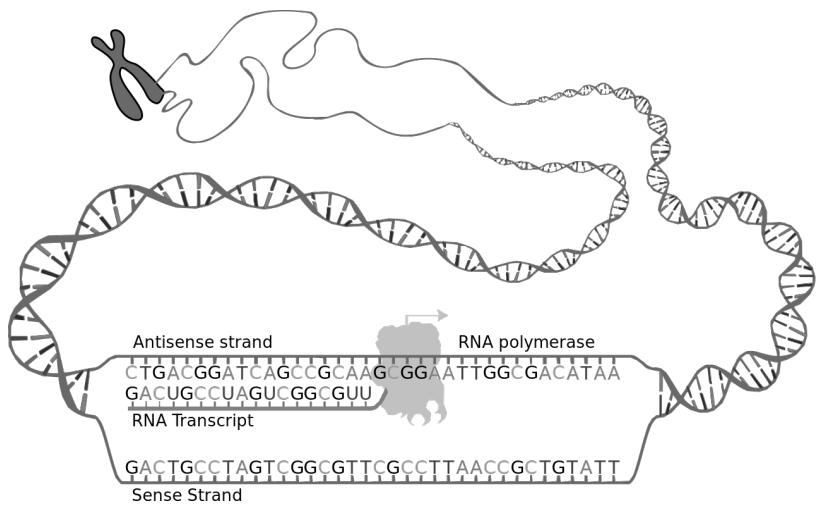
→ The semiconservative nature of the DNA replication process is thought to decrease the mutation rate. How might this method of replication reduce errors?

## DNA -> mRNA (transcription)

RNA is a single-stranded nucleic acid. Although DNA is the nucleic acid used for long-term data storage in the cell, turning the information stored in DNA into the proteins used by the cell requires several steps. The first of these is transcription, in which the two strands of the DNA separate and a single-stranded molecule of **mRNA** is assembled against the template (antisense) DNA strand. The resulting mRNA molecule has the same sequence as the coding (sense) DNA strand, but with all the thymine bases replaced by uracil bases.

## Lab 9: DNA and Gene Expression

The enzyme that assembles the mRNA molecule, which is called **RNA polymerase**, travels in the direction going from the 5' end to the 3' end. It attaches to the DNA molecule at a particular sequence of nucleotides upstream of the gene, copies out the coding region of the gene, and stops copying and detaches from the DNA molecule when it reaches another specific sequence of nucleotides. Both the promoter and terminator are called **noncoding** DNA because they do not get transcribed or translated. The part between them, which gets transcribed into mRNA and taken outside the nucleus for translation, is called **coding** DNA. (There is no noncoding DNA represented in your sequence.)



- Transcribe your model DNA sequence into an mRNA sequence. The instructions for how to do this are included with the DNA/RNA model kit. Remember to start assembling the mRNA molecule from its 5' end.
- Show your mRNA strand to the instructor once you've completed it and answer the questions below before continuing.

Notes: In this step, you will be the RNA polymerase. Use the template strand of your model DNA to build a complementary model mRNA strand. The mRNA strand will use the other shape of “sugar molecule”, indicating that it is made of ribose instead of deoxyribose, and will be a copy of the *coding* strand: that is, it will contain the same bases as the sequence on your paper, except with U instead of T because it's RNA.

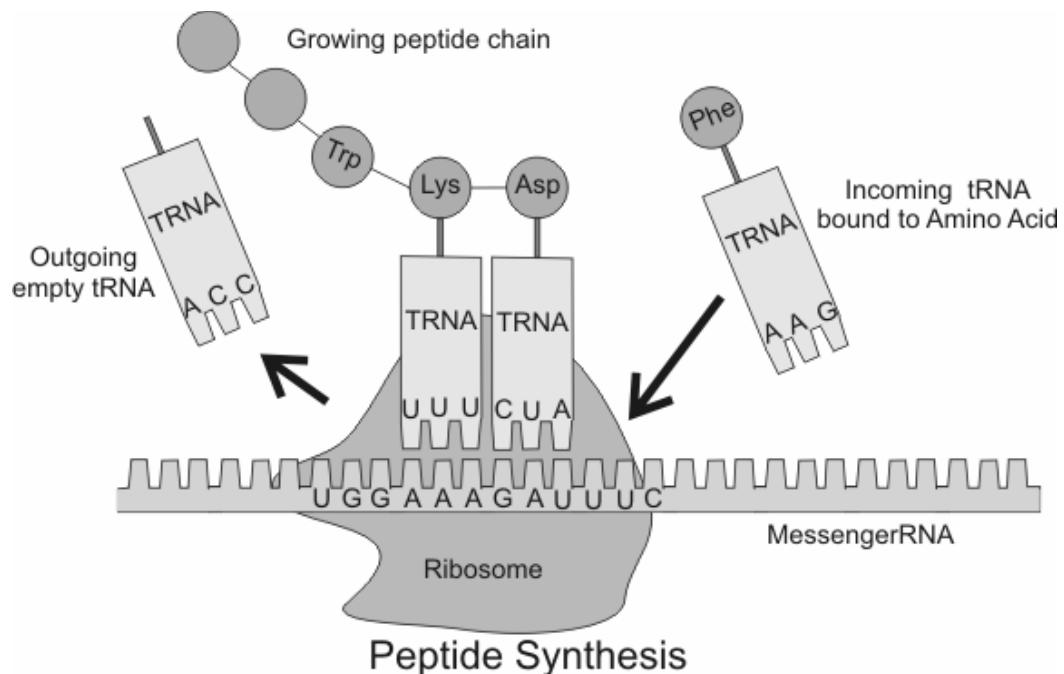
Questions:

- Write out the nucleic acid sequence of your mRNA molecule.
- Eukaryotes keep the DNA inside the nucleus and send mRNA transcripts out into the cytoplasm. Prokaryotes don't have a nucleus, so the whole process happens right out in the cytoplasm. What advantages might there be to each of these approaches?

## RNA → protein (translation)

The process that turns an mRNA transcript into a chain of amino acids is called translation. Every codon (group of three bases) in the mRNA corresponds to a particular amino acid. Since the bases in the mRNA transcript are the same as in the DNA it came from, the two sequences describe the same sequence of amino acids. Thus, the sequence of amino acids represented by the sequence of codons is the “message” that the mRNA carries. For a usable protein to be assembled from this “recipe”, those amino acids need to be assembled into a peptide (an amino acid polymer) that can be folded up into the right shape in the endoplasmic reticulum.

## Lab 9: DNA and Gene Expression



**Translation of mRNA to an amino acid chain by tRNA and ribosome.**  
 (Credit: Wikimedia Commons under CC-BY 3.0)

Ribosomes, which are very small and numerous organelles made up of rRNA and protein, use mRNA and tRNA to translate the mRNA transcript into a peptide by repeating the following steps for every codon in the mRNA, as illustrated in the figure above:

1. The ribosome finds the next codon on the mRNA molecule and grabs the matching tRNA anticodon from the cytoplasm. (The tRNA anticodon that matches the mRNA codon has the same base pairs as the *template* strand in the original DNA sequence, except with U's instead of T's.)
2. The rRNA in the ribosome, acting like an enzyme, attaches the amino acid to the end of the peptide and releases the tRNA back into the cell.

The table on p. 9 gives the relationship between **mRNA codons** (not tRNA anticodons or DNA sequences!) and amino acids. The three-letter and one-letter abbreviations are sometimes used instead of the names to save space.

→ Using the amino acid code chart, translate the mRNA into an amino acid sequence by filling out the following table, as follows.

1. Write down the mRNA codons in order in the first column.
2. Figure out the corresponding tRNA anticodons and write them in the second column.
3. Look up the **mRNA codons** in the above table and write down the corresponding amino acids in the last column to get your amino acid sequence. You should use either the full names or the three-letter abbreviations.

→ All translated sequences begin with the **Start codon** (AUG), which tells the ribosome where to attach to the mRNA transcript and start translating. This means that all amino acid sequences begin with methionine (Met). The first row of the table is already filled in with this information. When the ribosome reaches a **Stop codon** (UGA, UAA, or UAG), it stops translating and releases the peptide.

mRNA codon						
tRNA codon						
Amino acid	Methionine					STOP

# Lab 9: DNA and Gene Expression

## mRNA - amino acid translation table

	U		C		A		G	
U	UUU	Phenylalanine (Phe, F)	UCU	Serine (Ser, S)	UAU	Tyrosine (Tyr, Y)	UGU	Cysteine (Cys, C)
	UUC		UCC		UAC		UGC	
	UUA	Leucine (Leu, L)	UCA		UAA	STOP	UGA	STOP
	UUG		UCG		UAG		UGG	Tryptophan (Trp, W)
C	CUU		CCU	Proline (Pro, P)	CAU	Histidine (His, H)	CGU	Arginine (Arg, R)
	CUC		CCC		CAC		CGC	
	CUA		CCA		CAA	Glutamine (Gln, Q)	CGA	
	CUG		CCG		CAG		CGG	
A	AUU	Isoleucine (Ile, I)	ACU	Threonine (Thr, T)	AAU	Asparagine (Asn, N)	AGU	Serine (Ser, S)
	AUC		ACC		AAC		AGC	
	AUA		ACA		AAA	Lysine (Lys, K)	AGA	Arginine (Arg, R)
	AUG	Methionine (Met, M) START	ACG		AAG		AGG	
G	GUU	Valine (Val, V)	GCU	Alanine (Ala, A)	GAU	Aspartic acid (Asp, D)	GGU	Glycine (Gly, G)
	GUC		GCC		GAC		GGC	
	GUА		GCA		GAA	Glutamic acid (Glu, E)	GGA	
	GUG		GCG		GAG		GGG	

You have now encountered all three of the different kinds of RNA involved in DNA transcription and translation. Their differences are summarized in the following table.

Name	Short for...	Function
mRNA	“messenger RNA”	Copies gene from DNA and takes it to the ribosome
tRNA	“transfer RNA”	Attaches to amino acids and takes them to the ribosome
rRNA	“ribosome RNA”	Matches up tRNA and mRNA, then bonds amino acids together

→ Answer the questions below before moving on to the DNA extraction.

# Lab 9: DNA and Gene Expression

## Questions:

- Explain, in your own words or a diagram, the whole process by which a gene is used to make a protein.
  - Explain how complementary base pairing makes replication, transcription, and translation work.
  - If the average protein is about 500 amino acids long, how many base pairs are there in the coding region of the average gene? Remember that each amino acid corresponds to a codon 3 base pairs long.
  - Given the previous answer, if the average gene is 8000 base pairs long, how many of those base pairs are noncoding (i.e. never translated into protein)? What percentage of the gene's length is that?

# Mutations

Any change in a DNA sequence is called a mutation. Depending on exactly what changed and what gene it happened in, a mutation can have any size effect, ranging from none at all to a huge change in the organism's growth or functioning. In this section we will discuss only **point mutations**, which consist of a change in a single nucleotide. However, mutations can also insert, delete, or repeat more than one base pair. These non-point mutations usually produce a major change in the function of the resulting protein.

## Lab 9: DNA and Gene Expression

A **substitution** is the simplest kind of mutation, consisting of changing one nucleotide in the DNA sequence to a different nucleotide (e.g. AACATGATGC → CACATGATGC). Since many amino acids are coded for by several different codons, you can imagine that such a mutation may or may not have an effect. In a **missense mutation**, the codon is changed so that it encodes a different amino acid. For example, if the codon GCU is changed to GAU, then the amino acid will be changed from Alanine to Aspartic Acid (see genetic code table on previous page). In a **silent mutation**, the codon is changed, but the amino acid is not. For example, if the same GCU codon is change to GCC, it will still code for Alanine. The third type of base substitution is a **nonsense mutation**, where a codon that encodes for an amino acid is changed to a stop codon, creating a truncate (shorter) protein. For example, if the codon UAC is changed to UAG, then the codon is changed from Tyrosine to a stop codon.

→ Name one silent, one missense and one nonsense mutation using your gene sequence.

Silent Mutation:

Missense Mutation:

Nonsense Mutation:

Other point mutations make a bigger difference. A **frameshift** happens when a single nucleotide is deleted or added in a sequence (e.g. AACATGATGC → ACATGATGC or AACATGATGC → AGACATGATGC). As a result, *all* the codons “downstream” of the mutation are affected. This substantially changes the peptide that is produced, usually making the resulting protein totally useless.

→ Write out your group’s starting sequence. Then write it out again with a frame-shift mutation -- either an insertion or a deletion -- in the first codon.

Original DNA sequence:

Frame-shifted mutant DNA sequence:

→ What sequence of amino acids would be produced by this mutant version of your gene? Write out the original and mutant amino acid sequences.

Original (copy from your results in part C above):

Frame-shifted mutant:

## Cleanup

1. Pour the contents of your test tube down the sink.
2. Scrub the test tube, beakers, and stirring rods carefully and return them to the appropriate places.
3. Put away your DNA kits. Make sure they are fully taken apart, the pieces sorted, and the instruction sheet replaced.
4. Spray and wipe your lab bench.

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# Lab 10 - Mitosis and Meiosis

## Learning Goals

1. Understand the function of mitosis and meiosis and its role in the cell life cycle.
2. Describe the phases of mitosis and meiosis and identify them in plant and animal cells.
3. Understand how nondisjunction leads to chromosomal disorders.
4. Define karyotype and use one to diagnose chromosomal disorders.

## Agenda

1. Mitosis
  - a. Pop bead model
  - b. Microscopy: onion root and fish embryo cells undergoing mitosis
2. Meiosis: pop bead model
3. Karyotyping

## Mitosis

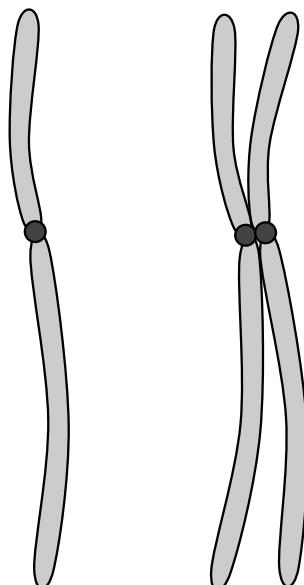
For a cell to clone itself, it must split everything in it into two equal parts. For most organelles, this means half of them can go to each **daughter cell**, but there is typically only one copy of the **genome**. That means it has to make a copy of its genome to pass to the new daughter cell. The simplest form of this process produces a single exact copy of the cell's whole genome. This occurs in all cells, but in this lab we will concentrate on eukaryotes. In eukaryotic cells, the process of exactly duplicating the genome is called **mitosis**.

In eukaryotes, DNA is stored in rolled-up structures called **chromosomes** that normally stay inside the nucleus. To produce a copy of the chromosomes, the chromosomes must be unrolled and copied, then rolled back up and moved outside the nucleus when the cell divides in half, and finally enclosed in the nucleus again to resume their normal function. As an analogy, you can think of the nucleus as the cell's library building, the chromosomes as the collection of books, and the genome itself as the words in the books. Usually one gene is copied at a time: the correct book is opened, the correct section of the book is copied out onto a loose sheet of paper that can be taken out of the library (an mRNA molecule), and then the book is closed and put away. During mitosis, however, the entire book collection gets copied out into a second set of books and then transported into a new building.

The entire cycle of mitosis, which turns one parent cell into two identical daughter cells, takes about 24 hours. The phases of mitosis are illustrated in the table below.

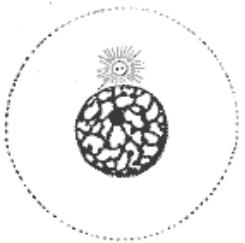
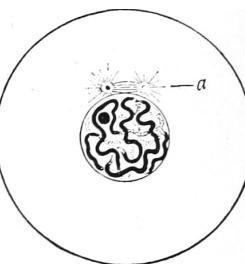
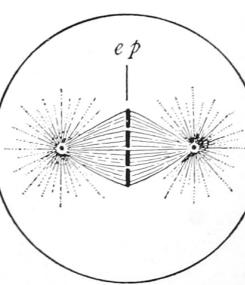
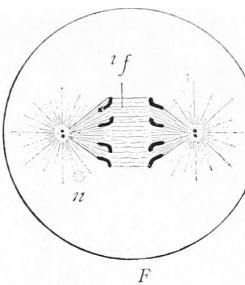
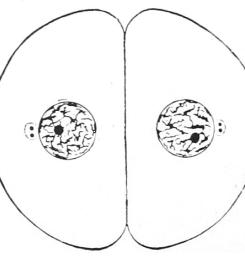
## Mitosis model

In this section, we will use pop beads and string to simulate what happens to the chromosomes during mitosis. Your instructor will demonstrate how to use the pop beads models.



Left: Unduplicated chromosome. 1 chromatid.  
Right: Duplicated chromosome. 2 identical chromatids.

## Lab 10 - Mitosis and Meiosis

	Interphase	The cell's chromosomes are unrolled and copied.
	Prophase	Both copies of each chromosome ( <b>sister chromatids</b> ) are rolled up again and attach to each other at the <b>centromere</b> , the <b>nuclear envelope</b> dissolves, and the <b>centrioles</b> move to opposite sides.
	Metaphase	The centromeres of the chromosomes line up along the center of the cell, forming the <b>metaphase plate</b> , and the centrioles send out fibers that attach to the centromeres.
	Anaphase	The centrioles pull the fibers in, separating each pair of sister chromatids and dragging a single set of chromosomes to each end of the cell.
	Telophase	A new nuclear envelope forms around each set of chromosomes, the fibers detach, and the rest of the cell pinches itself in half to form two new cells ( <b>cytokinesis</b> ).

**All images of stages in mitotic cell division from "The germ-cell cycle in animals", R. W. Hegner, 1914, Figure 3 (p. 32). Public domain.**

### Procedure:

1. Construct two pairs of chromosomes. Each pair should have one red and one yellow unduplicated chromosome (see figure on page 1), exactly the same size, each with a single magnetic “centromere” attached to two different length strings of pop beads. Make the two pairs different lengths so you can tell them apart. This is your starting cell. Your cell is **diploid**, with one copy of each chromosome from each parent (represented by red and yellow), and its **chromosome number** is 4.

## Lab 10 - Mitosis and Meiosis

2. Construct an exact copy of each of the chromosomes you made in part 1. This is what happens in interphase.
3. Using the chromosomes you just made, and using pieces of string to represent the cell membrane and nuclear envelope, lay out a picture of a cell in prophase, then move the chromosomes and membranes around through each stage, all the way to telophase.
4. When you're satisfied that you have the process down, call your instructor over to watch as you walk through it.
5. Someone in your group should make a stop motion video of the mitosis process, just as we did for the enzymes in lab 6. You can add labels, take more than one photo per stage, whatever you like -- this video is intended as a study aid for you and your group.

### Mitosis slides

Take one of each of the slides labeled "Onion Root Tip Mitosis" and "Whitefish Blastodisc Mitosis" from the slide tray. These each contain fixed (not living) tissue from a part of the organism that is actively growing by increasing the number of cells.

→ On each of the two slides, find and draw cells in each of the four states of mitosis. Draw them in the tables below.

Notes:

- Start with the onion root tip slide; it's easier to see because the stain is better.
- Make sure your cells look like the cells under the slide (shape, orientation, etc).
- We can tell when you faked the drawing without looking at the cell. Draw what you see, not what you're expecting to see!

Onion: Prophase	Onion: Metaphase	Onion: Anaphase	Onion: Telophase

Whitefish: Prophase	Whitefish: Metaphase	Whitefish: Anaphase	Whitefish: Telophase

# Lab 10 - Mitosis and Meiosis

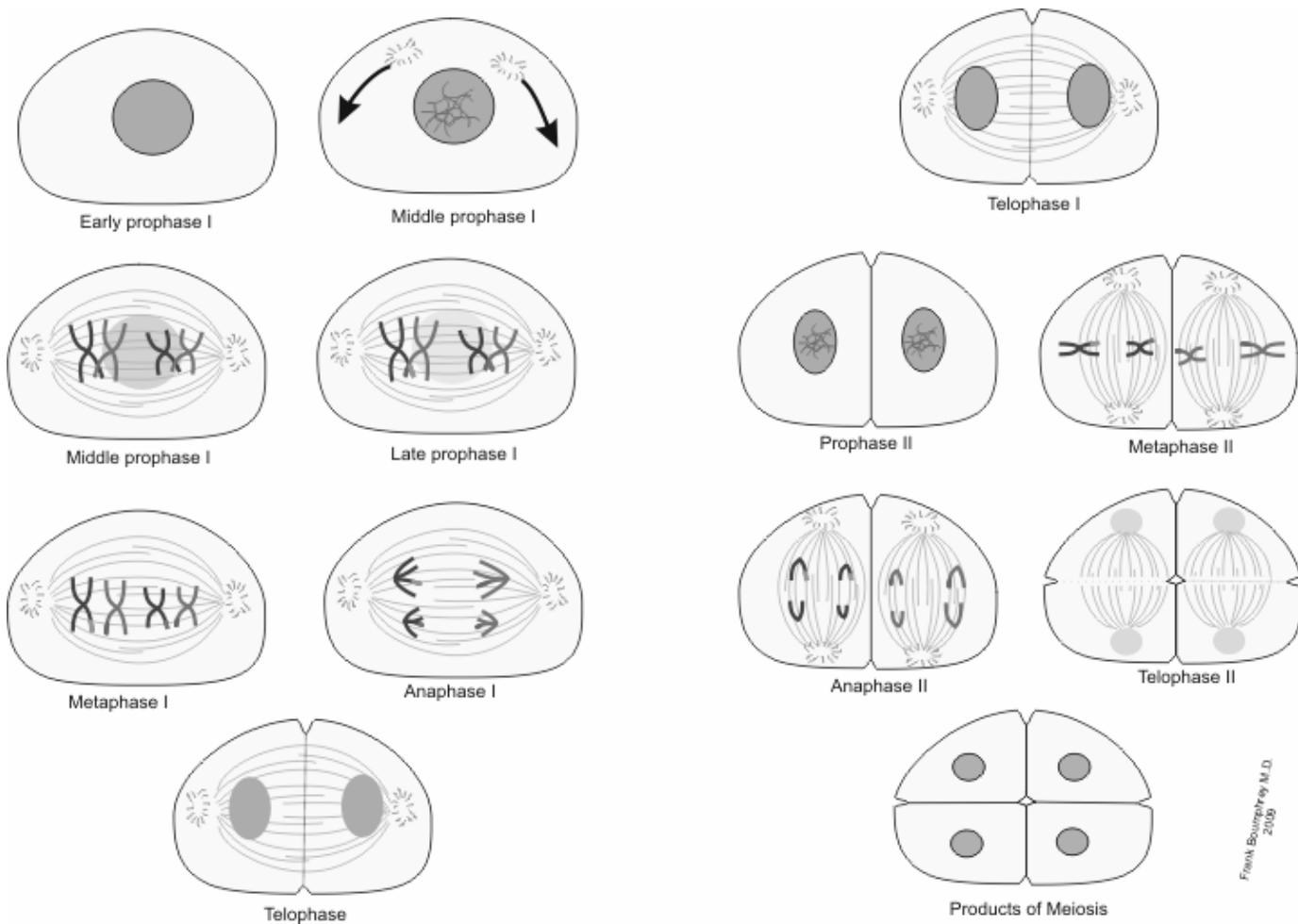
Questions:

- What is the purpose of mitosis?

## Meiosis

Cells can reproduce by either asexual or sexual reproduction. **Asexual reproduction**, such as mitosis, produces new cells genetically identical to the parent cell, while **sexual reproduction** mixes and separates genetic material from multiple parent cells (usually two) to produce cells that are genetically different from their parents. This genetic remixing requires a more complex process of cell reproduction.

In all animals and most plants, the cells of the body are **diploid**; that is, they contain two different versions of each chromosome. When such a diploid cell undergoes mitosis, the two daughter cells it produces are also diploid. However, sexual reproduction requires the production of **haploid** cells, which have only one version of each chromosome. This process typically happens in specialized organs, which in animals are called the **gonads**. Two of these specialized haploid cells (**gametes** or **sex cells**), one from each parent, can then fuse together to form a new diploid cell different from those of either parent (a **zygote**). The production of gametes is the purpose of **meiosis**, which is illustrated in the figure below.



Frank Boumphrey, M.D.  
2009

Left: Meiosis I. Right: Meiosis II. Credit: Dr. Frank Boumphrey (2009) via Wikimedia Commons under CC-BY 3.0

## Lab 10 - Mitosis and Meiosis

The phases of meiosis have the same names as the phases of mitosis, but each of them happens twice. A cell undergoing meiosis first doubles its chromosomes, just as in mitosis. Then it goes through a process called **crossing-over** in which each pair of duplicated chromosomes physically stick themselves together and swap equivalent regions of DNA between them (this happens in prophase I). The resulting four chromatids are thoroughly mixed up: none of them are genetically identical to any of the others or to either of the parent chromosomes. Crossing-over therefore is the step that generates **genetic diversity** among offspring. After this, the newly mixed chromosomes are separated into two cells, each containing two different copies of each chromosome. Then they do it again, splitting into the four haploid sex cells that are the final products of meiosis.

### Meiosis model

Next, we will use pop beads and string again to simulate meiosis.

Procedure:

1. Construct the same starting set of chromosomes that you had at the start of your mitosis video: 4 chromosomes, of two different sizes, one of each size red and the other one yellow.
2. As in the mitosis model, make an exact duplicate of each of the chromosomes you just made. (Interphase is essentially the same in meiosis as in mitosis.)
3. Now go through all the stages of meiosis with your pop beads model. Don't forget to mix together all four of each size of chromosome during crossing over!
4. When you're satisfied that you have the process down, call your instructor over to watch as you walk through it.
5. Again, make a stop motion video of the process.

Questions:

- On the figure on page 90, label the following: homologous chromosomes, sister chromatids, centromere, nuclear membrane, spindles, spindle fibers, cytokinesis, diploid cell, haploid cells.
- What is the purpose of meiosis?

### Karyotypes and chromosomal disorders

A **karyotype** (picture of chromosomes) is made by stopping the division of a cell when it's in metaphase, staining and extracting the chromosomes, spreading them out and taking a photograph, and then physically cutting them out of the photograph and sorting them. The next page shows normal XX and XY karyotypes after sorting. Notice that in a normal human karyotype there are 2 of each of the first 22 **autosomal chromosomes**, and then 2 **sex chromosomes** (either 2 X or 1 X and one Y), for a total of 46 chromosomes. 46 is the **diploid chromosome number** for humans. A haploid cell has half that, so the **haploid chromosome number** in humans is 23. Chromosome number varies among species.

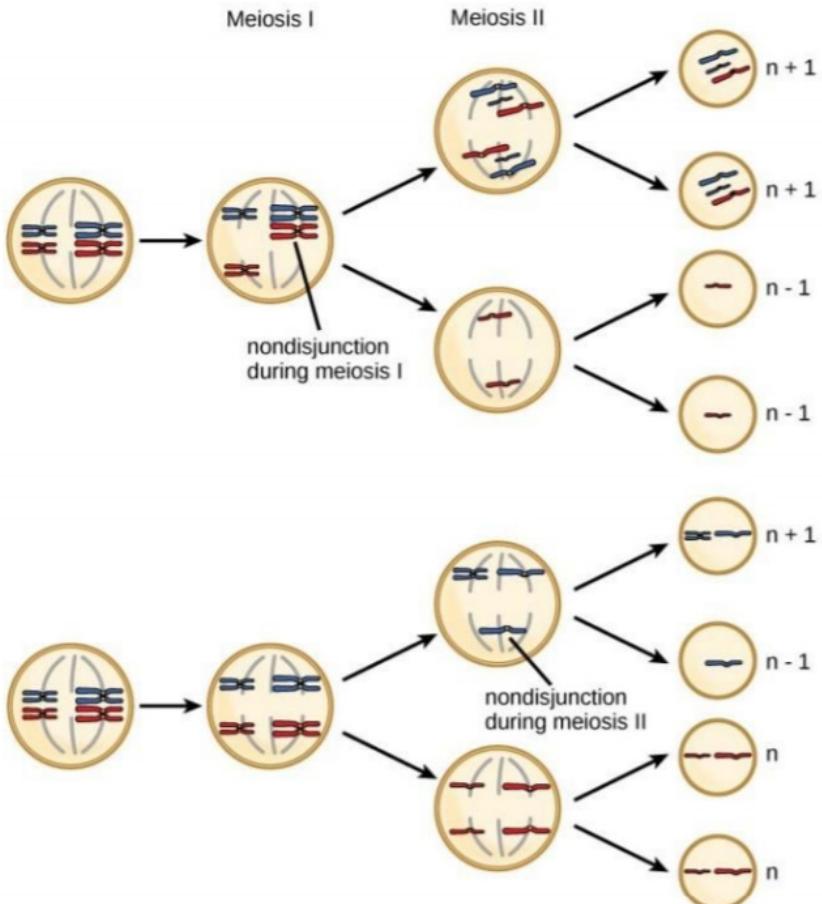
# Lab 10 - Mitosis and Meiosis

If the chromosomes don't get pulled apart correctly during either phase of meiosis (an error called **meiotic nondisjunction**; see illustration at right), the gametes can end up with either more or less than one copy of each chromosome. When the gamete contains an extra copy of a chromosome, after fertilization the cell will have three copies of that chromosome; this is called **trisomy** (tri = three, soma = body). When it completely lacks that chromosome, the cell will have only one copy; this is called **monosomy**. All autosomal monosomies are fatal to the developing embryo, because lacking a second copy of a vital gene causes the embryo to fail to develop. The only survivable monosomy known in humans is in the sex chromosomes. Most trisomies are also fatal to the embryo. However, trisomies in the smaller chromosomes or the sex chromosomes are survivable, and are the cause of several different disorders. The table below describes all the known survivable nondisjunction disorders in humans.

Syndrome	Chromosome affected	Type
Patau	13	Trisomy
Edward's	18	Trisomy
Down's	21	Trisomy
Klinefelter's	Sex (XXY)	Trisomy
Triple X	Sex (XXX)	Trisomy
XYY	Sex (XYY)	Trisomy
Turner's	Sex (X)	Monosomy

Questions:

- Use the table above, the images of normal XX and XY karyotypes on the next page, and the pictures of abnormal karyotypes on the next two pages to answer the following questions for each of the abnormal karyotypes:
- What chromosomal abnormality is visible in this karyotype? Identify the affected chromosome and specify whether it's a trisomy or a monosomy.
  - What disorder does this correspond to?

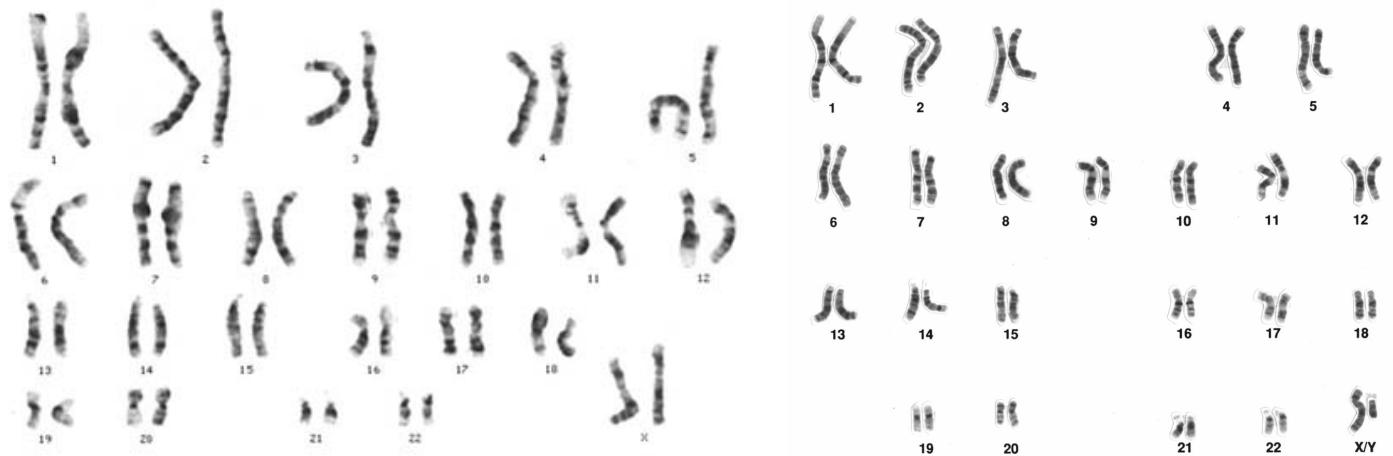


**Meiotic nondisjunction errors occur when homologous chromosomes fail to separate correctly during anaphase I or anaphase II.**

Credit: Concepts of Biology, ch. 7.4.1.1 (Open Textbooks of Hong Kong) under CC-BY SA 4.0 International

Karyotype	Chromosome affected	Type	Disorder name
A			
B			
C			

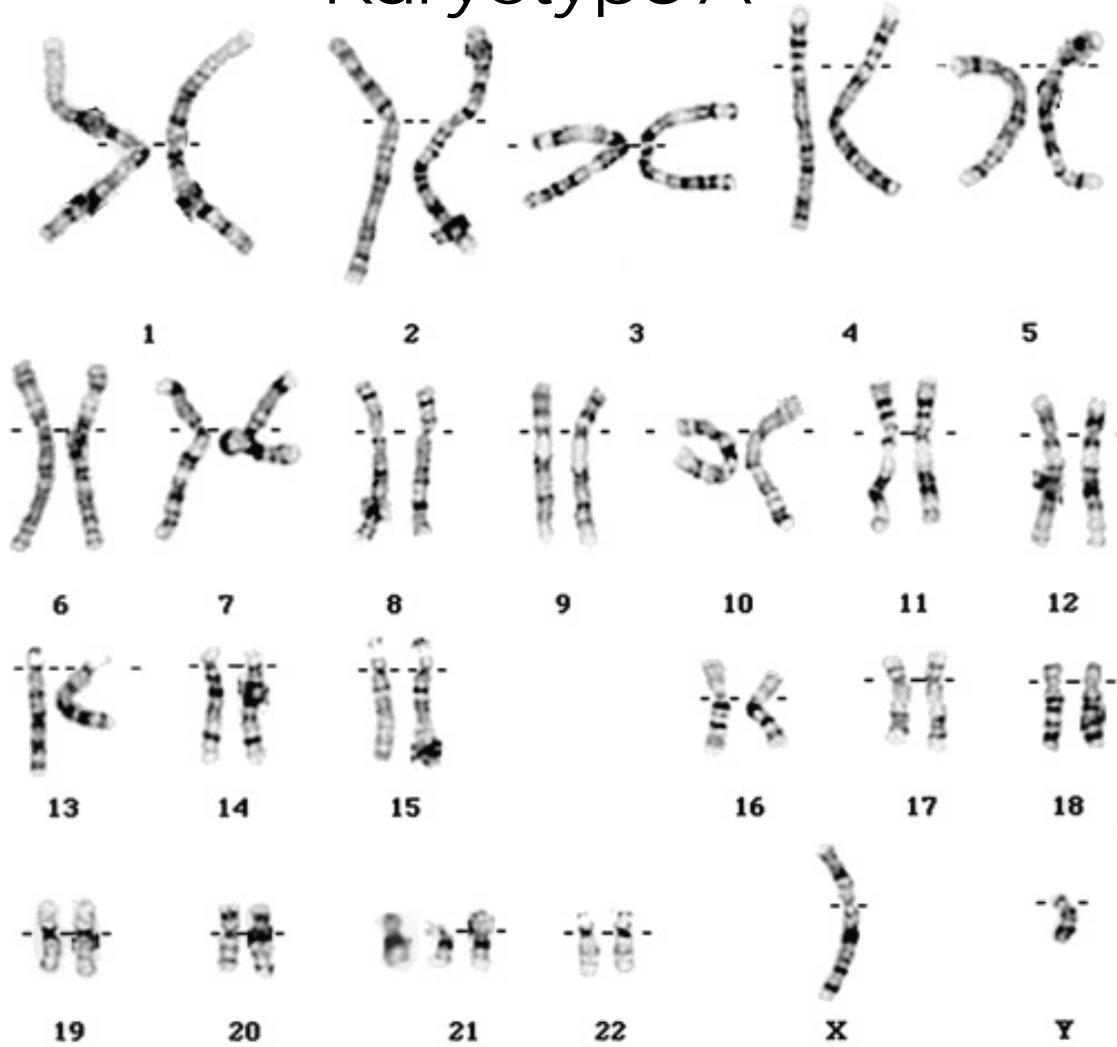
## Lab 10 - Mitosis and Meiosis



Above: Normal human karyotypes. Left: chromosomal female (XX). Right: chromosomal male (XY)

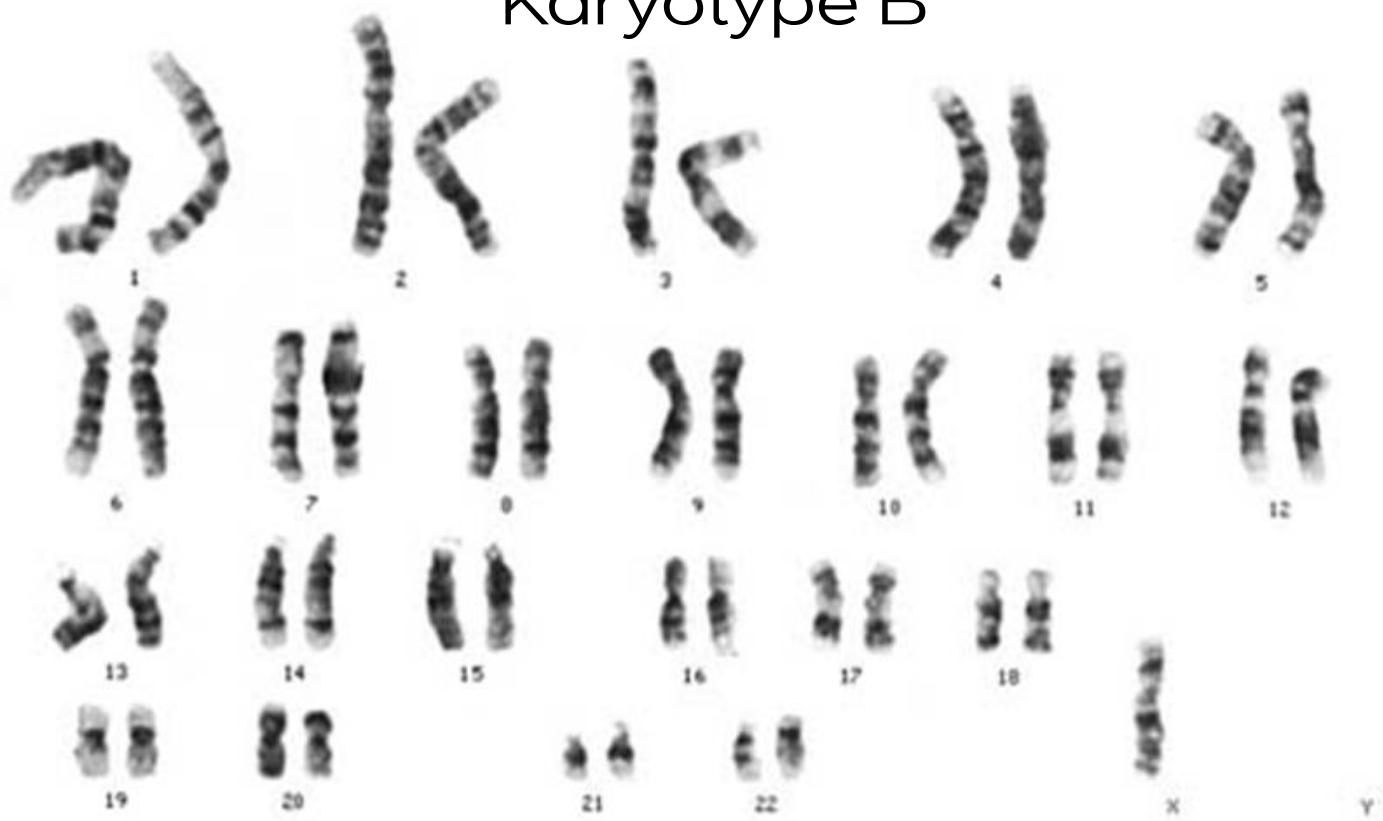
Credit for all karyotypes: Wikimedia Commons under CC-BY SA 3.0

### Karyotype A

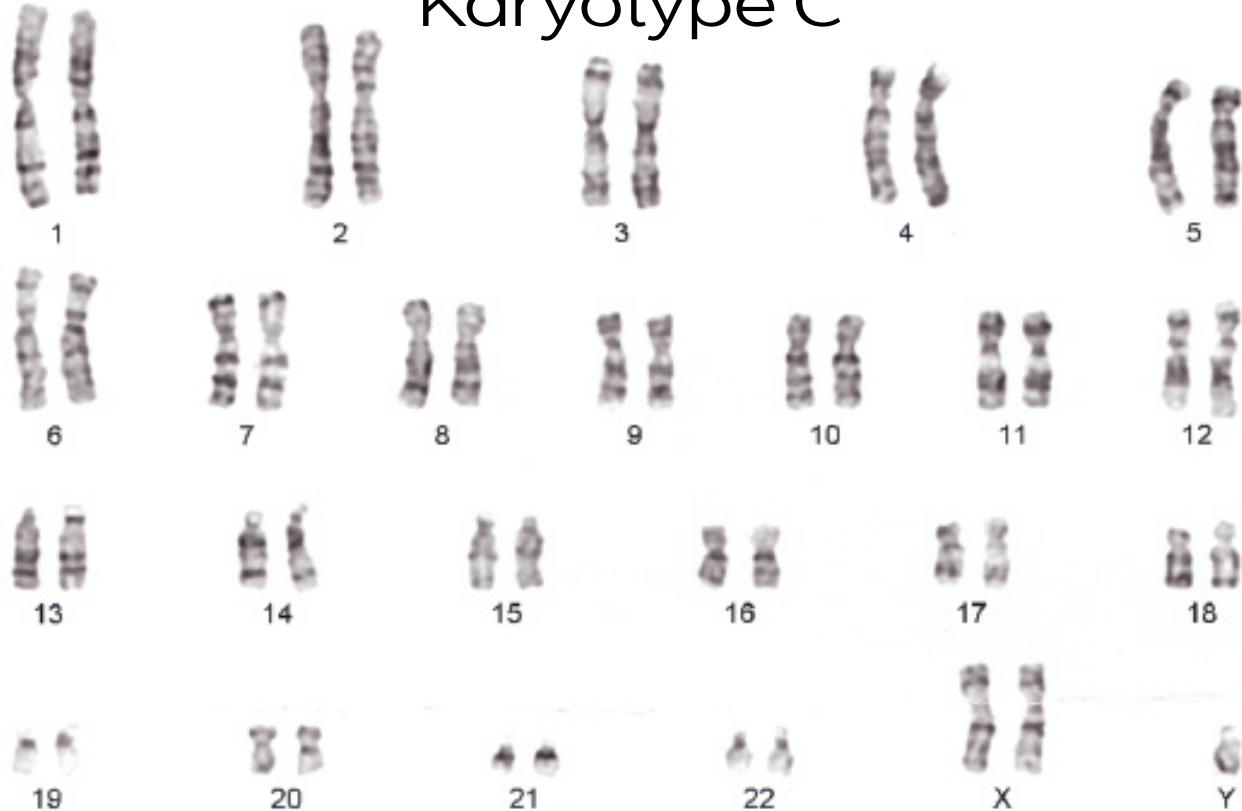


Lab 10 - Mitosis and Meiosis

Karyotype B



Karyotype C



# Lab 11: Genetics and Inheritance

## Learning Goals

1. Define: gene, allele, genotype, phenotype, homozygous, heterozygous, recessive, dominant.
2. Predict the outcomes of monohybrid and dihybrid crosses in corn using a Punnett square, and validate the predictions by observing corn phenotypes.
3. Describe how sex-linked and codominant traits are inherited and expressed.
4. Describe the three alleles in the ABO blood type and their relationship to one another.

## Agenda

### 5. Mendelian genetics

- a. Monohybrid crosses
- b. Dihybrid crosses
- c. Sex-linked traits

### 6. Blood type tests

- a. Genetics of blood type
- b. Example scenario

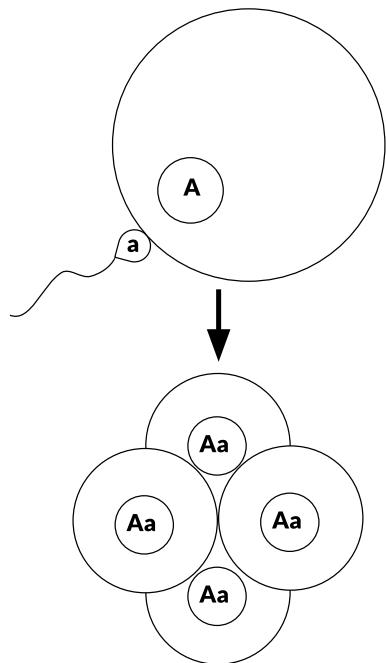
## Mendelian genetics

A gene is the instructions for the production of a single protein. The genes are arranged on the chromosomes in a predictable order: any single locus (the same position along the same chromosome; plural loci) in two different individuals will contain the gene that encodes the same protein. The two individuals may have different alleles (versions of the same gene), but they will affect the same trait (physical attribute of the organism). The relationship between a gene and the associated trait is described in terms of the individual's genotype (the two alleles they have for that gene, one from each parent) and the resulting phenotype (the way the trait is expressed in their body).

Almost all traits in any organism are produced by the complex interaction of many, many genes. However, there are a few traits which are controlled by a single gene that is present on one of the 22 autosomes (chromosomes other than X or Y). These single-gene autosomal traits are the first ones that were discovered, and the simplest to understand; therefore, these are the ones we will explore in the first section.

When a zygote forms from the union of a male gamete and a female gamete, each gamete contributes one allele for each gene in the genome. (Remember, gametes are haploid -- they contain only one copy of each chromosome.) The new individual that grows from that zygote thus has one allele from each parent. An individual's genotype for a particular gene is the product of the interaction between their two alleles for that gene.

Within any single gene locus, some alleles are dominant (they are expressed regardless of what other alleles are present) and others are recessive (they are expressed only if no dominant allele is present). By convention, the dominant allele for a gene is written with a capital letter ("A") and the recessive allele with a lower-case letter ("a"). An individual who is homozygous recessive (they have two identical copies of a recessive



## Lab 11: Genetics and Inheritance

allele of the gene) will have the recessive phenotype, while if they are homozygous dominant (two identical copies of a dominant allele) or heterozygous (having two different alleles, one recessive and one dominant), they will have the dominant phenotype.

A Punnett square, an example of which is depicted at right, is a tool for predicting the distribution of genotypes in all the possible offspring of a cross (i.e. when two organisms reproduce together). The possible gamete genotypes from each parent go at the top and left, then get added together in each box to produce the offspring genotypes. In this example, the gene shown is the *agouti* gene in cats, which controls whether they have black tabby stripes (dominant) or a solid coat (recessive). This example shows a cross between a homozygous dominant parent and a homozygous recessive parent. The offspring of this cross all have the same genotype.

→ Fill in the following table for this cross:

Individual	Genotype	Description of genotype	Phenotype: tabby or solid
Parent 1	AA		
Parent 2	aa		
Offspring	100% Aa		

Using the blank Punnett square at right, write out the results of a cross between two cats that are both heterozygous for the *agouti* gene.

→ What genotypes are present in the possible offspring? How many of each are there? (Express your results as a ratio.)

\_\_\_\_\_ AA : \_\_\_\_\_ Aa : \_\_\_\_\_ aa

→ What phenotypes are present in the possible offspring? How many of each are there? (Express your results as a ratio.)

\_\_\_\_\_ tabby: \_\_\_\_\_ solid

The numbers you just wrote down are the genotypic ratio and the phenotypic ratio. You can see that the ratios you just wrote down will always be the result of a cross between two heterozygous organisms. Heterozygous individuals are sometimes called carriers, because they don't have a recessive phenotype but can pass on a recessive allele to their offspring.

## Dihybrid crosses

The example above is a monohybrid cross; that is, we considered the effects of only a single gene (*agouti*) on only a single trait (tabby vs. solid color). The more traits you examine, as you might imagine, the more complex the statistical outcomes become. If you examine two traits (a dihybrid cross), you need a four-by-four Punnett square to account for all the possible offspring. In this exercise, we will consider genes for the color and texture of corn kernels.

First, take a look at the purebred ears of corn. Notice their uniform appearance: each ear has a uniform phenotype, which is either purple+smooth, purple+rough, yellow+smooth, or yellow+rough. Each kernel in an ear of corn is the fruit of a separate flower, and can grow into its own separate and genetically unique plant; that is, each kernel is an individual offspring. Thus, an ear of corn contains enough offspring to provide a statistically meaningful look at its two parents'

	A	A
a	Aa	Aa
a	Aa	Aa

Parent 1 genotype: AA (homozygous dominant)

Parent 2 genotype: aa (homozygous recessive)

Offspring genotypes: 100% Aa (heterozygous)

## Lab 11: Genetics and Inheritance

genotypes. All the kernels in each of these ears are homozygous for both traits. For the color trait, purple (P) is dominant, and yellow (p) is recessive; for the texture trait, smooth (S) is dominant and wrinkled (s) is recessive.

Then examine one of the hybrid ears. Notice that each kernel has its own phenotype: each one can be either purple or yellow, and either smooth or wrinkled. These hybrid ears are the product of a cross between two heterozygous individuals. Take one of them with you to your table for the next part. You will compare their real phenotype ratios to the phenotype ratios predicted by a Punnett square.

- First, find the predicted phenotype ratios by writing out a dihybrid Punnett square for two parents which both have the genotype PpSs (i.e. heterozygous for both traits).
  - Remember that the gametes have one allele of each gene, so each of the outside boxes should have one **P** or **p** and one **S** or **s**, and the offspring have two alleles of each gene.
  - There are four possible ways to combine the alleles in the parent genotype (PpSs): PS, Ps, pS, and ps. Place these along the outside edges.
- Then combine them just like you did for the monohybrid crosses. Each box in the grid should end up with four letters in it, two of each kind. These are usually written with the dominant alleles first (for example, **PpSs** and not **pPsS**).


## Lab 11: Genetics and Inheritance

- Once you have completed the square, count how many different genotypes there are. Record each genotype and the number of times it occurs in your grid.
- Now figure out how many different phenotypes there are. For each of the genotypes you counted, figure out its phenotype -- that is, what does the actual kernel look like? Record the number of each phenotype in the table below; then find the percentage of each phenotype by dividing each number by 16.

	Purple smooth	Purple wrinkled	Yellow smooth	Yellow wrinkled
Number				
Percentage				

The first row is the ideal phenotype ratio for a dihybrid cross.

- Next, get the real phenotype data for your ear of corn by counting kernels on your hybrid ear of corn. Count how many kernels of each phenotype are present over at least six full rows, and record the data here. Then calculate the percentage of each phenotype by dividing each number by the total number of kernels you counted.

	Purple smooth	Purple wrinkled	Yellow smooth	Yellow wrinkled
Number				
Percentage				

Questions:

- How close are your real data ratios to the expected ideal ratio?
- If your real data are not close to the expected phenotype ratio, why do you think this might be? (For example, if you counted more ears of corn, do you think your data would get closer to the ideal ratio?)
- Do you think that the simple dominant/recessive model is a good description of the genetics of this cross, or is it more complicated than that? Why?

Once you understand the genetics of a system, you can use offspring phenotype ratios to infer the genotypes of the parents.

## Lab 11: Genetics and Inheritance

→ Take one from the other pile of hybrid corn cobs and count the phenotype ratios, then calculate the percentages.

	Purple smooth	Purple wrinkled	Yellow smooth	Yellow wrinkled
Number				
Percentage				

→ Approximate the values in the “Number” line of the table as a ratio of small numbers:

\_\_\_\_\_ : \_\_\_\_\_ : \_\_\_\_\_ : \_\_\_\_\_

→ Which of the following combinations of parent genotypes would produce this phenotype ratio in the offspring?  
(Circle the right answer.)

**PpSs × PpSs**

**PPSS × ppss**

**PpSs × ppss**

**ppSs × Ppss**

## Sex-linked traits

Humans have 23 pairs of chromosomes. For 22 of them, every human with a normal karyotype receives one copy from each parent; these are the autosomes. The last pair are the sex chromosomes, which are called X and Y. A chromosomally female human has two X chromosomes, while a chromosomally male human has one X and one Y chromosome. We share this system of sex determination with the rest of the mammals, although a huge variety of other systems (some chromosomal, some not) occur among the various animal and plant species which have distinct male and female individuals. In mammals, the X chromosome is as big as an autosome and contains a large number of genes that control non-sex-related traits, while the Y chromosome is much smaller and contains only sex-determining genes. This is also the case in the fruit fly *Drosophila melanogaster*, the most common model organism used in labs.

The non-sex-related traits controlled by genes on the X chromosome are called **sex-linked** traits. In humans and other mammals, recessive genes for sex-linked traits are always expressed in males that have them, because they have only one X chromosome, but females may be carriers, because their second X chromosome may contain a dominant allele for that gene. One well-known example of a sex-linked trait in humans is red/green colorblindness. Color vision depends on pigment proteins produced in the cells of the retina, at the back of the eye. The two alleles for this gene are written as  $X^B$  (gene for functioning green pigment, the dominant allele) and  $X^b$  (gene for broken green pigment, the recessive allele). In this lab, you will model the inheritance pattern of sex linked genes.

Materials: popsicle sticks with alleles written on the front and back, one XX, one XY

Instructions: Work in pairs. One of you will “flip for” the XX parent, one for the XY parent. Flip both of the sticks and write down the offspring genotype from the two alleles that are lying face-up. Do this 20 times, recording the data in the table below. When you’re done, count up the frequency of each of the genotypes.

#	Genotype	#	Genotype	#	Genotype	#	Genotype	#	Genotype
1		5		9		13		17	
2		6		10		14		18	
3		7		11		15		19	
4		8		12		16		20	

## Lab 11: Genetics and Inheritance

When you're done, summarize your results in the following table.

Genotype	Sex	Colorblind?	Number	Proportion

Questions:

- What proportion of females express the recessive (colorblind) trait? What proportion of the males express it? Why are these numbers different?


- Fill out the Punnett square at right to show the genetic cross in your experiment.

- What is the predicted phenotype ratio of the male offspring?


- What is the predicted phenotype ratio of the female offspring?

- Which of the offspring genotypes could potentially pass on the  $X^b$  colorblindness gene to their own children?

## Observable human traits

In other familiar plants and animals, a number of highly visible traits are subject to the simple dominant/recessive kind of inheritance we have been discussing, and many such traits have been discovered by breeders. Many traits that are easily observed in humans were formerly thought to be inherited in the same simple Mendelian manner. However, most of the human traits that are commonly used as examples in introductory biology texts (eye color, tongue rolling, widow's peak, etc.) turn out to be much more genetically complicated. Most of them have multiple alleles or multiple genes involved in producing the phenotype. However, among all the classic examples, two stand out as really, truly, cases of single-gene simple dominance, both of them mildly icky: the texture of an individual's earwax (sticky vs. dry) and whether or not their urine smells funny after they eat asparagus. If you are interested in this, you could start here: <http://udel.edu/~mcdonald/mythintro.html>. However, in the interests of time and hygiene, we will not explore this topic further in lab.

## Blood type testing and genetics

The ABO blood type system is the major blood type classification system that determines which type of blood can safely be used for a transfusion. In this section, you will get familiar with the genetics that control ABO blood type in humans,

## Lab 11: Genetics and Inheritance

and then use it to interpret the results of a (simulated) blood type test. This section will not address the “positive/negative” part of blood type, which is controlled by an entirely different gene called the Rh factor.

The four blood types in the ABO system refer to different versions of carbohydrate molecules which are present on the surface of red blood cells. These molecules, which act like flags in the immune system, are called **antigens**.

Blood type	Carbohydrate molecules expressed on red blood cells
A	Antigen A
B	Antigen B
AB	Both antigen A and antigen B
O	Neither antigen A nor antigen B

These different blood types result from different alleles of a gene in the DNA that gives directions for how to make a particular enzyme that makes the antigens and puts them on the surfaces of the red blood cells. Each of the alleles gives a different version of those directions, so each allele results in the presence of a different antigen. The following table summarizes what each of the blood type alleles do.

Allele	Gives the directions for making a version of the enzyme that:
I <sub>A</sub>	puts antigen A molecules on the surface of red blood cells
I <sub>B</sub>	puts antigen B molecules on the surface of red blood cells
i	doesn't work -- doesn't put any antigens on the surface of red blood cells

Each person has two copies of this gene, one inherited from each parent. The I<sub>A</sub> and I<sub>B</sub> alleles are **codominant** -- that is, the two alleles each have a different observable effect on the phenotype of a heterozygous individual, and both of them are expressed at the same time. Thus, in codominance, neither allele is recessive — both alleles are dominant.

→ Write out the phenotype that results from each of the possible genotypes. From the table you just filled out, circle the genotype that results in a blood type that provides clear evidence of codominance in the genetics of blood type.

	Genotype					
	ii	I <sub>A</sub> i	I <sub>B</sub> i	I <sub>A</sub> I <sub>A</sub>	I <sub>B</sub> I <sub>B</sub>	I <sub>A</sub> I <sub>B</sub>
Antigens present (A and/or B)						
Phenotype (A, B, or O)						

→ In the blank Punnett square at right, write out the results of a monohybrid cross where one parent has genotype I<sub>A</sub>i and the other has genotype I<sub>A</sub>I<sub>B</sub>.

The type A and type B carbohydrate molecules are called antigens because they can stimulate the body to produce an immune response, which is controlled by the production of **antibodies**. Each specific type of antibody binds to a specific antigen. For example, anti-B antibodies bind to type B antigens, but not to type A antigens.

In general, your body does not make antibodies against any antigens that are part of your body. For example, a person with type A blood does not make anti-A antibodies against the type A antigen on their red blood cells. However, they do make anti-B antibodies against the type B antigen which is not present on their red blood cells.

If you are given a blood transfusion that does not match your blood type, antibodies in your blood can react with the antigens on the donated red blood cells. (The opposite reaction can occur, but does not typically cause any problems, because transfused blood


## Lab 11: Genetics and Inheritance

or plasma does not contain enough antibodies to produce a large reaction.) This reaction causes the donated red blood cells to burst and/or clump together and block blood vessels. A transfusion reaction can be fatal. To prevent this from happening, doctors test whether donated blood is compatible with a person's blood before they give a blood transfusion.

→ Fill out the last two rows in the following table, based on the rest of the information.

Genotype	I <sub>Ai</sub> or I <sub>AIA</sub>	I <sub>Bi</sub> or I <sub>IBIB</sub>	I <sub>AI</sub> B	ii
Blood type	A	B	AB	O
Antigens on blood cells	A	B	A and B	none
Antibodies in blood	anti-B	anti-A	none	anti-A and anti-B
Can give blood transfusions to types...				
Can receive blood transfusions from types...				

To test a person's blood type, you take a blood sample from the person, then mix it (separately) with a solution of anti-A antibody and with a solution of anti-B antibody to see if it clumps with either antibody. The following table describes the results of mixing a blood sample of each type with the anti-A and anti-B antibodies. You will use this table to interpret your results from the experiment in the next section.

Blood type	Does it clump when combined with...	
	anti-A?	anti-B?
A	yes	no
B	no	yes
AB	yes	yes
O	no	no

## Blood type case study

"Blood type case study" was modified from "Were the babies switched?" by Drs. Jennifer Doherty and Ingrid Waldron, U. of Penn. Biology Department, 2016, shared under a CC-BY-NC 4.0 license.

Scenario: Two couples had babies on the same day in the same hospital. Denise and Ernest had a girl, Tonja. Danielle and Michael had twins, a boy, Michael, Jr., and a girl, Michelle. Danielle was convinced that there had been a mix-up and she had the wrong baby girl, since Michelle had light skin, while Michael Jr. and Tonja looked more like twins since they both had dark skin. Danielle insisted on blood type tests for both families to check whether there had been a mix-up.

→ Your group will need a blood-typing kit from the back of the room. For each of the people assigned to your group, you will test for the A antigen and the B antigen separately.

1. Place one drop of the person's blood in the A well on the testing dish.
2. Place one drop of the anti-A antibody solution on the blood sample.
3. Take a clean toothpick and mix the blood sample with the antibody solution. Discard the toothpick after you have used it, so you don't cross-contaminate the samples.
4. For each person, record the results of both tests in the table below. Clean and dry your blood typing tray.
5. Repeat with anti-B antibody solution.
6. Test each person assigned to your group and write down the blood type and possible genotypes of each person. When the class combines its results, get the others too.

## Lab 11: Genetics and Inheritance

	Reacts with anti-A?	Reacts with anti-B?	Blood type (A, B, AB, O)	Possible genotype(s)
Michael (father of twins)				
Danielle (mother of twins)				
Ernest (father of single daughter)				
Denise (mother of single daughter)				
Michael Jr. (boy twin)				
Baby girl 1 (girl twin, according to hospital)				
Baby girl 2 (single girl, according to hospital)				

### Interpretation

Now you can use the results of your blood tests to evaluate whether Michael and Danielle's baby girl was switched with Ernest and Denise's baby girl.

- Fill out a Punnett square (or several) to show the possible genotypes for Michael and Danielle and their children. Write in the blood type for each possible child genotype.
- Fill out a Punnett square (or several) to show the possible genotypes for Ernest and Denise and their children. Write in the blood type for each possible child genotype.
- Who are the parents of each baby girl? How do you know?
- Were the babies switched? How do you know?

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# Lab 12: Natural Selection

## Learning Goals:

1. Model genetic drift and natural selection.
2. Understand the difference between natural selection and genetic drift
3. Model the Hardy-Weinberg equilibrium state of a Mendelian genetic locus.
4. Understand the idea of allele frequency and how it responds to natural selection.

## Agenda:

1. Modeling natural selection
2. Modeling Hardy-Weinberg equilibrium
3. Natural selection in the real world

## Modeling drift and natural selection

The basic idea of natural selection is the simple observation that heritable phenotypic traits that increase reproductive success (**fitness**) are more likely to be passed on to the next generation, and therefore they will increase in frequency over time. This observation, which was most famously formulated by Charles Darwin in *On the Origin of Species*, is the primary mechanism of adaptive evolution.

Three factors need to be present in a system for natural selection to occur:

1. **heritability**,
2. **variation**, and
3. **selection**.

Heritability is the capacity of phenotypic traits to be passed on to the next generation. Variation is the range of phenotypes in a population, on which natural selection operates. Selection is anything that makes one phenotype more able to survive and reproduce than another.

The only type of selective force that Darwin considered was competition for limited resources, but later it became obvious that many other forces can produce selection. If any of these three factors is not present, natural selection will not take place, but if they are all present, the phenotypes which are more favorable to survival and reproduction will become more common.

In this lab exercise, you will use paper butterflies to simulate the results of both genetic drift and natural selection.  
**Assemble yourself into groups of TWO or THREE students for this exercise -- four players is too many for this game and one is too few.**

*Materials:* Each group of two or three students will need a bag of butterflies, a piece of colorful cloth, and a copy of the lab to record results.

## Genetic drift

Genetic drift happens in populations where variation and heritability are present, but no selection is taking place.

## Lab 12: Natural Selection

### Instructions:

1. Count out 4 butterflies of each of 5 colors, for a total of 20 in your first generation. Write down the distribution of colors (e.g. “4 blue, 4 yellow...”) 20 is the *carrying capacity* of this area, i.e. the maximum population of butterflies.
2. Shake them all together in your hands and then dump them out on the table.
3. Pick up the first 10 butterflies on the left side of the group. These have been eliminated randomly from the gene pool and will not reproduce, so put them back in the bag.
4. Pick out another butterfly from the bag to match each of the “surviving” butterflies (those that remain on the table). Each of these has successfully reproduced.
5. You should have 20 butterflies again at this point. This is generation 2. Write down the new color distribution.
6. Repeat steps 2-5 five more times. When you’re done, plot your results on page 2.

	# butterflies at start of generation					
Color	1	2	3	4	5	6 (end)
	4					
	4					
	4					
	4					
	4					

### Questions:

→ What happened to the distribution of color phenotypes in your group?

→ Did it ever stabilize? If so, how?

→ Did the same thing happen in all the groups? Why or why not?

## Lab 12: Natural Selection

### Natural selection

To simulate natural selection, you will do the same thing as in the genetic drift experiment, but you will add a component of selection by a “visual predator” -- i.e. you!

#### Instructions:

1. This time, spread your cloth out on your lab table. This is your butterflies' *environment*.
2. Again, count out 4 butterflies of each of 5 colors for your first generation and write down the distribution of color phenotypes. You may wish to pick butterfly colors that match the colors in your cloth.
3. Pick one player to be the predator. They should face away from the table while the others sprinkle the butterflies over the cloth.
4. The predator should now capture ten butterflies by picking up 10 of them, one at a time, looking away in between. Be sure to completely break eye contact between captures, and pick the very first butterfly you notice each time you look at the “environment” again. Put the “eaten” butterflies back in your bag; they don’t get to reproduce.
5. As before, duplicate each of the surviving butterflies to make generation 2.
6. Record the new color distribution.
7. Repeat steps 3-6 four times. When you’re done, plot your results on page 2.

	# butterflies at start of generation					
Color	1	2	3	4	5	6 (end)
	4					
	4					
	4					
	4					
	4					

#### Questions:

- What was the selective pressure acting on your butterfly population? How did this emerge from the interaction of the environment and the predator?

## Lab 12: Natural Selection

- What colors were most and least fit in your environment?

*Most fit:*

*Least fit:*

- If the colors in the starting population were less variable (five different shades of red and orange, for example), would you still expect the results to be similar? Explain what you would expect to happen under these circumstances.

- In this experiment, the only heritable trait was color. Were there any non-heritable factors (e.g. shape, location) that affected the results? If so, what did they do?

## Natural selection in a changing environment

Instructions:

8. Keeping the same 20 butterflies that you just finished with, trade cloths with another group to get a different print.
9. If you were down to only 1 or 2 colors, you may three butterflies at random and replace them with “mutants” of other colors to return genetic diversity to your population.
10. Repeat the process up to generation 6, just as you did in the previous run. When you’re done, record the results next to your first set of natural selection results by drawing a vertical line and then continuing the graph.

	# butterflies at start of generation					
Color	1	2	3	4	5	6 (end)

## Lab 12: Natural Selection

### Questions:

→ What colors were most and least fit in this environment?

*Most fit:*

*Least fit:*

→ Was the highest-fitness color in the new environment the same as the highest-fitness color in the previous environment?

→ If no, did 6 generations give enough time for the most fit color in the new environment to become the most abundant?

→ Under what circumstances could an environmental change that affected selective pressure result in the extinction of a species?

## Modeling gene frequencies

*Materials:* 4 cards per person with “A” or “a” on one side and “W” or “w” on the other side

### A. “Pure” Hardy-Weinberg equilibrium

In the Mendelian model of genetics that you have already studied in this lab, each individual is assumed to carry two copies of each gene in the genome. Each allele of a gene is described using a single letter, which is either dominant (capital letter) or recessive (lowercase letter), and an individual’s diploid genotype at a particular genetic locus is represented as a pair of letters. Meiosis and fertilization then take one copy of each gene at random from each parent and put them together to produce an offspring genome; that is, each of the two alleles carried by the parent has 50% probability of ending up in any one of its offspring. This is the basis of the genotype ratios that are calculated with a Punnett square.

Now let’s extend this thinking to genotype frequencies in whole populations. For a gene on which natural selection is not operating (called a **neutral** gene), the population will reach a stable genotype ratio, which is called a **genetic equilibrium**.

Using the same example of the tabby-stripe *agouti* (**A/a**) gene that we used in Lab 9. Let’s first specify that we’re dealing with an isolated population of cats that can’t interbreed with other populations -- maybe on an island. Now let’s say that 85% of the cats are tabby **phenotype** (Aa or AA) and 40% are solid-color **phenotype** (aa). How can you figure out what the **genotype** frequency distribution is?

## Lab 12: Natural Selection

In this experiment you and your classmates will represent an isolated breeding population of cats. Each of you will receive four allele cards, two A and two a. (Ignore the W's for now.) The four cards represent the products of meiosis. You will play a “parent” in each generation, contributing a haploid set of chromosomes to the next generation; then you will become the offspring and do it again. You will go through the simulation for six generations.

### Instructions:

1. Everyone will begin this simulation as a heterozygote (Aa). Hold one A card and one a card; put the other two cards away in your pocket for now.
2. Begin simulation by walking around the room. Pick a mate *at random* (this is important!) and show them your mating dance.
3. Once your mating request is accepted, it’s time to reproduce. You and your mate will produce two offspring together.
  - a. Shuffle your two cards behind your back and take the card on top to contribute to the production of the first offspring. Your partner should do the same. Put the two cards together to find the genotype of the first offspring. One of you will assume the genotype of this child for the next round of mating.
  - b. Shuffle again to determine the genotype of the second offspring the same way. The other parent will assume the genotype of this child for the next round of mating.
4. Once you’ve both got all four of your cards back, pick out the two that reflect your new genotype and put the other two away in your pocket. Record your new genotype in the table below under “1”.
5. Repeat steps 2-4 five more times.

Your genotypes:

Generation	1	2	3	4	5	6
Genotype						

Class genotype distribution (Total people in simulation = \_\_\_\_\_)

Generation	1	2	3	4	5	6
% AA						
% Aa						
% aa						

→ Did the class numbers eventually stabilize? If so, what was the genotype ratio when it reached equilibrium?

→ What were the final percentages of dominant (A) and recessive (a) alleles?

A: \_\_\_\_\_ %    a: \_\_\_\_\_ %

→ Did these percentages change? (i.e. are they different from the initial 50/50 ratio)?

## Lab 12: Natural Selection

The **Hardy-Weinberg principle** is a simple mathematical model for the relationship between allele frequencies and genotype frequencies. In order for this model to be correct, the population must be diploid, genetically isolated, sexually reproducing, and randomly mating. The Hardy-Weinberg equation is usually stated as follows:

For a gene with two alleles **A** and **a**, call the proportion of allele **A** in the population  $p$  and the proportion of allele **a**  $q$ . ( $p + q$  always adds up to 1.) Then, if mating happens at random, the equilibrium genotype proportions in the population will be as follows:

$$\mathbf{AA} = p^2, \mathbf{aa} = q^2, \mathbf{Aa} = 2pq$$

For this simulation, in which  $p$  and  $q$  both equaled 0.5, the predicted equilibrium values are:

$$\mathbf{AA} = 0.5^2 = \underline{0.25}, \mathbf{aa} = 0.5^2 = \underline{0.25}, \mathbf{Aa} = 2 * 0.5 * 0.5 = \underline{0.5}$$

In the next section, we will experiment with how natural selection affects the equilibrium by selecting against one of the homozygote phenotypes (removing it from the gene pool).

## B. Under selective pressure

If natural selection acts on a gene, favoring one phenotype and eliminating another, then the equilibrium point will shift as one of the genes gets removed from the population. The *dominant white* gene (**W/w**) in horses is an example of a gene that is under selection. This gene is one of several that can produce a white coat. Heterozygote (**Ww**) horses are white, and homozygote recessive (**ww**) horses are colored. However, due to non-color-related effects of the gene, homozygote dominant (**WW**) embryos stop developing at an early stage, so there are no adult horses with the **WW** genotype.

Instructions:

1. Your allele cards from the first round have **W**'s on the back. Make sure everyone has two **W** and two **w** cards. Once again, start with a heterozygote genotype (**Ww**) and put the other two cards in your pocket.
2. Just as you did the first time, choose a mate at random.
3. Shuffle your cards to produce the offspring, just like before, but with one important change: **Every offspring with a WW genotype dies**. That is, if your shuffling process produces a **WW** offspring, try again. Shuffle and draw as many times as necessary until you get two viable offspring genotypes.
4. Write down your new genotype, pick out the correct allele cards, and put the other two cards back in your pocket.
5. Repeat steps 2-4 five more times.

Your genotypes:

Generation	1	2	3	4	5	6
Genotype						

Class genotype distribution (Total people in simulation = \_\_\_\_\_)

Generation	1	2	3	4	5	6
% WW						
% Ww						
% ww						

## Lab 12: Natural Selection

→ Did the class numbers eventually stabilize? If so, what was the equilibrium genotype ratio? If not, how many more generations do you think it would take to stabilize?

→ What were the final percentages of dominant (W) and recessive (w) alleles?

W: \_\_\_\_\_ %   w: \_\_\_\_\_ %

→ Did these percentages change? (Are they different from the initial 50/50 ratio)? If so, what happened, and would they keep changing if you continued the experiment?

→ In a larger population, do you think the W allele (and therefore the white phenotype) would eventually disappear from the population completely? Why or why not?

### C. Heterozygote advantage

Some genes have an effect where the heterozygote genotype has higher fitness than either of the homozygotes, called a **heterozygote advantage**. A classic example of this in humans is the gene for sickle-cell anemia: the homozygous dominant genotype has no deformed red blood cells, the homozygous recessive genotype has too many deformed red blood cells (resulting in severe health problems), but the heterozygote genotype has just enough of the deformed cells to confer resistance to malaria. In this run, we will add another rule to represent this scenario.

Instructions:

1. Your allele cards from the first round have W's on the back. Make sure everyone has two W and two w cards. Once again, start with a heterozygote genotype (**Ww**) and put the other two cards in your pocket. This time, though, get out a coin as well.
2. Just as you did the first time, choose a mate at random and show them a card.
3. This time, ***every offspring with a ww genotype dies***. That is, if your shuffling process produces a **ww** offspring, try again.
4. The new rule is this: If you have a **WW** genotype, flip a coin. If it comes up tails, the offspring dies from the condition that the heterozygote advantage protects against (e.g. malaria). Again, huffle and draw as many times as necessary until you get two living offspring.
5. Write down your new genotype, pick out the correct allele cards, and put the other two allele cards back in your pocket.
6. Repeat steps 2-4 five more times.

Your genotypes:

Generation	1	2	3	4	5	6
Genotype						

## Lab 12: Natural Selection

Class genotype distribution (Total people in simulation = \_\_\_\_\_)

Generation	1	2	3	4	5	6
% WW						
% Ww						
% ww						

- Did the class numbers eventually stabilize? If so, what was the equilibrium genotype ratio? If not, how many more generations do you think it would take to stabilize?
- What were the final percentages of dominant (W) and recessive (w) alleles?  
W: \_\_\_\_\_ %   w: \_\_\_\_\_ %
- Did these percentages change? (Are they different from the initial 50/50 ratio)? If so, what happened, and would they keep changing if you continued the experiment?
- In a larger population, do you think the W allele (and therefore the white phenotype) would eventually disappear from the population completely? Why or why not?

## Natural selection in the real world

Although natural selection is constantly at work, it is not always easy to observe, because the traits under selection may be hard to measure or interacting gene effects may produce a complex effect. However, one place where natural selection produces a highly important and visible effect is in the evolution of antibiotic resistance in bacteria.

The discovery of antibiotics (as well as other antimicrobial agents and immunization) have tremendously reduced the danger to humans of diseases caused by pathogenic bacteria. Antibiotics also boost the growth speed of livestock, which has led to even greater antibiotic use in the meat industry than in medicine. However, every use of antibiotics applies selection to the affected bacteria. Any bacterial population may contain a few individuals with genetic mutations giving them higher resistance to any given antibiotic. When an antibiotic is given, if any resistant individuals are present, they will reproduce successfully, leading to the growth and multiplication of a new population of bacteria resistant to that antibiotic.

## Lab 12: Natural Selection

- Explain how antibiotic resistance in bacteria can result from the use of antibiotics. Make sure to note the parts where **heritability**, **variation**, and **selection** are involved.
  - By your understanding of natural selection, would you expect the antibiotic resistance mutations to show up in the population before or after the antibiotic is given? Why?
  - Is it reasonable to describe the selective force in this example as “competition for limited resources”? Why or why not?
  - When you get an antibiotic prescription, it typically contains strict instructions on dosage, timing, and length of treatment. Why does following these instructions help to decrease the risk of developing an antibiotic-resistant bacterial infection?

# Lab 13: Evolutionary Relationships

## Learning Goals

1. Learn how to read and build phylogenetic trees.
2. Define phylogeny, homology, analogy, clade, and outgroup.
3. Explain the evidence provided by each of the following topics in support of the idea that modern species evolved from common ancestors:
  - a. Comparative anatomy
  - b. Embryology
  - c. The fossil record
  - d. Molecular evidence

## Agenda

1. Introduction to macroevolution, taxonomy, and phylogeny
2. Comparative anatomy
  - a. Classify and draw an animal specimen
  - b. Examine homologous and analogous features in skeletons
3. Embryology: examine and draw chick embryos at different stages
4. Fossils: analyze what fossils tell us about evolution
5. Assemble a molecular phylogeny based on Cytochrome C sequence

## Introduction

Natural selection is the main method by which evolution occurs, but when it keeps going for a long time, populations can become much more different than just different colored butterflies: single species can split into multiple species, eventually producing organisms so different from each other that it requires dedicated observation to determine their relationships. People have been making such observations for millennia, but a detailed scientific picture of the interrelatedness of all life only started to be assembled in the last 150 years. The discovery of natural selection and genetics in the mid-1800s was the key that unlocked our new understanding of how the relationships between organisms are structured.

Evolution is responsible for the vast diversity of living things on Earth both now and throughout its history. Many different lines of evidence from all the different branches of biology all lead to the conclusion that all living things on Earth are descended from a single common ancestor by the splitting of populations and modification of gene frequencies. This profound insight is so crucial to modern biology that it has been said that “nothing in biology makes sense except in the light of evolution”. We continue to discover new details about the multitude of ways that evolution can operate at different timescales and in different kinds of organisms, but the fundamental insight that new life forms arise from existing ones by *descent with modification* remains a governing principle of biology.

In this lab, you will explore how biologists describe the evolutionary relationships between organisms, as well as explore some of the classical examples that first inspired the development of the theory of evolution and some of the methods used to study it today. We will focus on the evolution of animals, because, although all living things evolve, animals were the first subject of evolutionary biology and the example on which the science was first built.

## Lab 13: Evolutionary Relationships

# Taxonomy and phylogenetic trees

To talk about the relationships between groups of organisms, we must be able to define those groups in a scientifically supportable way. **Taxonomy** is the science of naming groups of related organisms, which are called **taxa** (singular **taxon**). These taxa can be at different **taxonomic levels**, where multiple lower-level taxa combined make up a larger taxon. Some of these taxonomic levels are named: in order of increasing level, these are **species**, **genus**, **family**, **order**, **class**, **phylum**, **kingdom**, and **domain**. Although the **population** is the level at which natural selection operates, the boundaries between populations can be fluid; therefore, the **species**, which is easier to define, is treated as the fundamental unit of evolutionary biology. An organism is referred to by a formal taxonomic name called a **binomial**, which contains the **genus name** and the **species name** (plus any subgenus, subspecies, or variety names that may apply). If the species name is not known, the genus name can stand alone.

- ❖ Italicized
- ❖ Genus name is always present
- ❖ Genus name is capitalized
- ❖ Species name is never capitalized
- ❖ Species name never appears alone

**Tyrannosaurus rex** or **Tyrannosaurus sp.** or just **Tyrannosaurus**

genus              species              genus              (species unknown)              genus

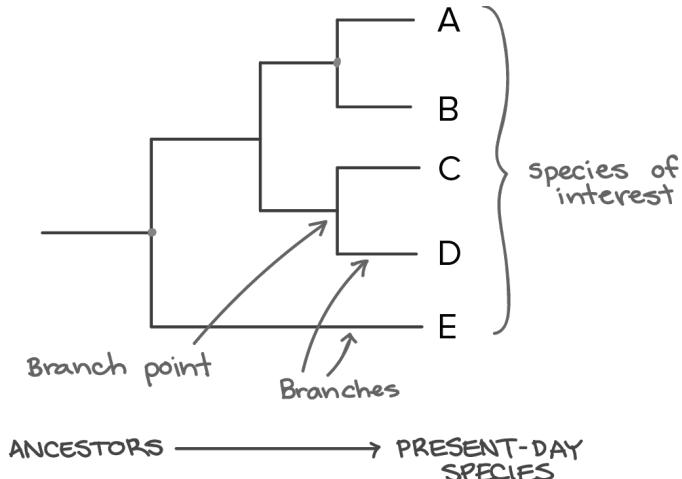
For the purposes of evolutionary biology, we are mainly interested in the relationships between organisms. Therefore, we think in terms of which taxa are most closely related to each other. Two taxa are more closely related if they have a recent common ancestor and less closely related if their common ancestor is a long way back.

A **phylogeny** is a hypothesis about the relationships between organisms expressed in terms of their common ancestors, and a **phylogenetic tree** is a way of visually representing a phylogeny. Thus, in the example phylogenetic tree shown at right, you can see the following relationships:

- Species A and B, and species C and D, are each more closely related to each other than they are to anything else on the tree (**sister** taxa). A group of organisms more closely related to each other than to anything else is called a **clade**.
- The taxon (A,B) and the taxon (C,D) are more closely related to each other than they are to species E, so ((A,B),(C,D)) is also a clade. That makes species E the **outgroup**.

Questions:

- What is the purpose of a phylogenetic tree? What information does it contain?



## Lab 13: Evolutionary Relationships

# Comparative anatomy

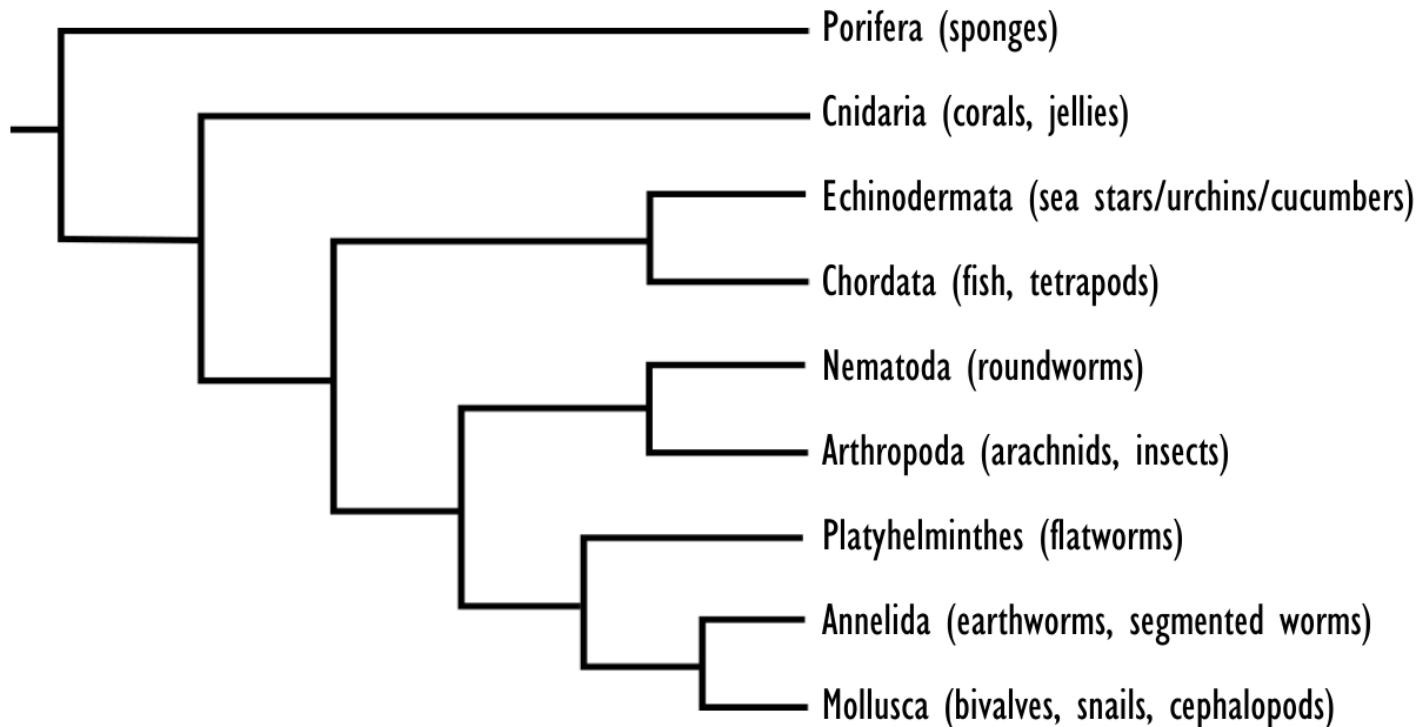
The discipline of comparative anatomy was one of the first and most important parts of biology that let us begin interpreting relationships among organisms, using observations on the origin and function of physical structures in different animals. Detailed observation of anatomical structures in animals was important in the initial recognition of phylogenetic relationships in nature, and continues to be the foundation of most phylogenetic reconstructions. Although the variety of forms in nature is vast, careful comparison of specimens can allow us to recognize anatomical structures that are distinctive to each taxon, and thereby determine their relationships.

## Distinguishing features of taxa

Taxa are defined on the basis of features that all the members of the taxon have in common with each other and that are not shared with any other organisms outside the group. These key characteristics are the basis of a phylogeny.

Choose one of the preserved animal specimens in the lab.

- Circle its phylum on the phylogeny of kingdom Animalia, below. (The capitalized names on the tips are the phyla.) Then circle the phylum or group of phyla most closely related to it.



- Write out its full taxonomic definition by giving the taxon to which it belongs at each of the following taxonomic ranks, starting with the phylum name from the tree above.
- The ranks above phylum have been filled out for you, because they're the same for all the specimens available.
  - Make sure to format the binomial name correctly in the last line! See the discussion on page 2 if you need a reminder.

## Lab 13: Evolutionary Relationships

**Domain:** Eukaryota

**Kingdom:** Animalia

**Phylum:**

**Class:**

**Order:**

**Family:**

**Genus and species:**

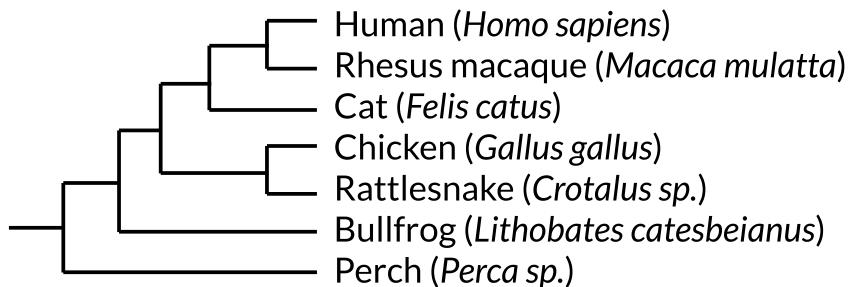
- At the bottom of this page, make a large and detailed anatomical drawing of your specimen. Make sure to clearly draw and label all its parts.
- Find a second preserved specimen that belongs to the same phylum. (You don't need to draw this one.) Give its name. What visible features do the two specimens share in common that might be distinguishing features of the phylum?

[Anatomical drawing goes below here]

## Lab 13: Evolutionary Relationships

### Homology and analogy

Structures are said to be **homologous** if they have similar origins and **analogous** if they are similar only in function. The wings of birds and the wings of flies are examples of analogous structures. They serve the same function, but obviously have different origins (one is made of bone and flesh, the other is composed largely of non-living chitin, and they originate from completely different structures in the embryo). On the other hand, the wings of birds and the forelegs of frogs are homologous structures (although these limbs have different functions, their embryonic origins are similar). To comparative anatomists, homologous structures are important because they imply an evolutionary linkage between two species.



Examine the labeled skeletons and other body parts at this station. All of these specimens are from the phylum **Chordata**, the phylum whose distinguishing feature is having a spinal column. This group includes sharks, bony fish, amphibians, reptiles, birds, and mammals. They also share a number of other similarities. As you answer the questions, pay special attention to the similarities and differences between homologous structures in different vertebrates.

- Examine the front limbs of the vertebrates listed below. Compare the shapes of the bones in each limb according to the given example. Then describe the function of the limb, and think about how the two are related.

Name	Limb shape	Limb function
Human	Arm bones long and thin. Wrist and hand bones short, strong, and flexible. Five fingers with the front one set back toward the wrist.	Grasping, lifting, carrying, manipulating
Rhesus macaque		
Cat		
Chicken		
Bullfrog		
Perch		

## Lab 13: Evolutionary Relationships

# Embryology

The embryos of many animals look similar and have similar features at early stages. For instance, features called *pharyngeal pouches* are present early in both fish and human embryo development, but as the human embryo grows they turn into internal parts of the head and neck, while in the fish they develop into gills. As they develop, they get less and less similar as each develops the distinctive features of its species. Many features are also gained and lost again before the embryo finishes maturing; for example, a human embryo grows three separate sets of kidneys, but only one set develops into the actual human kidney, while the other two vanish very early in development.

At one time it was hypothesized that “ontogeny recapitulates phylogeny” -- that is, that as an organism goes through its embryonic development (ontogeny) it repeats (recapitulates) the stages in its evolutionary history (phylogeny). This notion of embryology as an “instant re-play” of evolution was called the “biogenetic law”. Although the relationship between evolution and embryonic development has proven to be more complex than that, related organisms do show similarities in their embryonic development. These similarities can be traced to the conservative nature of embryology: small changes in early in development can have drastic consequences in later stages (through a “domino” effect). From an evolutionary perspective, similarities in embryology are valuable because they give us information about homology and reveal our kinship with other members of our phylum.

### Questions:

→ Draw the 33-hour embryo in the box at right and label the head and the spine.

Can you recognize any other body parts at this very early stage of embryonic development?

	33-hour chicken embryo
	72-hour chicken embryo

→ Draw the 72-hour embryo in the box at right. Label the head and spine, plus at least two new features that you couldn't see in the 33-hour embryo. Is there anything about this embryo that's identifiably birdlike yet?

→ What similarities do all embryos have very early in their development? What does this tell you about their evolutionary origin?

## Lab 13: Evolutionary Relationships

# Fossils

Animals and recognizable plants have existed for only the last half-billion years of the Earth's 4.6 billion year history. During that time, a tremendous number of different species have come and gone; in fact, it is estimated that more than 99.9% of all the species that have ever existed are now extinct. Some of these fossil organisms are the extinct ancestors of living species, and therefore have combinations of features that are still present in living species but are no longer found together. For instance, the early bird *Archaeopteryx* from about 150 million years ago had both feathers like a bird and teeth like a crocodile, and the mammal *Ambulocetus* from about 45 million years ago had a long narrow skull like a whale and big webbed feet like a hippopotamus. These combinations of traits allow the reconstruction of relationships between living organisms that would otherwise be very difficult to determine.

The fossil record of extinct life also demonstrates that evolution is a continual process by providing clear evidence that species constantly originate and go extinct. Most fossil species have no living descendants. Some of them are clearly similar to some living relative, but others are so different from any modern organism that it requires substantial taxonomic expertise to figure out where they fit in the tree of life. Examples of the latter include such notoriously strange creatures of the Cambrian explosion about 500 million years ago as *Hallucigenia* and *Opabinia* (look them up!) and the mysterious fungal "tree" *Prototaxites* from about 400 million years ago, as well as later organisms like the randomly-coiled cephalopod *Nipponites* from around 80 million years ago. The forms of fossil organisms tell us about the evolutionary pressures at the time they lived.

### Questions:

Examine the large trilobite cast and the small trilobites in the fossil sets. Trilobites are an extinct group of **arthropods** (i.e. phylum **Arthropoda**).

→ What are some modern arthropods?

→ What features do these extinct trilobites share with modern arthropods that allow you to recognize them as relatives?

Examine the fossil and modern shark teeth. Sharks have existed, in more or less the same forms, for hundreds of millions of years. Notice that the shark tooth 300 million years old, the one 30 million years old, and the one from a modern shark all look very similar.

→ What does this tell you about the past and present selective pressures that have influenced the evolution of sharks?

## Lab 13: Evolutionary Relationships

Examine the fossil and modern ferns. Ferns are one of the earliest groups of land plants. Like sharks, modern-looking ferns existed long before the dinosaurs. However, their ecological role has changed: before the evolution of flowering plants, ferns made up a large proportion of the forest canopy, whereas now they live mainly in understory and in weird marginal habitats.

- If the ferns themselves didn't change, what might have changed around them that made them lose their ecological dominance?

## Molecular phylogeny

The invention of sequencing technologies has allowed us to read the information contained in DNA and protein. As a result, we have discovered the technique of **molecular phylogeny**: that is, the comparison of DNA and protein sequences. If a DNA sequence or a protein sequence is similar enough between related organisms, the sequences from the two organisms can be compared to find the number of changes that have occurred in that gene or protein between one individual and another. This provides not only a phylogeny, but also a divergence "clock": the more changes have taken place, the longer the two species must have been separated.

A particular protein in the mitochondrial genome is called cytochrome C. This protein is part of the mitochondrial electron transport chain; it is present in all eukaryotic cells, and any mutation that causes it to stop working is likely to be fatal. As a result, this gene changes extremely slowly, so that only a few amino acids in it are different even between very distantly related organisms. In this section, you will compare part of the gene sequence that codes for cytochrome C from a variety of organisms and use the resulting list of changes to reconstruct a molecular phylogenetic tree.

- Compare the cytochrome C amino acid sequences of the different organisms shown on the laminated poster. The organisms whose sequences you will use in your exercise are the ones whose names are highlighted in yellow.

### Questions:

First, you will count the differences between one pair of cytochrome C sequences using the poster, so that you can become familiar with the data that are used for molecular phylogeny.

- Using the washable marker on the laminated sheet, find, mark, and count the amino acids which are different between the cytochrome C sequences for humans and dogs, as shown in the following example:

- - - G D V A K G K K T F V Q K C A Q C H T V E N G G K H K V G P N L W G L  
G V P A G D V E K G K K I F V Q R C A Q C H T V E A G G K H K V G P N L H G L

(Note that some of the amino acids are the same in all the species on the sheet. These have been printed in blue/light gray.)

- How many differences are there? \_\_\_\_\_

Next, you will use similar data for ten species to make a phylogeny. The differences have been counted for you and are summarized in the table on the following page.

- In the following table, write in the differences between each of the following pairs of species, as given in the spreadsheet. (Write in the white cells only; skip the grey ones.)

# Number of Inter-Species Differences in Cytochrome-C Amino Acid Sequence

	Human, chimpanzee	Rhesus monkey	Horse	Zebra	Pig, cow, sheep	Dog	Gray whale	Rabbit	Kangaroo	Chicken, turkey	Penguin	Pekin duck	Rattlesnake	Snapping turtle	Bullfrog	Tuna	Tomato hornworm moth	Screwworm fly	Silkworm moth	Wheat	Rice	Neurospora	Baker's yeast	Candida
Human, chimpanzee	0	1	12	11	10	11	10	9	10	13	14	11	14	15	18	21	31	27	31	43	44	48	45	51
Rhesus monkey	1	0	11	10	9	10	9	8	11	12	13	10	15	14	17	21	30	26	30	43	45	47	45	51
Horse	12	11	0	1	3	6	5	6	7	11	13	10	21	11	14	19	28	22	29	46	47	46	46	51
Zebra	11	10	1	0	2	5	4	5	8	10	12	9	20	10	13	18	27	22	28	45	46	46	45	50
Pig, cow, sheep	10	9	3	2	0	3	2	4	6	9	11	8	19	9	11	17	27	22	27	45	46	46	45	50
Dog	11	10	6	5	3	0	3	5	7	10	11	8	20	9	12	18	25	21	25	44	45	46	45	49
Gray whale	10	9	5	4	2	3	0	2	6	9	10	7	18	8	11	17	27	22	27	44	46	46	45	50
Rabbit	9	8	6	5	4	5	2	0	6	8	9	6	17	9	11	17	26	21	26	44	46	46	45	50
Kangaroo	10	11	7	8	6	7	6	6	0	12	11	10	20	11	13	18	28	24	28	47	45	49	46	51
Chicken, turkey	13	12	11	10	9	10	9	8	12	0	3	3	18	8	11	17	28	23	28	46	47	47	46	51
Penguin	14	13	13	12	11	11	10	9	11	3	0	4	19	9	13	19	28	25	28	47	48	48	45	50
Pekin duck	11	10	10	9	8	8	7	6	10	3	4	0	16	7	11	17	27	22	27	46	47	46	46	51
Rattlesnake	14	15	21	20	19	20	18	17	20	18	19	16	0	20	22	24	31	27	30	45	46	45	46	49
Snapping turtle	15	14	11	10	9	9	8	9	11	8	9	7	20	0	10	18	29	24	28	46	45	49	49	53
Bullfrog	18	17	14	13	11	12	11	11	13	11	13	11	22	10	0	15	30	22	29	48	49	49	47	51
Tuna	21	21	19	18	17	18	17	17	18	17	19	17	24	18	15	0	30	24	32	49	49	48	47	48
Tomato hornworm moth	31	30	28	27	27	25	27	26	28	28	28	27	31	29	30	30	0	12	5	42	44	46	45	47
Screwworm fly	27	26	22	22	22	21	22	21	24	23	25	22	27	24	22	24	12	0	14	45	48	41	45	48
Silkworm moth	31	30	29	28	27	25	27	26	28	28	28	27	30	28	29	32	5	14	0	45	45	47	47	48
Wheat	43	43	46	45	45	44	44	44	47	46	47	46	45	46	48	49	42	45	45	0	12	54	47	51
Rice	44	45	47	46	46	45	46	46	45	47	48	47	46	45	49	49	44	48	45	12	0	55	49	53
Neurospora	48	47	46	46	46	46	46	46	49	47	48	46	45	49	49	48	46	41	47	54	55	0	41	43
Baker's yeast	45	45	46	45	45	45	45	46	46	45	46	46	49	47	47	47	49	41	41	0	28			
Candida	51	51	51	50	50	49	50	50	51	51	50	51	49	53	51	48	47	48	48	51	53	43	28	0

## Lab 13: Evolutionary Relationships

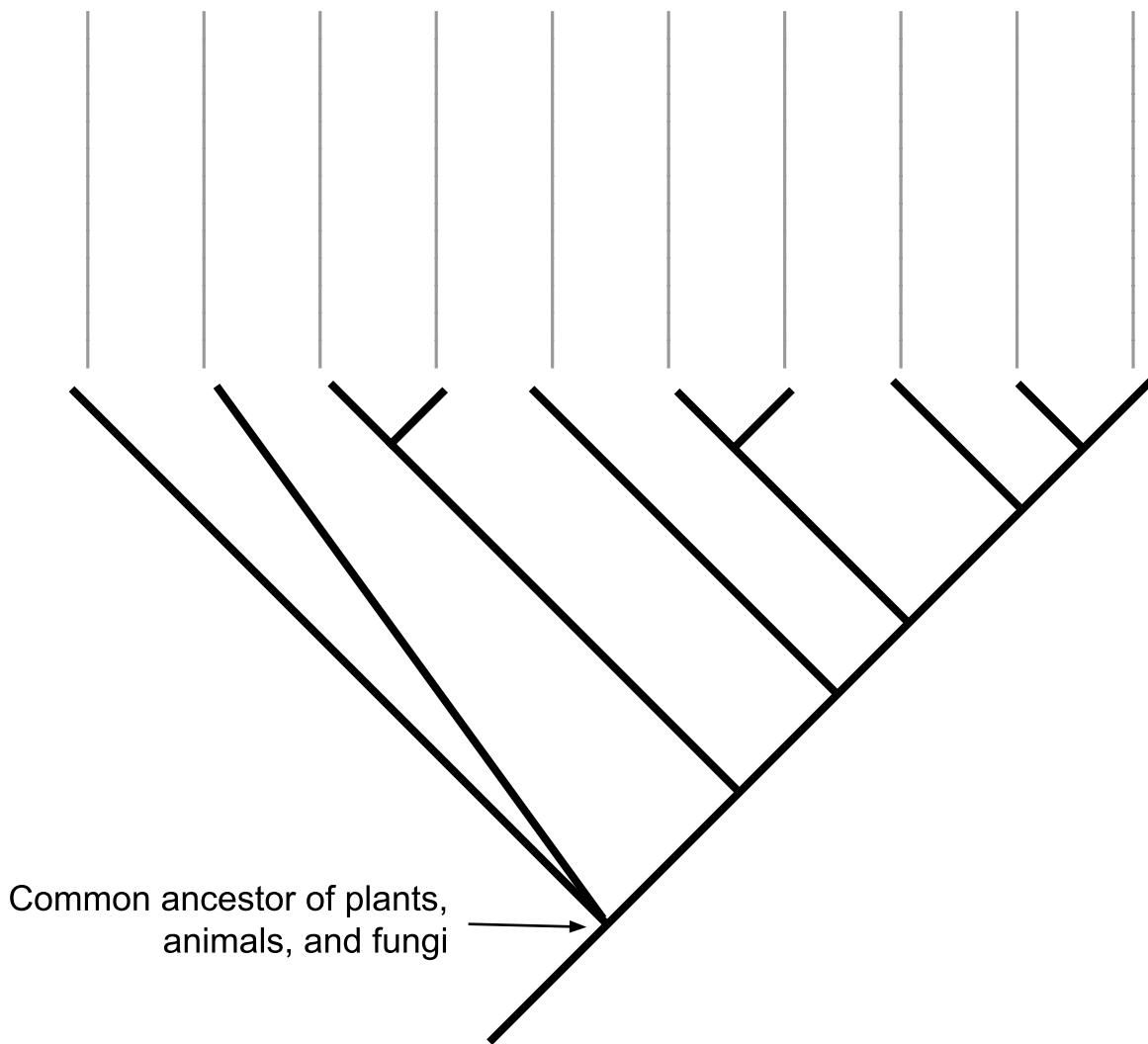
	Human, chimp	Dog	Chicken, turkey	Penguin	Snapping turtle	Tuna	Screw- worm fly	Silkworm moth	Wheat	Baker's yeast
Human, chimp	0									
Dog		0								
Chicken, turkey			0							
Penguin				0						
Snapping turtle					0					
Tuna						0				
Screw- worm fly							0			
Silkworm moth								0		
Wheat									0	
Baker's yeast										0

→ Why do more closely related species have more similar cytochrome C sequences?

Next, using the data you just wrote down, label the tips of the following molecular phylogenetic tree with the ten named taxa, according to the following instructions:

- The two most distantly related species are those with the most differences in their amino acid sequences. Write the names of the two species most distant from the rest on the deepest branches (on the left side of the tree).
- Notice that the next group on the tree is a pair of species that are closely related to each other, but distant from everything else. Find these two species in the data set and write their names on the tree in the right place.
- Continue until all the species are on the tree. When you're done, show your instructor.

## Lab 13: Evolutionary Relationships



The cytochrome C amino acid sequence does not give you enough information to tell whether fungi are more closely related to plants or to animals. (The three-way join at the base of this tree, called a **polytomy**, is the way we depict such an ambiguous relationship in a phylogenetic tree.) However, other gene sequences and homologous structures do make it clear that fungi are closer to animals than to plants.

→ Why might different genes and structures give different information about the relationships between organisms?

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# Lab 14: Plants

## Learning Goals

1. Identify how land plants have adapted to life on land and to specific environments.
2. Understand how plants alternate between sporophyte and gametophyte generations.
3. Observe the anatomy of flowers and its function in angiosperm reproduction.
4. Identify homologous reproductive structures in various flowers.
5. Use a dichotomous key to identify things.

## Agenda

1. Plant adaptations and plant sex
  - a. Observe living plants
  - b. Label flower model
  - c. Flower dissections and drawings
2. Learn to use dichotomous keys

## Plant adaptations

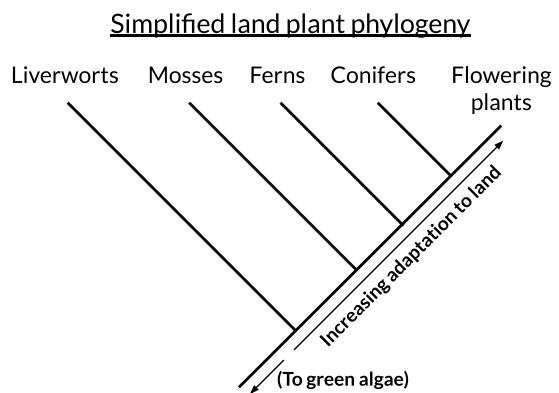
It's easy to take plants for granted, as part of the scenery. Don't be fooled -- they're getting up to all kinds of stuff while you're not looking.

Land plants have a huge variety of specialized **adaptations** to their environments. Cacti have reduced their leaves to dry little spikes, and use their thick, waxy stems for photosynthesis as well as water storage. Many flowering plants have oddly-shaped flowers that tuck their nectar-secreting parts away into pockets only accessible by their favorite pollinator, or that trap, trick, or confuse their pollinators into visiting. Some plants are parasites, stealing water or nutrients from a host plant rather than collecting their own, or live entirely without roots, collecting their water from the air with their leaves instead. Carnivorous plants have even adapted to nitrogen-poor environments by capturing and digesting insects, and in a couple of cases by becoming toilets for small mammals (search *Nepenthes hemsleyana* or *Nepenthes lowii*). The most important adaptations, though, are the basic ones that allow life on land.

## Adapting to life on land

Like everything else living on Earth, plants started out in the water and gradually adapted to drier and drier environments as the Paleozoic went on. The main problems they had to deal with in adapting to land were similar to those that animals had: how to avoid drying out, how to reproduce without water, and how to get up off the ground.

The most primitive living land plants are the **non-vascular plants** (aka **bryophytes**), which includes the mosses and liverworts. Bryophytes are the amphibians of the plant world: they need to stay damp to live, although they can live mostly out of the water. Mosses and liverworts lack roots, instead having thin threads called rhizoids; their leaves are only one cell thick (like a green alga); and they rely on the presence of water to keep them from drying out and to carry their reproductive cells.



## Lab 14: Plants

The **vascular plants** (ferns, conifers, & flowering plants) carry water inside their tissues, so they can stay out of water entirely, only taking it up when it's available; and their roots and stems are made up of thick bundles of water vessels. Ferns reproduce by spores which must be fertilized in water. By contrast, the **seed plants** (conifers & flowering plants) have pollen and ovules and reproduce by internal fertilization, which means their life cycle is fully terrestrial -- they don't require water even for reproduction.

## Alternation of generations

All land plants, including liverworts, ferns, conifers, flowering plants, and everything in between, have a life cycle in which they alternate between forms with diploid cells and forms with haploid cells. This is, if you stop to think about it, deeply weird and unfamiliar -- it's completely unlike animals, which have haploid sex cells but otherwise spend their whole life cycle being entirely made up of diploid cells. The haploid stage is called the **gametophyte** because it produces gametes (diploid zygotes that develop into a diploid individual); the diploid stage is called the **sporophyte**, because it produces spores (haploid zygotes that develop into a haploid individual).

In the non-vascular plants, such as mosses, the main visible life stage is the gametophyte. All vascular plants are sporophyte-dominant. In ferns the gametophyte is a tiny free-living individual. In the **seed plants** (i.e. gymnosperms and angiosperms), though, the process has been outwardly simplified: the gametophytes are tiny things that stay inside the anther or the ovary and only become active during the fertilization process. Luckily for you, in this lab we're going to focus on flowering plants. (And if you felt disappointed reading that sentence, sign up for a botany class next term.)

**Instructions:** Examine the living plants on the counter and answer the questions below.

### Questions:

→ What adaptations to living on land do each of these plants have?

Type of plant	Adaptations to land
Flowering plants	
Seed plants (flowering plants + gymnosperms)	
Vascular plants (seed plants + ferns)	
Land plants (vascular plants + mosses and liverworts)	

# Lab 14: Plants

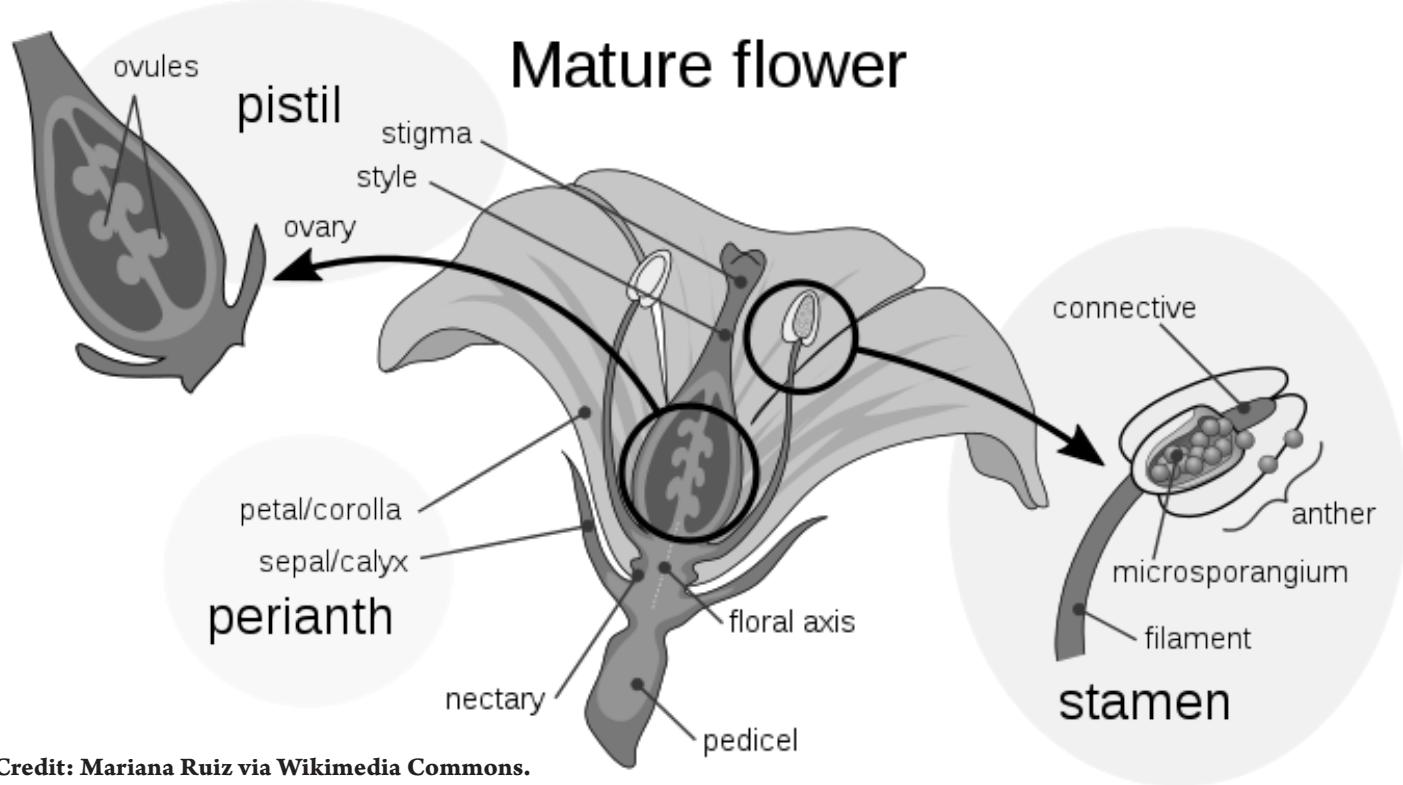
- What environment does each of the seed plants come from, and what visible adaptations do they have to surviving in that specific environment?

## Plant sex

Flowers are the sex organs of flowering plants: their purpose is to get the zygotes together. They're hugely variable, which is why they're often used to distinguish plant species, but they all perform basically the same function -- they release pollen, capture pollen, and fertilize ovules. The flowers of some plants have only male or only female parts (*imperfect* or *monosexual* flowers), while others have both (*perfect* or *bisexual* flowers). Some plants even have an overall sex to the whole organism (*monoecious*), with each individual bearing only one kind of monosexual flowers, while most plants have both male and female parts on each individual (*dioecious*). Today you'll look at a general model of a flower, then dissect several real ones.

## Flower models

- On the big model of the flower, label a;; the parts named in the table on the next page. The image on this page should help you locate them. (You only need to label the parts that are in the table.)



Credit: Mariana Ruiz via Wikimedia Commons.  
Released by author into public domain.

## Lab 14: Plants

Name	Description	Function
Receptacle	Thickened part of the stem adjacent to the reproductive structures	Attachment point of flower organs. In some plants, produces an accessory fruit
Sepals	Outer ring of leaf-like structures. Often green, but not always.	Protect the flower in bud, support the petals after bloom
Petals	Inner ring of leaf-like structures. Often colored and fragrant, but not always. May contain nectar-producing tissue.	Protect interior parts of flower, make flower attractive to some pollinators but not others
Stamen	Thin stalk topped with a larger head. Usually more than one is present.	Male reproductive structure
Filament	Thin stalk connecting anther to receptacle	Supports and feeds the anther
Anther	Head of stamen, with pollen inside and/or on its surface	Hosts the structures in which the pollen develops
Pollen	Small grains, often yellowish, variable in shape. Usually a loose powder but sometimes fused into a round mass	Male gametophytes
Pistil	Tube with a round structure at the bottom and a sticky or feathery cap at the top. There may be one or several	Female reproductive structure
Stigma	Rounded mushroom-shaped cap on the pistil, often sticky or feathery	Captures and germinates pollen grains
Style	Hollow stalk below the stigma	Guides pollen tubes from germinating pollen to the ovary
Ovary	Enlarged capsule at the base of the pistil	Protects and feeds the ovules as they develop, and later the seeds. Develops into a fruit
Ovules	Small round structures inside the ovary	Female gametophytes

When you're finished, ask the instructor to look. Take your labels off for the next group.

## Flower dissections

The model you just labeled is a generalized and simplified flower. Next you'll examine, dissect, and draw several different real flowers to see the variety of shapes that all those structures take in living plants.

Choose a flower from the supply, snip it off the plant, leaving about 1" of stem attached to the flower, and take it back to your lab bench. **Follow these steps** to draw and dissect it:

1. Put the flower down on the cutting board or dissecting board. Don't pin or cut it yet.
2. Begin by drawing the whole flower in the space on p. 131 or 132, using the colored pencils provided. Find out the scientific name of the flower and label your drawing with it. Label the same parts that you labeled on the model if you can see them.
  - If you're having trouble identifying them, remember to look for homologous features! (If you're really stuck, ask the instructor.)

## Lab 14: Plants

3. Gently remove the sepals, then the petals, by pulling them down toward the base to detach them from the receptacle. Cut across a petal to see its vascular system.
  - How many are there? What shapes do they have? The petals are the part of a flower that carry its smell -- does this flower have a smell?
  - Is the flower perfect (bisexual) or imperfect (unisexual)?
4. If the flower is large, pin the style (at the top near the stigma) and the bottom of the stem to the dissecting board so that they don't move around. The male reproductive parts (the stamens) will now be exposed.
  - a. If your main drawing doesn't show the stamens because they were concealed by the petals or sepals, draw and label them now next to your main drawing.
  - b. Slice a stamen in half with a scalpel to look at the inside. Be very gentle -- it's very small and fragile! You will see pollen grains; these are the male spores (microgametophytes), which fertilize the ovules.
  - c. Pick off one anther (or several if they're very small) with the tweezers and place it on a slide. You will use this later to examine the pollen under the microscope.
  - d. Examine the pollen under the microscope. If you can't see the pollen grains on the surface of the anther, crush the anther open onto the slide and find them. Draw a pollen grain next to your main drawing of the flower at whatever magnification you need to see it clearly. Don't forget to write down the magnification!
5. Now you'll do the same to expose the female reproductive parts (the pistil).
  - a. If you can't see the pistil and stigma in your main drawing, draw and label them now next to the main drawing. Add an arrow indicating where they're hiding.
  - b. With the scalpel, starting just below the stigma, slice through the side of the pistil all the way to the bottom of the calyx. Don't cut all the way through to the other side -- just open up one side of the style so that you can see the ovary and the pollen tube. Gently peel away the bottom of the style from the ovary.
  - c. Slice the ovary in half. Inside you will see the ovules. These are the female gametophytes (megagametophytes), which develop into seeds after fertilization. The outside layer of the ovary develops into the fruit.

When you're done, go get two more flowers. Choose ones that are as different in shape from the first one as possible. Draw and dissect your other two flowers the same way, noting the differences between them.

[Flower dissection drawings go below here]

## Lab 14: Plants

[Flower dissection drawings go on this page]

## Lab 14: Plants

# Dichotomous keys

A **dichotomous key** is a tool used for identifying things that have already been described. It consists of a series of questions, each of which has only two possible answers, and you follow the path of correct answers to the correct ID at the end.

## A silly example: cryptids

The following dichotomous key allows identification of a short list of mythical creatures. You have been provided with a drawing and a photo of a “preserved specimen” of one cryptid. Use the dichotomous key to ID the creature shown in the pictures below. Then look up the ID in the table to see more information about it.



### Dichotomous key to imaginary creatures

1. Does the creature live on land or in water?
  - Land: go to question 2
  - Water: go to question 5
2. Is the creature humanoid?
  - Yes: go to question 3
  - No: go to question 4
3. Is the creature brown or white?
  - Brown: **Sasquatch**
  - White: **Yeti**
4. Is the creature scaly or furry?
  - a. Scaly: **Chupacabra**
  - b. Furry: **Jackalope**
5. Does the creature have tentacles?
  - Yes: **Kraken**
  - No: go to question 6
6. Does the creature live in a lake or in the ocean?
  - Lake: **Nessie**
  - Ocean: **Sea serpent**

## Lab 14: Plants

The following table gives the full description of each cryptid, organized in alphabetical order. Note that these descriptions contain all sorts of extra information that isn't used in the dichotomous key -- the key only asks the necessary questions to get you to an unambiguous ID.

Name	Description
Chupacabra	Large, hopping reptile with scales and dorsal spines. Sucks the blood of goats. Ranges from southern North America to the Caribbean.
Jackalope	Jackrabbit with horns like an antelope. Native to North America, but similar to the Egyptian <i>mi'raj</i> and German <i>wolpertinger</i> . Aggressive when disturbed.
Kraken	Giant octopus- or squid-like creature. Lives in open ocean habitats worldwide. Reported to sink ships and eat entire whales.
Nessie	Lake monster with a long neck and tail, reminiscent of a plesiosaur. Type specimen is from Scotland, but similar creatures are reported worldwide.
Sasquatch	Pacific Northwest giant humanoid, covered in brown fur, reportedly with a distinctive odor; lives in forests.
Sea serpent	Large aquatic reptile with a long snakelike body and a dorsal frill. Hunts both terrestrial and marine prey.
Yeti	Himalayan giant humanoid, covered in white fur; lives above snowline.

### A real example: leaf ID

Dichotomous keys are frequently used in field identification guides, such as those for plants or birds. In this example, you will use them to identify leaves from several North American trees.

A selection of tree leaves are provided in the box. Please leave them in their plastic bags, and be careful not to damage them -- they're dry and crunchy. Three of the sets are labeled with the common name of the species; the other three have only a key number and the word "Alternate", "Opposite", or "Unknown". The "Opposite", "Alternate", or "Unknown" at the top of the label refers to the position of the leaves on the twig, which is useful for identification but not visible on the leaf alone.

First, take one of the **named** leaves.

- Use the dichotomous key to identify it (even if you already know what it is).
- The purpose of this is to make sure you can get a correct result from the key.
  - The meanings of the words used to describe leaf characters are described on the page behind the key.
  - If you don't get it right, figure out what character you misread or what mistake you made in keying it out; then put it back, take another one and try again.

Next, take three different **unlabeled** leaves.

- Use the key to identify them. Below, give the number on the card and the name you found for each one.

Leaf number (from card)	Tree name (from key)

## Lab 14: Plants

Last, take a look at one of the small green tree ID booklets that are in the box with the leaves. This is a dichotomous key for whole trees, not just leaves. Answer the following questions:

→ What kinds of non-leaf characteristics are used to identify trees in the book?

→ Which of the characteristics used in the tree ID key could you use to identify a deciduous tree in winter when its leaves are gone?

## Plants of California and the U.S.

### Native plants of California

Examine the plant specimens in the “Native Plants of California” binder. For each one, look up its life history information. Record it along with your observations in the table on page 10.

### Wood samples from North American trees

Examine the wood samples in the “American Wood Blocks” collection. Look up the life history information for each of the listed tree types and record it along with your observations in the table on page 11.

## Cleanup

1. Discard your dissected flowers and pollen slides.
2. Clean your dissecting trays with soap and water; return the pins and tools.
3. Put the leaves back into the rubber-banded bundles that you got them from.
4. Return the “Native Plants” binder to where you got it from.

## Lab 14: Plants

# Native Plants of California

## Lab 14: Plants

# American Wood Blocks

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