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Principles of Biology I Lab Manual

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UNIVERSITY SYSTEM OF GEORGIA

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Principles of Biology I Lab Manual

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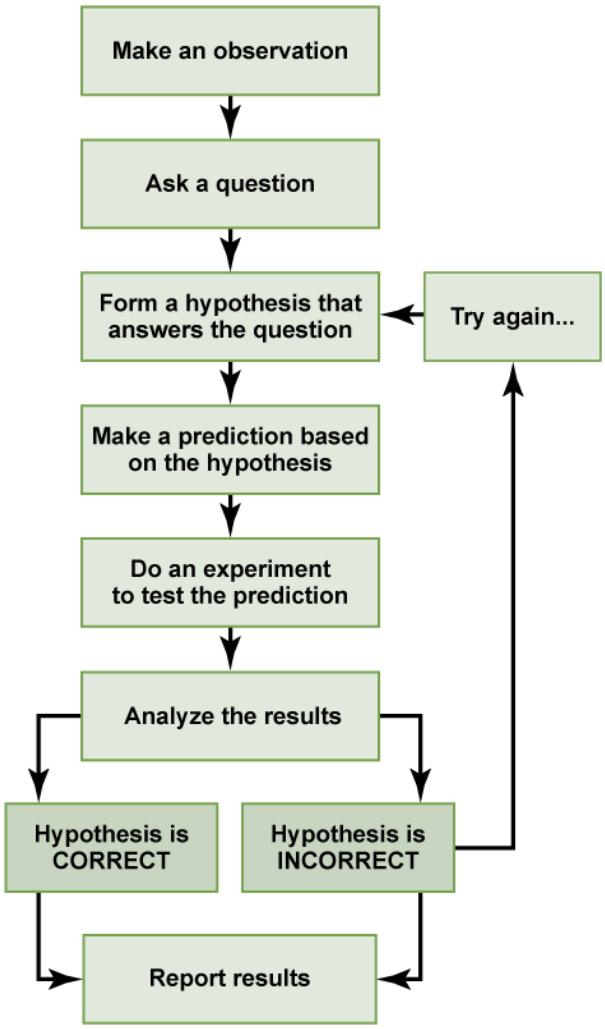
Scientific Method

(Adapted from http://www.biologycorner.com/)

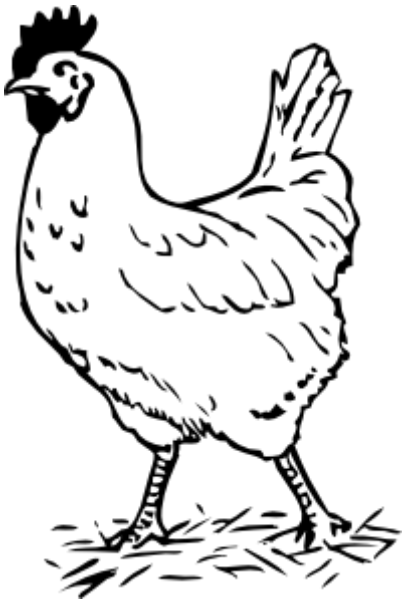
Introduction

The scientific method is central to the study of biology: it is a process of acquiring and verifying information through experimentation. The general steps of the scientific method are depicted in the figure below. The **hypothesis**, or suggested explanation for the observation, is the basis for setting up experiments. Good experimental design is essential to the scientific method. A few keys to good experimental design include effective use of controls, reproducibility, a large sample size, and multiple trials. In an experiment, in order to determine that any changes that occur are due to investigator manipulation only, there must be some basis for comparison. A **control group** is necessary to establish this basis of comparison. In the control group, everything is kept the same as the experimental group except for the **independent variable**. The experimental group is actually being experimented upon. For example, in a drug trial there will be a group that receives the drug (the experimental group) and a group that receives a placebo (the control group). The drug itself is considered the independent variable and any change(s) that occur because of the drug are considered the **dependent variable**. In order to ensure that it is only the drug causing changes, all other variables must be tightly controlled (such as diet, exercise, smoking, etc.). These are referred to as **controlled variables**.



The Scientific Method. *Biology. OpenStax College.*

Part 1: The Strange Case of BeriBeri



In 1887 a strange nerve disease attacked the people in the Dutch East Indies. The disease was beriberi. Symptoms of the disease included weakness and loss of appetite, victims often died of heart failure. Scientists thought the disease might be caused by bacteria. They injected chickens with bacteria from the blood of patients with beriberi. The injected chickens became sick. However, so did a group of chickens that were not injected with bacteria.

One of the scientists, Dr. Eijkman, noticed something. Before the experiment, all the chickens had eaten whole-grain rice, but during the experiment, the chickens were fed polished rice. Dr. Eijkman researched this interesting case and found that polished rice lacked thiamine, a vitamin necessary for good health.

1. State the Problem

2. What was the hypothesis?

3. How was the hypothesis tested?

4. Do the results indicate that the hypothesis should be rejected?

5. What should be the new hypothesis and how would you test it?

Part 2: How Penicillin Was Discovered

In 1928, Sir Alexander Fleming was studying Staphylococcus *bacteria growing in culture dishes. He noticed that a mold called* Penicillium *was also growing in some of the dishes. A clear area existed around the mold because all the bacteria that had grown in this area had died. In the culture dishes without the mold, no clear areas were present.*

Fleming hypothesized that the mold must be producing a chemical that killed the bacteria. He decided to isolate this substance and test it to see if it would kill bacteria. Fleming transferred the mold to a nutrient broth solution. This solution contained all the materials the mold needed to grow. After the mold grew, he removed it from the nutrient broth. Fleming then added the nutrient broth in which the mold had grown to a culture of bacteria. He observed that the bacteria died which was later used to develop antibiotics used to treat a variety of diseases.

1. Identify the problem.

2. What was Fleming's hypothesis?

3. How was the hypothesis tested?

4. Do the results indicate that the hypothesis should be rejected?

5. This experiment led to the development of what major medical advancement…?

Part 3: Identify the Controls and Variables

Smithers thinks that a special juice will increase the productivity of workers. He creates two groups of 50 workers each and assigns each group the same task (in this case, they're supposed to staple a set of papers). Group A is given the special juice to drink while they work. Group B is not given the special juice. After an hour, Smithers counts how many stacks of papers each group has made. Group A made 1,587 stacks; Group B made 2,113 stacks.

Identify the:

- Control Group
- Independent Variable
- Dependent Variable
- What should Smithers' conclusion be?

- How could this experiment be improved?

Homer notices that his shower is covered in a strange green slime. His friend Barney tells him that coconut juice will get rid of the green slime. Homer decides to check this out by spraying half of the shower with coconut juice. He sprays the other half of the shower with water. After 3 days of "treatment" there is no change in the appearance of the green slime on either side of the shower.

- What was the initial observation?

Identify the:

- Control Group
- Independent Variable
- Dependent Variable
- What should Homer's conclusion be?

Bart believes that mice exposed to radio waves will become extra strong (maybe he's been reading too much Radioactive Man). He decides to perform this experiment by placing 10 mice near a radio for 5 hours. He compared these 10 mice to another 10 mice that had not been exposed. His test consisted of a heavy block of wood that blocked the mouse food. He found that 8 out of 10 of the exposed mice were able to push the block away, while 7 out of 10 of the other mice were able to do the same.

Identify the:

- Control Group
- Independent Variable
- Dependent Variable
- What should Bart's conclusion be?

- How could Bart's experiment be improved?

Krusty was told that a certain itching powder was the newest best thing on the market: it even claims to cause 50% longer lasting itches. Interested in this product, he buys the itching powder and compares it to his usual product. One test subject (A) is sprinkled with the original itching powder, and another test subject (B) was sprinkled with the Experimental itching powder. Subject A reported having itches for 30 minutes. Subject B reported to have itches for 45 minutes

Identify the:

- Control Group
- Independent Variable
- Dependent Variable
- Explain whether the data supports the advertisements claims about its product.

- How could this experiment be improved?

Lisa is working on a science project. Her task is to answer the question: "Does Rogooti (which is a commercial hair product) affect the speed of hair growth". Her family is willing to volunteer for the experiment.

Design Lisa's experiment.

Taxonomy **Name: _____**

Introduction

Because the diversity of life on Earth is so vast, biologists use a general system of classification and naming organisms (taxonomy) to track and organize species based on evolutionary relatedness. The broadest taxon is the domain; organisms belong to one of the three domains (Bacteria, Archaea, and Eukarya). Within the domains are increasingly specific taxa, usually following the order in the table below.

The scientific name of an organism is given using binomial nomenclature; the genus and species of an organism give its specific scientific name. These names are usually derived from Greek or Latin, and therefore must be italicized when written. The genus is to be capitalized and the species is lower case. For example, the scientific name of a common wombat (top) is *Vombatus ursinus*.

Let us compare the wombat to a similar species, a quokka (bottom).

	Wombat	**Quokka**
Domain	Eukarya	Eukarya
Kingdom	Animalia	Animalia
Phylum	Chordata	Chordata
Class	Mammalia (Marsupialia)	Mammalia (Marsupialia)
Order	Diprotodontia	Diprotodontia
Family	*Vombatidae*	*Macropodidae*
Genus	*Vombatus*	*Setonix*
Species	*ursinus*	*brachyurus*

"Vombatus ursinus -Maria Island National Park" by JJ Harrison (jjharrison89@facebook.com) - Own work. Licensed under CC BY-SA 3.0 via Wikimedia Commons

"Quokka" by the Hotel Rottnest, WA, Rottnest Island" by Vicsandtheworld - Own work. Licensed under CC BY-SA 3.0 via Wikimedia Commons

Note that both animals differ only when we reach the family level. If you knew that a kangaroo was in the same family as a quokka, would you assume the quokka was more closely related to a kangaroo or a wombat?

Scientific names might seem confusing, but are useful for several reasons. Common names tend to vary according to region (crawfish, crayfish, mudbug, crawdad), but the scientific name is always the same.

Questions:

1. Llamas, alpacas, and camels are all in the same family*: Camelidae*.
   Therefore, it is reasonable to assume that these animals will also be in the same...

2. The scientific name of the brown-throated three-toed sloth is named *Bradypus variegatus*. What is the genus of the organism? The species?

Part 1: A Simple Dichotomous Key

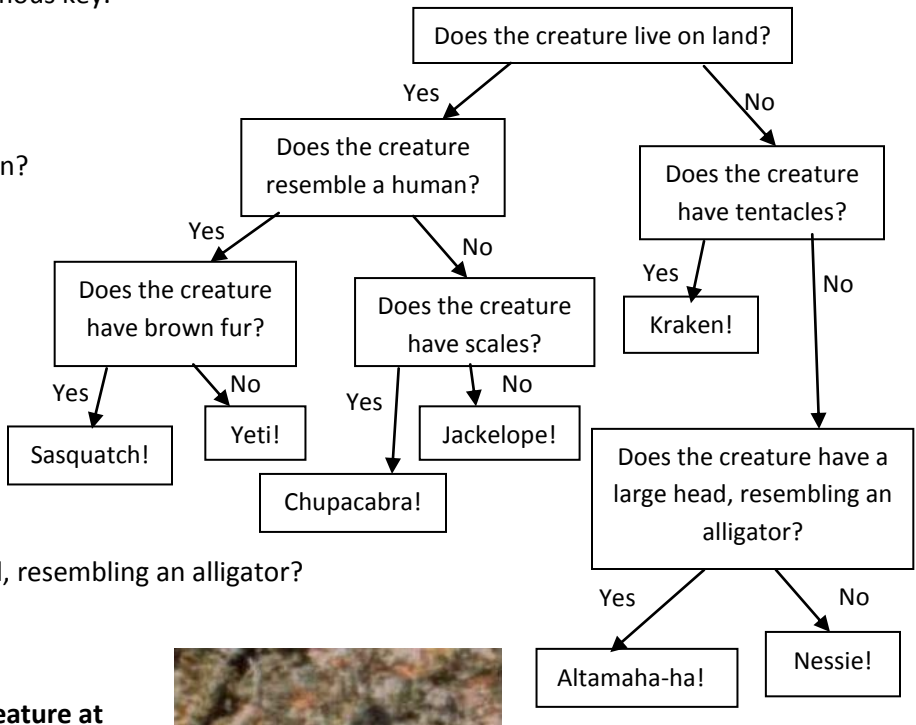
A dichotomous key is a tool used to determine the identity of species that have been previously described. You can think of it as a series of questions in which each question only has two possible answers.

In the table below, you have been given a list of creatures and their descriptions. The different characteristics, behaviors, and habitats of the creatures can be used in the dichotomous key to differentiate among them.

Creature	Description
Jackelope	Mean-spirited horned jack rabbit
Chupacabra	Reptilian creature covered in scales with spines along the dorsal ridge; likes to eat goats
Altamaha-ha	Water monster with an alligator-like head and long neck; lives in the marshes of Coastal Georgia
Sasquatch	Stinky giant humanoid covered in brown fur; found in the forests of North America
Yeti	Giant mountain humanoid covered in white fur; prefers the snow
Kraken	Giant octopus-like creature; takes down ships in the open ocean
Nessie	Water monster with a snake-like head and long neck; lives in Loch Ness, in the Scottish highlands

Below, you will find the dichotomous key used to identify a folkloric creature you may come across. On the left is the list of questions and on the right, the same list is represented as a flowchart. Both are useful representations of the same dichotomous key.

1. Does the creature live on land?
    a. Yes: go to question 2
    b. No: go to question 5
2. Does the creature resemble a human?
    a. Yes: go to question 3
    b. No: go to question 4
3. Does the creature have brown fur?
    a. Yes: Sasquatch!
    b. No: Yeti!
4. Does the creature have scales?
    a. Yes: Chupacabra!
    b. No: Jackelope!
5. Does the creature have tentacles?
    a. Yes: Kraken!
    b. No: go to question 6
6. Does the creature have a large head, resembling an alligator?
    a. Yes: Altamaha-ha!
    b. No: Nessie!

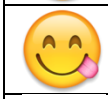
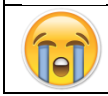


Using the dichotomous key, identify the creature at right.

"Patterson–Gimlin film frame 352" by Patterson-Gimlin film. Via Wikipedia

Part 2: Building a Dichotomous Key

In the table below, there are several different emojis. Your job is to build a dichotomous key that would help distinguish among them. There is space in the table to write out a description of each emoji, if necessary, as well as a name for each. Record your question series in the space below.

Emoji	Description	Name
		
		
		
		
		
		

Dichotomous Key Questions:

1.	
	a.
	b.
2.	
	a.
	b.
3.	
	a.
	b.
4.	
	a.
	b.
5.	
	a.
	b.

Part 3: Using a Dichotomous Key to Identify Trees

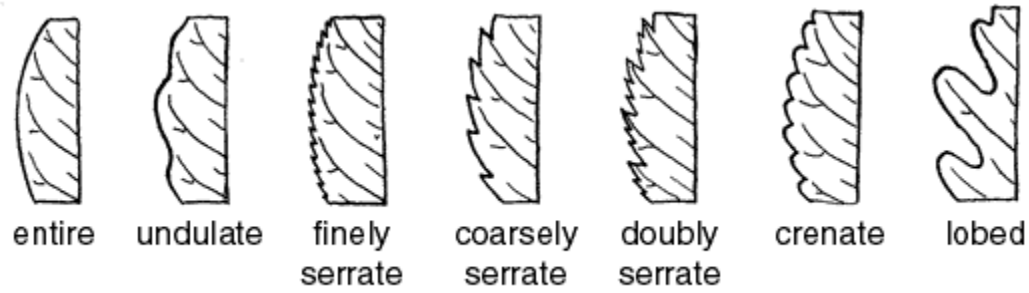
In this section, a dichotomous key will be used to identify tree species based on samples provided by your instructor. Refer to the guide that follows should any terms about the characteristics used to distinguish among trees be unfamiliar.

Materials

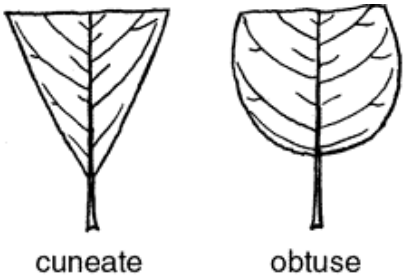
- Tree samples
- Dichotomous key

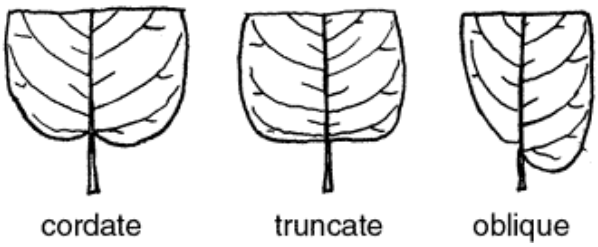
Tree Characteristics—Terminology Guide *(via Clemson Extension Office Bulletin 117)*

- **Petiole** — stalk of a leaf
- **Leaflet**—individual blade of a compound leaf
- **Apex** — the tip or distal end of a leaf
- **Sinus** — the space or indentation between the lobes of a leaf blade
- **Compound leaf** — a type of leaf that has three or more leaflets attached to a common stalk
    - **Palmately compound** — veins or lobes of a leaf radiating from a central point
    - **Pinnately compound** — arrangement of leaflets attached laterally along the middle of a compound leaf
- **Leaf Arrangement**
    - **Opposite** — leaves occurring in pairs at the nodes
    - **Alternate** — leaves arranged singly at intervals along the stems
- **Leaf Margins** (outer edge of a leaf blade)



- **Leaf Bases**





Biological Molecules

Introduction

(Modified from http://www.biology101.org/biologystudyguides/buildingblocksoflife.php)

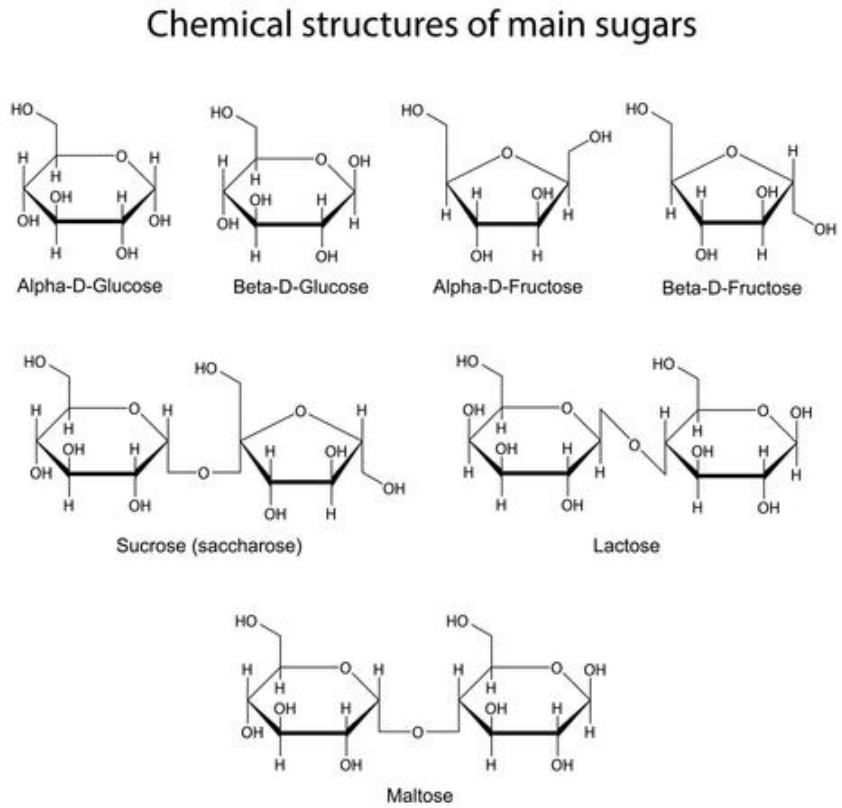
Biological systems are made up of four major classes of macromolecules: carbohydrates, lipids, proteins, and nucleic acids (nucleic acids will be covered separately later).

Carbohydrates

Carbohydrates are the most abundant macromolecules on earth, and the source of immediate energy needs in living systems. Carbohydrates also participate in defining the structure of cells and living systems. There are 3 general chemical grouping for carbohydrates: monosaccharides, disaccharides, and polysaccharides.

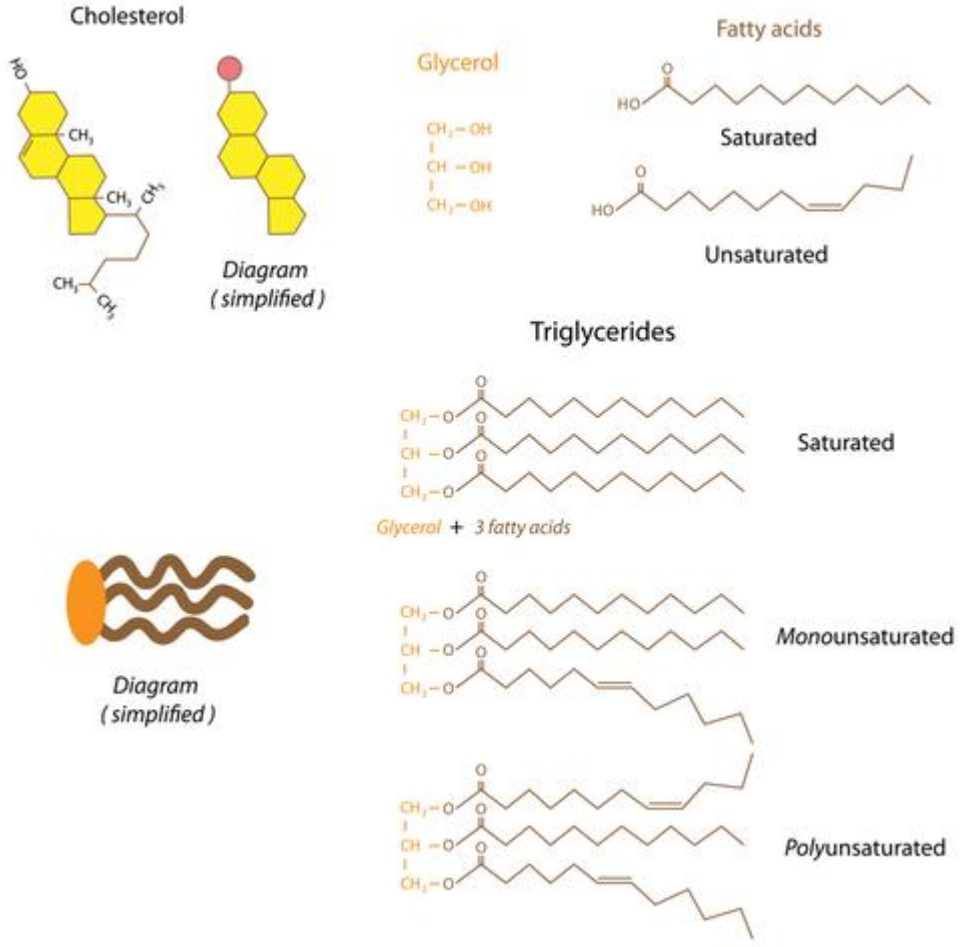
Monosaccharides, also referred to as simple sugars, are made up of a single sugar molecule. The major example of a monosaccharide is glucose ($C_6H_{12}O_6$). Other monosaccharides include isomers of glucose, such as fructose and galactose. Monosaccharides are transported in the blood of animals, broken down to produce chemical energy inside the cell, and can also be found within other macromolecules, such as nucleic acids.
Disaccharides are composed of two single monomers of sugar linked together. Examples of disaccharides are maltose (glucose + glucose) and sucrose (glucose + fructose). Disaccharides are broken down into their subunits for use inside living systems.

Polysaccharides are polymers, or long chains of sugar monomers linked together, and they are stored inside the cell for future energy use. In plants, the major storage polysaccharide is starch, while in animals it is glycogen. Plants also contain cellulose, which is the most abundant of all carbohydrates. Cellulose is the found in the plant cell wall, where it provides structure and support to the plant cell.



Lipids

Lipids are nonpolar macromolecules; thus they are insoluble in water. They include oils and fats, phospholipids, and steroids. Fats and oils are triglycerides, and are composed of glycerol and 3 fatty acids. A fatty acid is a long chain of carbon-hydrogen (C-H) bonds, with a carboxyl group (-COOH) at one end. Fatty acids can be classified as saturated or unsaturated. Fatty acids are saturated when they do not contain any double bonds between the carbons, and unsaturated when they contain double bonds. An example of a saturated fat is butter, while an example of an unsaturated fat is vegetable oil.



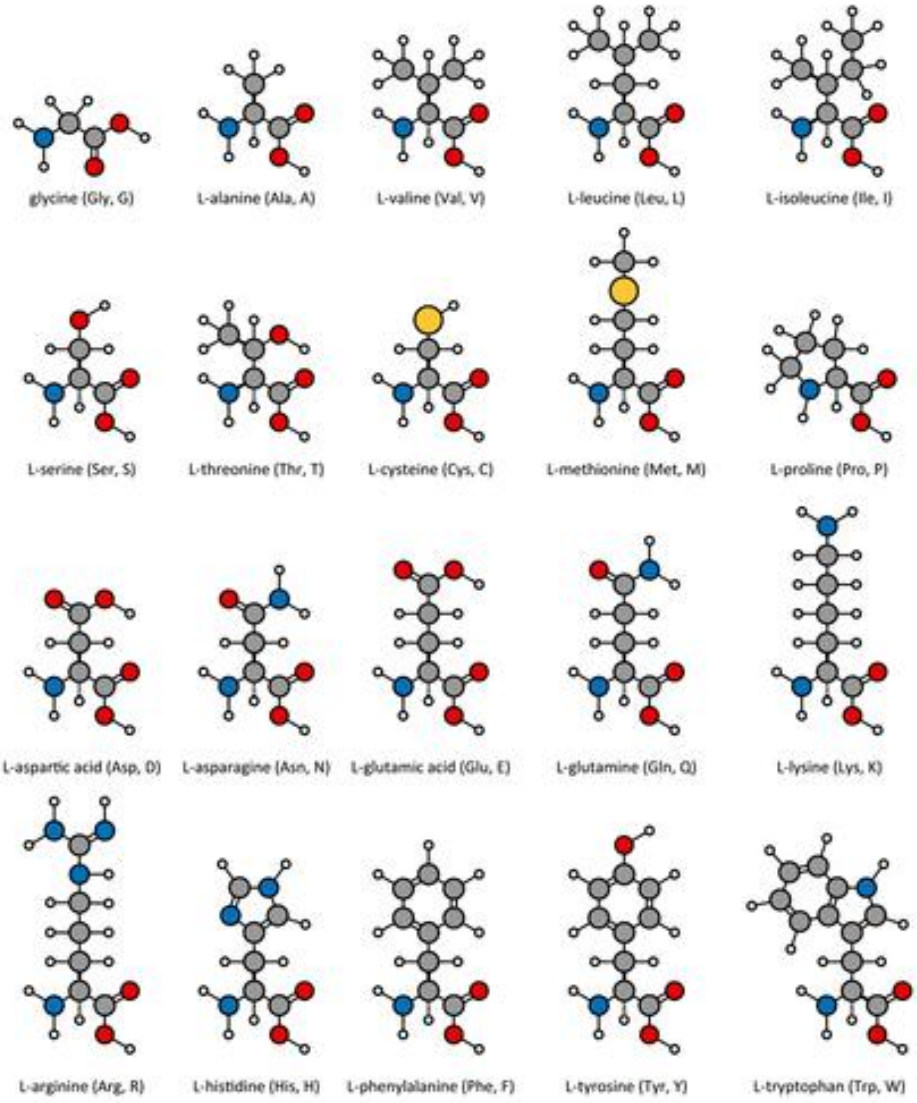
Phospholipids are found primarily in the cell membranes of living systems, of which they are the major component. Structurally, a phospholipid contains a hydrophilic head and a hydrophobic tail. The head of the phospholipid contains a phosphate group, while the tail is typically a diglyceride. The cell membrane is a double layer of phospholipids, in which the tails are turned inwards and the heads are exposed to the intracellular and extracellular environments.

Steroids are lipids which are typically made up of fused hydrocarbon rings. Each type of steroid is different in the type of chemically active functional groups that it contains. Examples of steroids include cholesterol, estrogen, and testosterone. Cholesterol in found in the cell membrane of animals, where it provides structural support. Cholesterol is also the precursor for other steroids, such as testosterone and estrogen. Some vitamins, such as Vitamin D, are also classified as steroids.

Proteins

Proteins are polymers of amino acids. Amino acids are small molecules that contain an amine ($-NH_2$), a carboxyl acid (-COOH), and a side chain (R). There are twenty naturally occurring amino acids, and each amino acid has a unique side-chain or R-group. Amino acids are connected by peptide bonds to form protein polymers. This gives rise to different levels of structure for proteins. The primary (1°) structure of a protein is a made up of a string of amino acids connected via peptide bonds. The secondary (2°) structure of a protein is formed by the coiling and folding of the 1° structure, due to hydrogen bonding. At this level, structures called alpha-helices and beta-sheets are visible. The tertiary (3°) structure of a protein is formed by interactions between the components of the 2° structure. Some proteins have quaternary (4°) structure, which includes the assembly of multiple individual subunits to form the functional protein.

Proteins have numerous biological functions. The common types of proteins that are found in biological systems are enzymes, antibodies, transport proteins, regulatory proteins, and structural proteins.



Part 1: Testing for simple sugars

Benedict's solution turns orange-brown in the presence of simple sugars

<u>Test samples</u>: distilled water, glucose, starch, orange juice, regular soda, diet soda, unknown

*Shake the starch before using it.

1. Fill a beaker about halfway with water and bring it to a gentle boil on the hot plate
2. Label tubes
3. Add ~2 ml sample to the tube (~0.5 inch)
4. Add ~2 ml (0.5 inch) Benedict's solution and swirl to mix.

   Record the color in table below
5. Put the tube in the water and heat for 3 minutes.
6. Use test tube clamps to remove tubes from heat and record color.

****Dispose of waste in waste beaker****

tube	sample	initial color	color after heat	conclusion
1	water			
2	glucose			
3	starch			
4	orange juice			
5	regular soda			
6	diet soda			
7	**unknown**			

Part 2: Testing for starch

Lugol's reagent (IKI solution) turns dark blue/black in presence of starch

<u>Test samples</u>: distilled water, glucose, starch, orange juice, regular soda, diet soda, unknown

*Shake starch before using

1. Label tubes
2. Add ~3 ml samples (~0.75 inch) and record color in chart.
3. Add 9 drops Lugol's reagent and swirl to mix.
4. Record color in table below.

tube	sample	initial color	color after IKI	conclusion
1	water			
2	glucose			
3	starch			
4	orange juice			
5	regular soda			
6	diet soda			
7	**unknown**			

Part 3: Testing for lipids

A. Paper test

Paper turns translucent (gets a grease spot) in presence of lipids

Test samples: distilled water, vegetable oil, cream, and unknown.

1. Put a drop of each substance on a brown paper towel. Rub in the cream.
2. Put the towel off to the side and let it sit for 10 minutes.
3. Record the appearance of the bag where you placed the spots in the table below.

sample	appearance after drying	conclusion
water		
oil		
cream		
unknown		

B. Sudan IV test

Two layers form in presence of lipids. Red dye concentrates in top layer.

Test samples: distilled water, vegetable oil, cream, and unknown.

1. Label tubes
2. Add 3 ml (0.75 inch) distilled water to each tube
3. Add 3 ml (0.75 inch) of sample to the tube (total of 6 ml per tube)
4. Add 9 drops of Sudan IV and shake (must mix well)
5. Add 2 ml (0.5 inch) water
6. Record results in the table below

tube	sample	appearance after Sudan IV	conclusion
1	water		
2	oil		
3	cream		
4	**unknown**		

Part 4: Testing for proteins

Biuret's reagent turns dark blue-purple in presence of peptide bonds

Test samples: distilled water, starch, egg albumin, glucose, regular soft drink, unknown

*Shake starch before using

1. Label tubes
2. Add 1 ml (0.25 inch) sample to tube
3. Add 10 drops Biuret's and swirl to mix
4. Wait 2 min, then record color
5. ****Dispose of Biuret waste in container****

tube	sample	initial color	color after Biuret	conclusion
1	water			
2	starch			
3	egg albumin			
4	glucose			
5	regular soda			
6	**unknown**			

Unknown

Look back over the results for the unknown sample from each of the previous tests. Based on the data, what combination of macromolecules is present in the unknown mixture?

Questions

1. The monomers that make up carbohydrates are:

2. The monomers that make up proteins are:

3. Which test could you use to distinguish between diet and regular soda? What would the test detect?

4. What is the Biuret test actually detecting? Be as specific as possible.

5. Each test included a sample that was just water. Why is it important to include a water-only sample for each test?

Microscopy

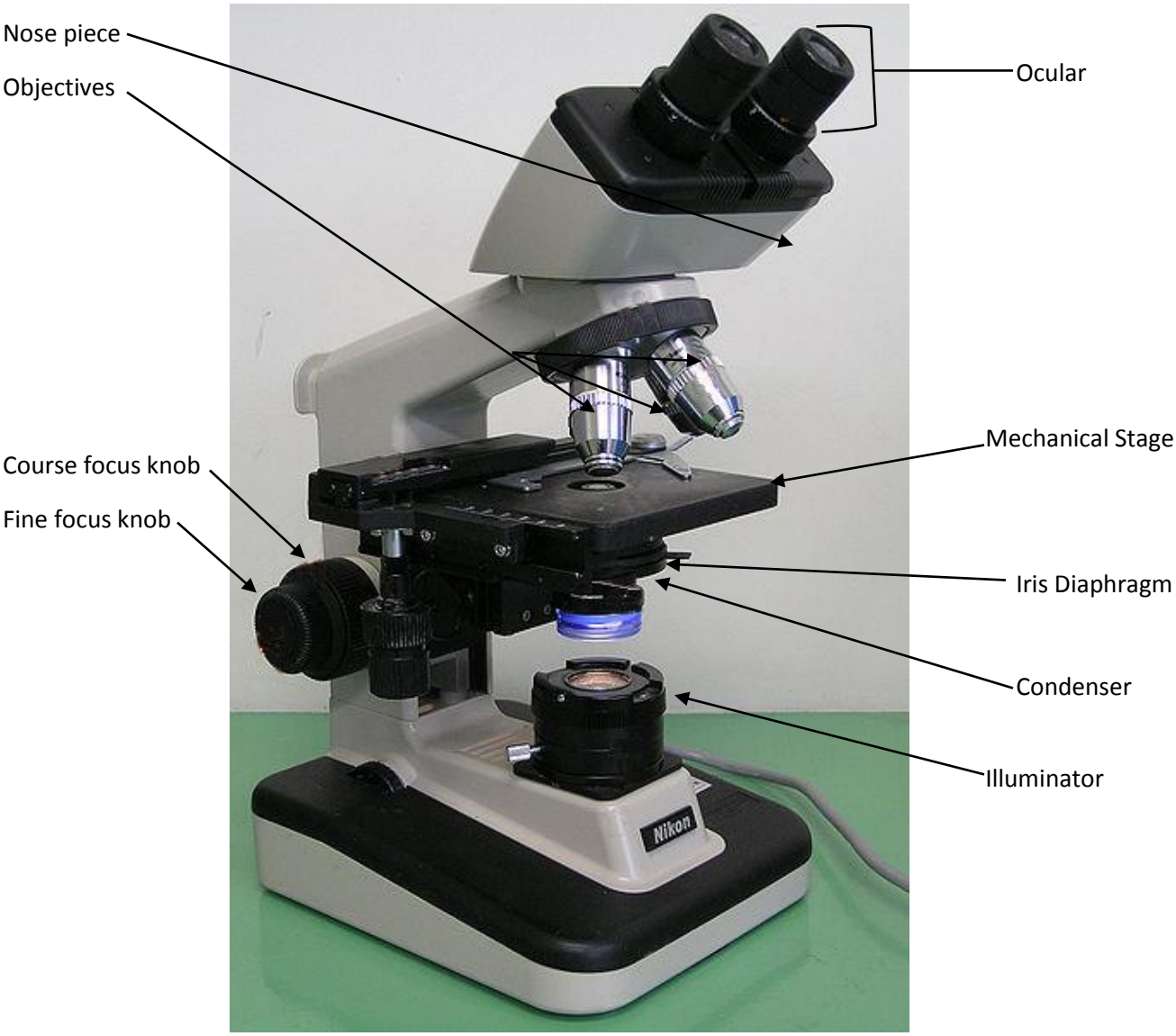
(Adapted from http://www.biologycorner.com/)

Introduction

A microscope is an instrument that magnifies an object so that it may be seen by the observer. Because cells are usually too small to see with the naked eye, a microscope is an essential tool in the field of biology. In addition to magnification, microscopes also provide resolution, which is the ability to distinguish two nearby objects as separate. A combination of magnification and resolution is necessary to clearly view specimens under the microscope. The light microscope bends a beam of light at the specimen using a series of lenses to provide a clear image of the specimen to the observer.

In this lab, parts of the microscope will be reviewed. Students will learn proper use and care of the microscope and observe samples from pond water.

Parts of the microscope



Magnification

Your microscope has 4 objective lenses: Scanning (4x), Low (10x), High (40x), and Oil Immersion (100x). In this lab you will not use the oil immersion lens; it is for viewing microorganisms and requires technical instructions not covered in this procedure.

In addition to the objective lenses, the ocular lens (eyepiece) has a magnification. **The total magnification is determined by multiplying the magnification of the ocular and objective lenses.**

	Magnification	Ocular lens	Total Magnification
Scanning	4x	10x	40x
Low Power	10x	10x	100x
High Power	40x	10x	400x
Oil Immersion	100x	10x	1000x

General Procedures

1. Make sure all backpacks, purses, etc. are off the benchtop.
2. Carry microscope by the base and arm with both hands.
3. Store with cord wrapped around microscope and the scanning objective clicked into place.

Focusing Specimens

1. Plug your microscope in to power supply and switch on illuminator.
2. Always start with the stage as low as possible and using scanning objective (4x). Odds are, you will be able to see *something* on this setting (sometimes it's only a color). Use the coarse knob to focus: the image may be small at this magnification, but you won't be able to find it on the higher powers without this first step. Move the mechanical stage until your focused image is also centered.
3. Once you've focused using the scanning objective, switch to the low power objective (10x). Use the coarse knob to refocus and move the mechanical stage to re-center your image. Again, if you haven't focused on this level, you will not be able to move to the next level.
4. Now switch to the high power objective (40x). At this point, ONLY use the fine adjustment knob to focus specimens.
5. If the specimen is too light or too dark, try adjusting the diaphragm.

Cleanup

1. Store microscope with the scanning objective in place and the stage in its lowest position.
2. Wrap cords around microscope.
3. Replace slides to original slide tray.

Troubleshooting

Occasionally you may have trouble with working your microscope. Here are some common problems and solutions.

1. Image is too dark!
   - Adjust the diaphragm, make sure your light is on.
2. There's a spot in my viewing field- even when I move the slide the spot stays in the same place!
   - Your lens is dirty. Use lens paper, and only lens paper to carefully clean the objective and ocular lens. The ocular lens can be removed to clean the inside.
3. I can't see anything under high power!
   - Remember the steps, if you can't focus under scanning and then low power, you won't be able to focus anything under high power.
4. Only half of my viewing field is lit, it looks like there's a half-moon in there!
   - You probably don't have your objective fully clicked into place.
5. I see my eyelashes!
   - You're too close to the objectives. Move your head back a little.
6. This is giving me a headache!
   - Relax. Try adjusting the ocular distance, check that the intensity of your light isn't too high or too low. Take breaks if needed!

BE PATIENT AND KEEP TRYING. USING A MICROSCOPE TAKES PRACTICE!!

Part 1: Orientation of Images in the Microscope

A large part of the learning process of microscopy is getting used to the orientation of images viewed through the oculars as opposed to with the naked eye. A common mistake is moving the mechanical stage the wrong way to find the specimen. This procedure is merely practice designed to make new users more comfortable with using the microscope.

Materials

- Compound microscope
- Microscope slide with the letter "e"

Procedure

1. Place the letter "e" slide onto the mechanical stage. Be sure to note the orientation of the letter "e" as it appears to your naked eye.

2. Use the SCANNING (4x) objective and course focus adjustment to focus, then move the mechanical stage around to find the letter "e". Note the orientation when viewed through the oculars.

Does the lens of the microscope reverse the image? _____
Does it flip the image? (upside down) _____

Part 2: Practice with Depth of Field in the Microscope

This portion of the procedure is another practice to demonstrate depth perception. Many new microscope users find it difficult to conceive that the specimen on the slide is in three dimensions. As the stage is moved up and down, different threads will be in focus.

Materials

- Compound microscope
- Microscope slide with 3 threads

Procedure

1. Place the thread slide onto the mechanical stage.

2. Use the SCANNING (4x) objective and course focus adjustment to focus, then move the mechanical stage around to find the threads.

3. If needed, switch to the low power (10x) objective and refocus.

4. Determine which thread is on the bottom, middle, and top of the slide.

TOP	**MIDDLE**	**BOTTOM**

Part 3: Investigation of Pond Water & Microorganisms

Materials

- Compound microscope
- Microscope slide
- Coverslip
- Transfer pipette
- Pond water sample

Procedure

1. Using the transfer pipette, transfer a drop of pond water onto a microscope slide. The best specimens usually come from the bottom and probably will contain chunks of algae or other debris that you can see with your naked eye.

2. Place coverslip onto the slide.

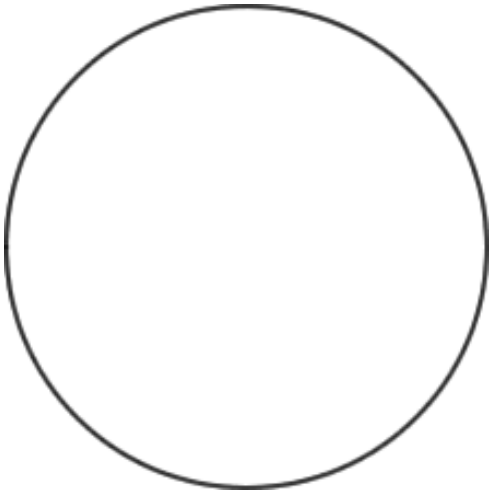
3. Use the SCANNING (4x) objective to focus, then move the mechanical stage around to scan the slide for live microorganisms. You are looking for tiny swimming beings- they may look green or clear and might be very small. Choose one to focus on and center it in your visual field.

   You may wish to use the ProtoSlo to keep your organisms from swimming to quickly!

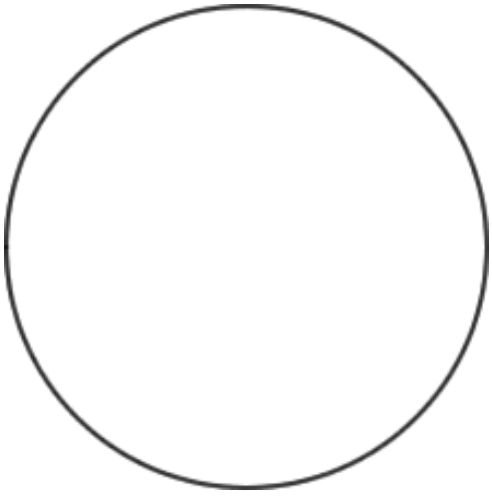
4. Switch to low power (10x). This may be sufficient to view your chosen organism. Try to note how it moves and do your best to draw it as you see it, unless you need more magnification.

5. Once you have centered and focused the image, switch to high power (40x) and refocus. Note movements and draw the organism as you see it.

   Remember, do NOT use the coarse adjustment knob at this point!



Pond water organism 1



Pond water organism 2

Questions:

1. Why is it important to begin focusing with the scanning objective?

2. If you're using the 40X objective and you know your ocular is 10X, what is the total magnification?

3. If you bump your microscope and lose focus, what do you do to refocus your specimen?

4. Why must you center your image before switching to a higher objective?

Parts of the Cell

Introduction

The cell theory states that all living things are composed of cells, cells are the basic units of life, and that all cells arise from existing cells. In this course, we closely study cells. There are 2 types of cells: prokaryotic and eukaryotic. Prokaryotes lack a nucleus and true organelles, and are typically significantly smaller than eukaryotic cells. Prokaryotic organisms are found within the domains Bacteria and Archaea. Eukaryotic cells do contain nuclei, as well as other organelles that work together to support homeostasis of the whole cell. Though eukaryotes are larger than prokaryotes, we must use a microscope to view all cells, which are typically too small to see with the naked eye.

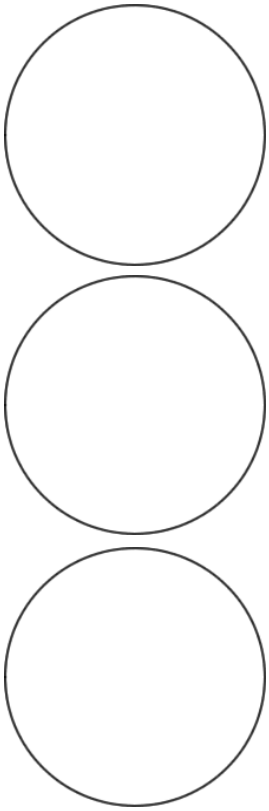
There are vast differences between cell types, but a few features are common to all cells: plasma membrane, cytoplasm, ribosomes, and cytoskeleton. All cells also use DNA for their genetic material; in eukaryotes this is within the nucleus and in prokaryotes it is found in the nucleoid region of the cytoplasm. Prokaryotes generally have a cell wall made of peptidoglycan and some have flagella or fimbriae, which are used for movement or attachment. Eukaryotes have several more organelles and are further differentiated into 2 categories: plant cells and animal cells.

Some organelles common to eukaryotes include mitochondria, peroxisomes, vesicles, lysosomes, smooth and rough endoplasmic reticula, and Golgi bodies. Animal cells tend to lack cell walls and chloroplasts, while plant cells do contain chloroplasts and have cellulose cell walls.

In this lab, bacterial, animal, and plant cells will be observed using the microscope. Students will draw what was visualized to record their observations.

Part 1: Bacterial Cell

View a prepared slide of common bacterial cell types; prepare a wet mount of cyanobacteria and observe under the microscope.



Materials

- Compound microscope
- Methylene blue
- Microscope slide
- Cover slip
- Transfer pipette
- Culture of *Oscillatoria*

Procedure

A. Fixed slide of bacterial types

1. Examine the demonstration slide of mixed bacteria. There are 3 common shapes: round (coccus), rod (bacillus), and spiral (spirillum). The slide should have several of each type of bacteria.

2. Draw each of the bacterial shapes in the spaces at right.

B. Wet mount of *Oscillatora*

1. Using the transfer pipette, transfer a drop of liquid culture onto a microscope slide.

2. Place coverslip onto the slide.

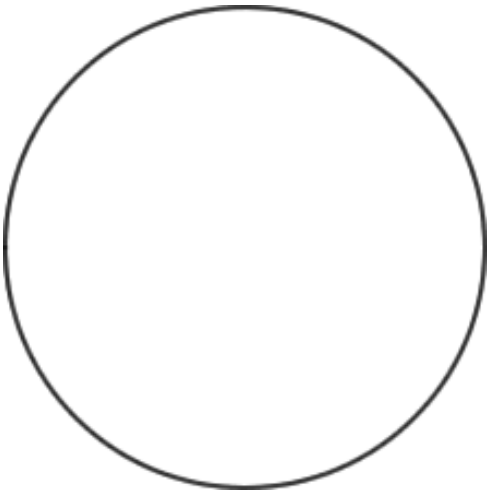
3. Use the SCANNING (4x) objective to focus. You are looking for very faint green thin filaments.

4. Switch to low power (10x). You may be able to see lines going across the filaments, but the image will likely just look like green floss.

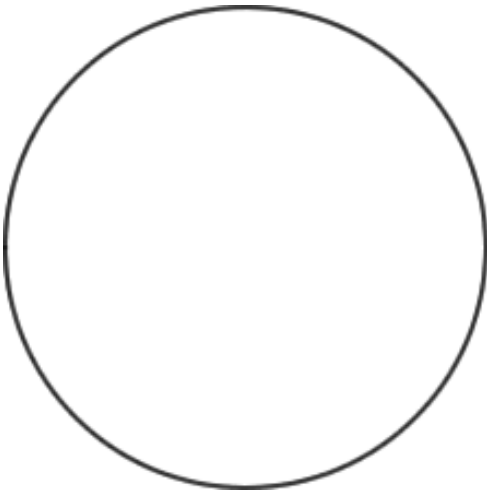
5. Once you have centered and focused the image, switch to high power (40x) and refocus. The individual cells should be visible at this magnification; each filament is composed of cells stacked on top of each other.

   Remember, do NOT use the coarse adjustment knob at this point!

6. Sketch the bacteria at low and high power. Label the cytoplasm and cell wall of a single cell. Draw your cells to scale.



Oscillatoria at low power (10x)



Oscillatoria at high power (40x)

Questions:

1. Why are nuclei not visible within the cells viewed?

2. The common name for species like *Oscillatoria* is blue-green algae. This group of bacteria are capable of photosynthesizing. Do they contain chloroplasts? Explain.

Part 2: Animal Cell

Prepare a wet mount of a human cheek cell and observe under the microscope

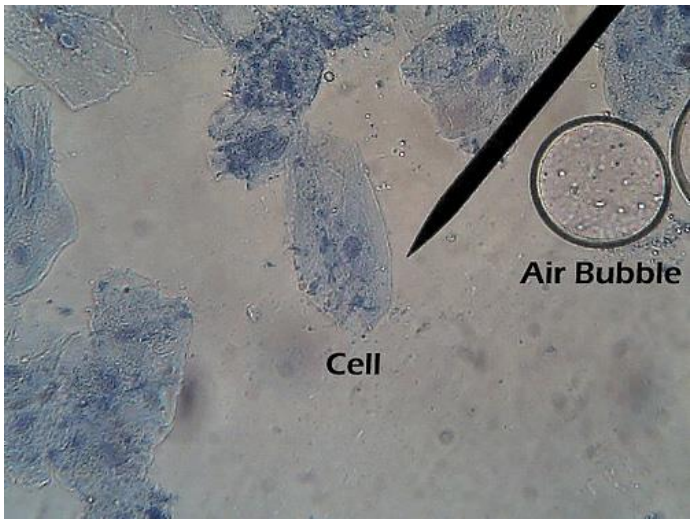


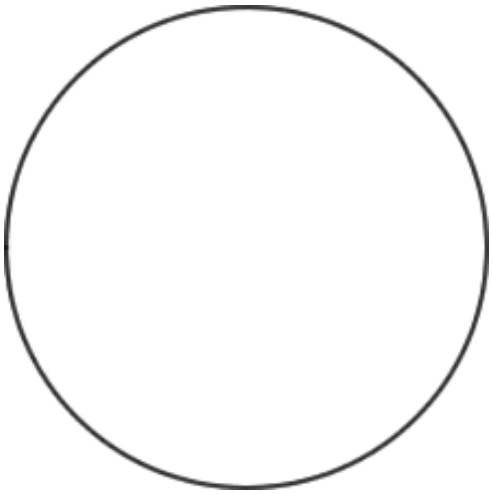
Figure 1: Human cheek cell stain at high power
"Cheek Cells Identified (400x)" by biologycorner, via Flickr

Materials

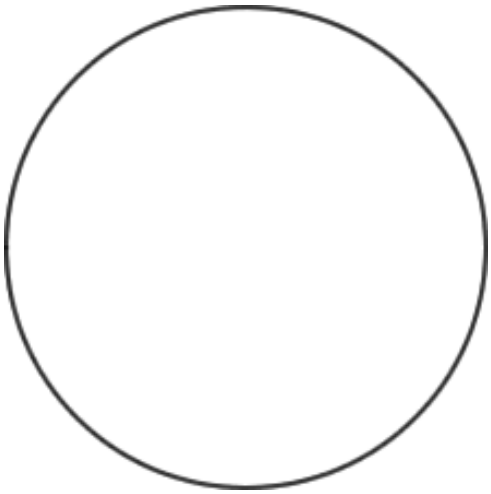
- Compound microscope
- Methylene blue
- Microscope slide
- Cover slip
- Toothpick

Procedure:

1. Put a drop of methylene blue on the slide.
2. Gently scrape the inside of your cheek with the flat side of a toothpick. *Scrape lightly!*
3. Stir the end of the toothpick in the stain and throw the toothpick away.
4. Place a coverslip onto the slide.
5. Use the SCANNING (4x) objective to focus. You probably will not see the cells at this power.
6. Switch to low power (10x). Cells should be visible, but they will be small and look like nearly clear purplish blobs. If you are looking at something very dark purple, it is probably not a cell.
7. Once you think you have located a cell, switch to high power (40x) and refocus.
   Remember, do NOT use the coarse adjustment knob at this point!
8. Sketch the cell at low and high power. Label the nucleus, cytoplasm, and cell membrane of a single cell. Draw your cells to scale.



Cheek cell at low power (10x)



Cheek cell at high power (40x)

Questions

1. Why is methylene blue added?

2. The light microscope used in the lab is not powerful enough to view other organelles in the cheek cell.

    a. What parts of the cell were visible?

    b. List 2 organelles that were NOT visible but should have been in the cheek cell.

3. Is the cheek cell a eukaryote or prokaryote? How do you know?

4. Keeping in mind that the mouth is the first site of chemical digestion in a human. Your saliva starts the process of breaking down the food you eat. Keeping this in mind, what organelle do you think would be numerous inside the cells of your mouth?

Part 3: Plant cells
Prepare wet mounts of an onion cell and an *Elodea* leaf cell and observe both under the microscope

Materials

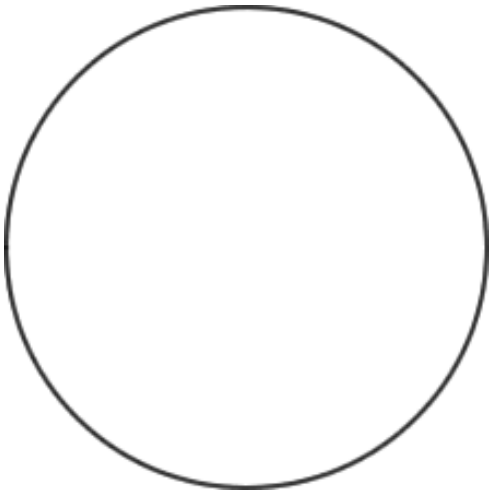
- Compound microscope
- Microscope slide
- Cover slip
- Dropper bottle with dH2O
- Forceps
- Pre-cut onion bulb
- Culture of Elodea
- Dissecting needle

Figure 2: Onion cells at high power
"Onion 100x" by biologycorner, via Flickr

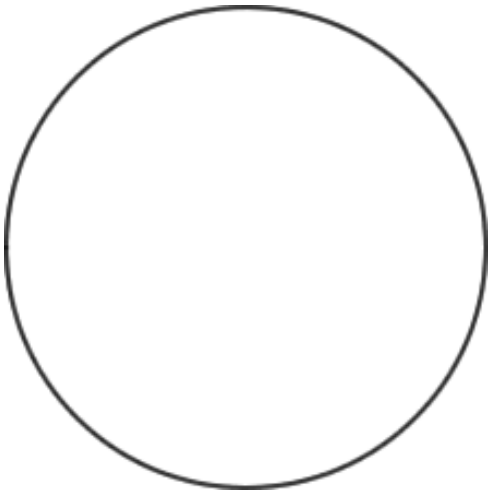
Procedure

A. Wet mount of an onion cell

1. Put a drop of water onto the microscope slide.
2. Using the forceps, gently peel off a small piece of the "membrane" of the onion (epidermis). It should be very thin and may curl up on itself.
3. Place the onion sample into the drop of water on your slide. Try to unroll/straighten out the sample to view a single layer of cells. You may need to use the dissecting needle to do this.
4. Place a coverslip onto the slide.
5. Use the SCANNING (4x) objective to focus. You probably will not see the cells at this power.
6. Switch to low power (10x). Cells walls should be visible: they will look like semi-clear grid lines.
7. Once you think you have located a cell, switch to high power (40x) and refocus.
8. Sketch the cell at low and high power. Label the nucleus, cytoplasm, and cell wall of a single cell. Draw your cells to scale.



Onion cell at low power (10x)



Onion cell at high power (40x)

B. Wet mount of an *Elodea* leaf cell

1. Put a drop of water onto the microscope slide.
2. Using the forceps, gently tear off a small piece of a leaf from *Elodea*.
3. Place the *Elodea* leaf into the drop of water on your slide.
4. Place a coverslip onto the slide.
5. Use the SCANNING (4x) objective to focus. You probably will not see the cells at this power.
6. Switch to low power (10x). Cells walls should be visible: they will look like dark grid lines.
7. Once you think you have located a cell, switch to high power (40x) and refocus.
8. Sketch the cell at low and high power. Label the chloroplasts, cytoplasm, and cell wall of a single cell. The nucleus may be visible as well- it will be a large, clear figure. Draw your cells to scale.

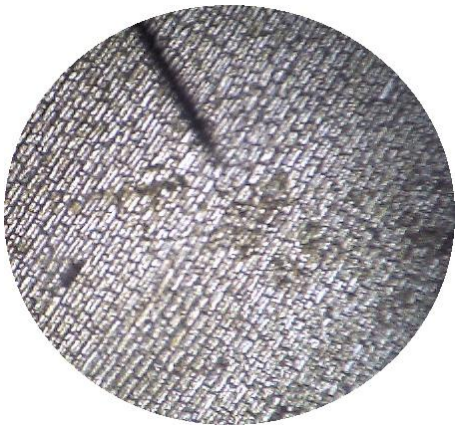


Figure 3: *Elodea* cells at low power
"Anacharis 40x" by biologycorner, via Flickr

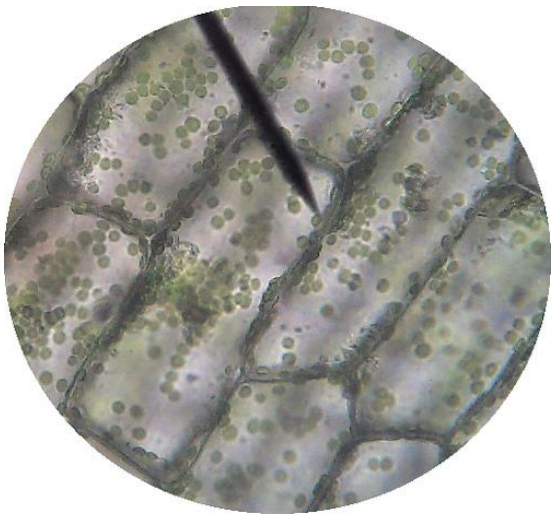
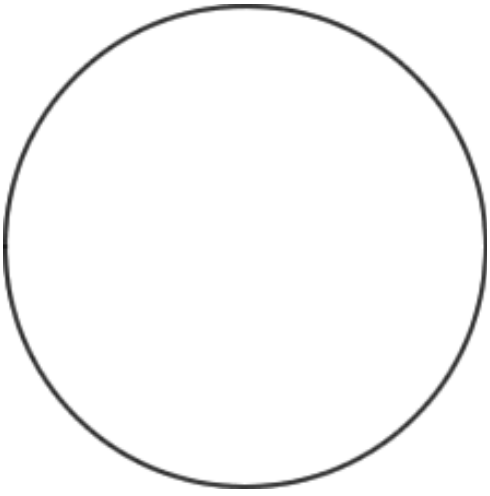
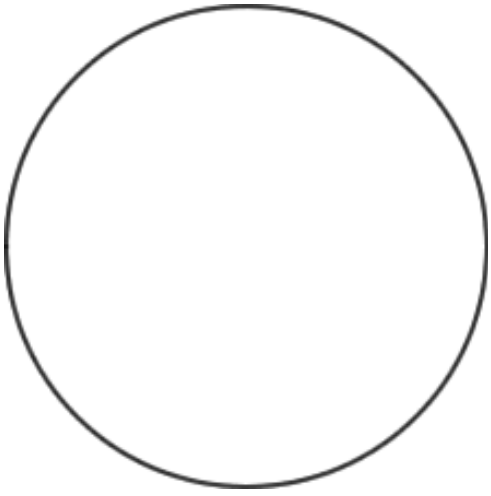


Figure 4: *Elodea* cells at high power
"Anacharis 400x" by biologycorner, via Flickr



Elodea cell at low power (10x)



Elodea cell at high power (40x)

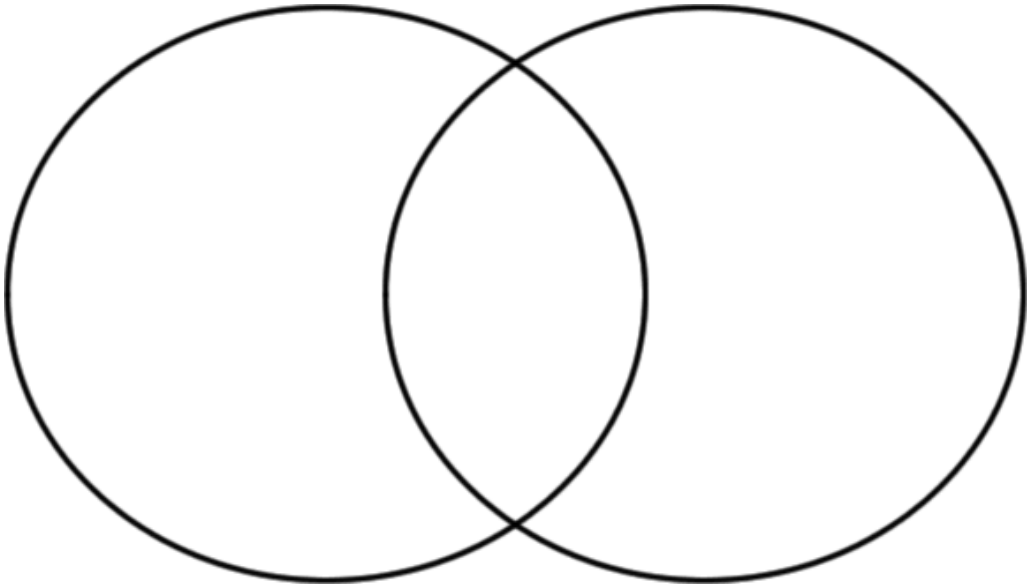
Questions

1. Describe the shape and the location of chloroplasts.

2. Were chloroplasts observed in the onion cells? Why or why not?

3. Is this statement true or false? “Animal cells have mitochondria; plant cells have chloroplasts.” Explain.

4. Fill out the Venn diagram below to show the differences and similarities between the onion cells and the *Elodea* cells.



Diffusion and Osmosis

(Adapted from biologycorner.com)

Introduction

Diffusion is the process by which molecules spread from areas of high concentration to areas of low concentration. This movement, down the **concentration gradient**, continues until molecules are evenly distributed. **Osmosis** is a special type of diffusion: the diffusion of water through a **semipermeable membrane**. The concentration of water is inversely related to the concentration of solute: more solute corresponds to less water and less solute corresponds to more water. This is important because osmotic vocabulary describes the solute and not the water. **Hypertonic** solutions contain a high concentration of solute and little water, relative to **hypotonic** solutions that have a low concentration of solute and therefore a higher concentration of water. The term "**isotonic**" is used when two areas have an equal concentration of solute: no net osmosis is occurring.

Exercise 1: Diffusion through a gel

One factor that can affect the rate of diffusion is the size of the molecule. Larger molecules tend to move more slowly than smaller molecules. In this experiment, students will compare the diffusion rates of two dyes traveling through agar.

Materials

- Pre-punched agar plates
- Potassium permanganate
- Janus green
- Ruler

Procedure

1. Using the dropper, drop a single drop of potassium permanganate into one of the wells on the plate.
2. Repeat with Janus green.
3. Allow the plates to sit undisturbed for 30 minutes.
4. Which dye do you think will have the faster diffusion rate? _____
5. After 30 minutes, measure the radius of the dye front from the middle of the well and record your results.
6. Calculate the diffusion rate (mm/hr) by dividing the dye front radius by 0.5.

	Potassium permanganate	**Janus Green**
Molecular weight	158g/mole	511g/mole
Radius (mm)		
Diffusion rate		

Questions:

1. Did your outcome match your expectation? Provide an explanation for your results.

2. What are other factors that can affect the rate of diffusion?

Exercise 2: Observation of Osmosis in a plant cell

Plants have cell walls that can prevent lysis if too much water flows into the cell. Plant cytoplasm tends to be hypertonic to the outside environment, which results in an inflow of water and a high amount of pressure (**turgor pressure**) inside the cell. When a plant is placed into a hypertonic environment, the water will leave the cell. This causes the cell to shrink and detaches the plasma membrane from the cell wall (**plasmolysis**). Turgor pressure can hold plants upright, while plasmolysis can cause plants to wilt.

Procedure

Observe the two *Elodea* leaves under the microscope. One slide is a leaf in isotonic solution: you should be able to identify the chloroplasts and an empty space in the middle of the cells which is the vacuole. The next leaf has been soaked in a salt water solution; compare the cells to the first slide.

Questions:

1. What is the difference between a hypertonic solution and a hypotonic solution?

2. What will happen to plant cells that are placed in a hypertonic solution?

3. What will happen to animal cells placed in hypotonic solution? Why should this be different from plant cells?

4. Why are dehydrated patients given saline intravenously instead of water?

Exercise 3: Osmosis Across a Membrane

Observe the movement of water across a semipermeable membrane.

Materials

- Dialysis bags (4 per group)
- Dental floss
- 15% sucrose solution
- 30% sucrose solution
- Triple beam balance
- Beakers (4 per bench)
- Graduated cylinder
- Stir rods

Procedure

1. Obtain 4 strips of dialysis tubing and tie a knot in one end of each using the dental floss.
2. Pour approximately 10ml of each solution into separate bags (see table below).

	Tube 1	**Tube 2**	**Tube 3**	**Tube 4**
Inside bag	water	15% sucrose	30% sucrose	water
Inside beaker	water	water	water	15% sucrose
Tonicity of bag?				
Predicted outcome?				

3. Remove most of the air from the bag (but leave a little bit of space) and tie the bag.
4. Blot the bags to remove any sugar that may have spilled; check the bags for leaks.
5. **Record the weight of each baggie in the data table.**
6. Place a bag in each beaker (*be sure to keep track of which bag is in which beaker*!). Fill the beakers with enough of the appropriate solution to cover your bags (refer to the above table).
7. **Predict what you think will happen during the experiment.**
8. Record weight every 10 minutes in data table.
9. After 30 minutes, remove the bags from solution and record the final weight.

Data	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**
Weight at 0 min				
Weight at 10 min				
Weight at 20 min				
Weight at 30 min				

Questions:

1. Did you results match your predictions? Propose an explanation for why your results (either overall or an individual bag) may have differed from what you were expecting.

2. Based on what you have observed, are the dialysis bags permeable to sucrose?

Enzymatic activity lab

Dr. Matt Chenoweth, Dalton State College

Introduction

Enzymes are biological catalysts and are usually proteins. They greatly increase the rate of chemical reactions by lowering the activation energy, which is the energy required to start a reaction. The metabolism of a cell depends upon enzymes in order to function correctly. Enzymes are sensitive to environmental conditions. If the conditions deviate too much, enzymes may stop functioning.

To examine the effects of environmental changes on enzymatic activity, we will work with the enzyme catalase. This is a very common enzyme that is present in most living organisms. Catalase catalyzes the breakdown of hydrogen peroxide to water and oxygen:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

We will measure catalase activity somewhat indirectly. The reaction tubes will contain a detergent, Triton X-100. When O_2 is generated in our reactions, it will create foam in the tubes because of the detergent. After the reactions are over, we will measure the height of the foam column as a way of determining how active the enzyme was under the conditions tested.

Materials

- Test tubes
- Filter paper discs (presoaked in catalase)
- Forceps
- TritonX-100
- Manual pipetter
- 1mL serological pipettes
- Hydrogen peroxide
- pH buffers

Procedure [1]

Part 1: Effect of temperature on catalase activity

1. Label 4 tubes A, B, C, and D. Also put your group name on each tube.

2. Add a catalase-soaked filter paper disc in the bottom of each tube
   Use forceps to handle the discs. Do not touch them with your hands as oil from your skin will interfere with the assay.

3. Pre-incubate the tubes for 5 minutes
   Place Tube A in a rack in an ice water bath
   Place Tube B in a rack at room temperature
   Place Tube C in a rack in the 37˚C water bath
   Place Tube D in a rack in the 80˚C water bath

[1] Protocol adapted from Iwase, T, et al. A Simple Assay for Measuring Catalase Activity: A Visual Approach. Scientific Reports 3:3081, 1-4 (2013).

4. After the pre-incubation step, add 1mL of Triton X-100 to each tube

5. Add 1mL of H_2O_2 to each tube and swirl to mix

6. Quickly return each tube to the appropriate rack

7. Allow the reactions to run for 10 minutes, then retrieve the tubes from their racks.
   While the reactions are in progress, answer the following questions.

   a. What is the independent variable in this experiment? _____

   b. What is the dependent variable in this experiment? _____

   c. How do you predict that the enzyme activity will be affected by the different incubation temperatures?

8. Measure the height of the foam in each tube and record your data.

Tube	Temperature	Foam height (mm)

9. Use your data to construct a graph.

Part 2: Effect of pH on catalase activity

1. Label 3 tubes A, B, and C. Also put your group name on each tube.

2. Add a catalase-soaked filter paper disc in the bottom of each tube
   Use forceps to handle the discs. Do not touch them with your hands as oil from your skin will interfere with the assay.

3. Add 1mL of the appropriate buffer to each tube
   Tube A – pH 4 buffer
   Tube B – pH 7 buffer
   Tube C – pH 10 buffer

4. Add 1mL of Triton X-100 to each tube

5. Add 1mL of H_2O_2 to each tube and swirl to mix

6. Place all 3 tubes in the 37˚C water bath

7. Allow the reactions to run for 10 minutes, then retrieve the tubes

While the reactions are in progress, answer the following questions.

a. What is the independent variable in this experiment? _____

b. What is the dependent variable in this experiment? _____

c. How do you predict that the enzyme activity will be affected by the different pH buffers?

8. Measure the height of the foam in each tube and record your data.

Tube	pH	Foam height (mm)

9. Use your data to construct a graph.

Questions

1. What was the optimal temperature for catalase activity? Was the prediction you made correct?

2. What happened to catalase activity at 80˚C? Based on what you know about protein structure, how would you explain that result?

3. What was the optimal pH for catalase activity? Was the prediction you made correct?

4. Can you identify any potential problems or sources of error with the experimental design? How could it be improved?

5. What is the substrate for catalase? What are the products of the reaction?

6. How is the substrate of an enzyme different from the active site?

7. The pH in the stomach is pH 2.0. What do you think the optimum pH is for pepsin, an enzyme that is secreted in the stomach?

Cell Respiration and Photosynthesis

Part 1: Photosynthesis and Floating Leaf Disks

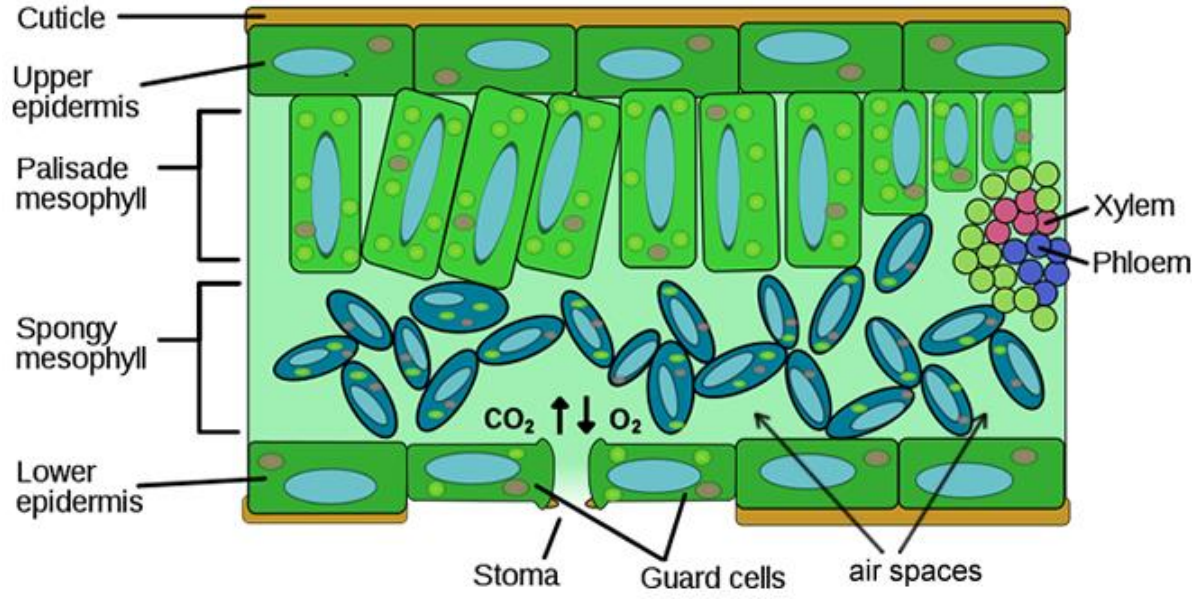
(Adapted from Biology Corner)

Introduction

Photosynthesis fuels ecosystems and replenishes the Earth's atmosphere with oxygen. Like all enzyme-driven reactions, the rate of photosynthesis can be measured by either the disappearance of substrate, or the accumulation of products. The equation for photosynthesis is:

$$6CO_2 + 6H_2O \text{ ------light--------> } C_6H_{12}O_6 + 6O_2 + H_20$$

In this investigation, you will use a system that measures the accumulation of oxygen in the leaf. Consider the anatomy of the leaf as shown below.



The leaf is composed of layers of cells. The spongy mesophyll layer is normally infused with gases (oxygen and carbon dioxide). Leaves will normally float in water because of these gases. If you draw the gases out from the spaces, then the leaves will sink because they become denser than water. If this leaf disk is placed in a solution with an alternate source of carbon dioxide in the form of bicarbonate ions, then photosynthesis can occur in a sunken leaf disk. As photosynthesis proceeds, oxygen accumulates in the air spaces of the spongy mesophyll and the leaf becomes buoyant and floats. Oxygen and carbon dioxide are exchanged through openings in the leaf called stoma.

While this is going on, the leaf is also carrying out cellular respiration. This respiration will consume the oxygen that has accumulated and possibly cause the plant disks to sink. The measurement tool that can be used to observe these counteracting processes is the floating (or sinking) of the plant disks. In other words, the buoyancy of the leaf disks is actually an indirect measurement of the net rate of photosynthesis occurring in the leaf tissue.

Materials:

- Syringe
- Desk lamp
- Hole punch
- Petri dishes (4 per group)
- Detergent and baking soda solution
- Detergent solution
- Fresh spinach leaves
- Forceps

Procedure:

1. Test the syringes by sealing the tip and pulling back on the plunger. When released, the plunger should snap back, indicating a good vacuum. Remove the plunger from a syringe.
2. Use a hole punch to punch out 40 disks from the leaves. The disks should be as uniform in size and mass as possible. Avoid the larger veins of the leaves. As you punch out the leaf disks, put them into the syringe. Continue until you have at least 40 disks.
3. Tap the side of the syringe so that the disks are at the bottom, and then reinsert the plunger—being careful not to crush the leaf disks.
4. Insert the tip of the syringe into the beaker and draw a small amount of the detergent solution into the syringe. Tap the syringe to dislodge disks that are stuck to the sides. There may be a couple of disks that you simply cannot dislodge.
5. Hold the syringe vertically, with the tip pointed upwards, and push in the plunger to expel the trapped air.
6. Close the tip of the syringe with your finger and pull on the plunger to create a vacuum. The vacuum removes gas from the leaf tissues. Hold the plunger in place for 10 seconds and release it. When you release the plunger, liquid infiltrates the tissue. Repeat this 3 times. As liquid infiltrates the leaf tissues, the density of the disks increases, and they begin to sink.
7. Use tweezers to transfer 10 disks to each petri dish and add enough solution to cover the disks; set up according to the table below:

	Dish A	**Dish B**	**Dish C**	**Dish D**
Solution	Baking soda	Baking soda	Baking soda	Detergent only
Placement	Directly under lamp	On benchtop	On benchtop, covered	Directly under lamp

** Make sure to fully submerge the leaf discs in solution before beginning the assay**

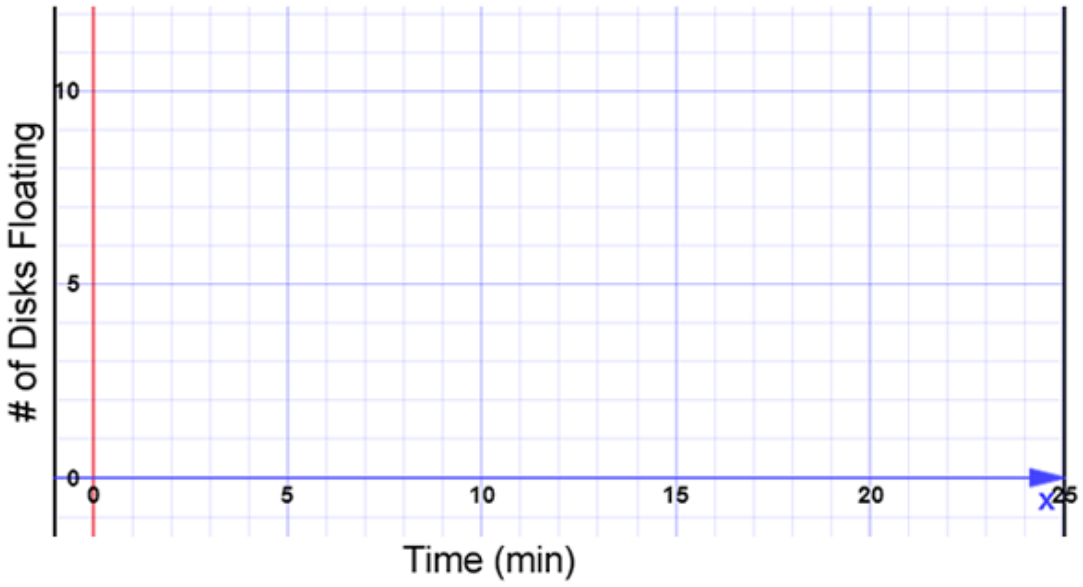
8. Turn on the light and begin recording the time. As oxygen is produced by photosynthesis, it comes out of solution and infiltrates the leaf tissue, replacing some of the water. This decreases the density of the disks, and they begin to float.
9. Record the number of floating disks in 5 minute intervals, continuing the experiment until all disks are floating. Record your data in the table on the following page.

	Number of Disks floating			
Time (min)	**Dish A**	**Dish B**	**Dish C**	**Dish D**
5				
10				
15				
20				
25				
30				

Analyzing Data

To make comparisons between experiments, a standard point of reference is needed. Repeated testing of this procedure has shown that the point at which 50% of the disks are floating (ET_{50}) is a reliable and repeatable point of reference. In this case, the disks floating are counted at the end of each time interval.

Graph your data for each experimental group. Determine the ET_{50} for your data. Did your outcome match your expectation?



Questions:

1. A mutation is capable of reducing the amount of chlorophyll in the leaf. Would this also reduce the rate of photosynthesis?

2. What about a plant that exhibits variegation… Do areas of the leaf with chlorophyll outperform areas that lack chlorophyll? Could you design an experiment to test this?

3. In this experiment, the amount of oxygen produced was observed to measure the rate of photosynthesis. What else could you measure to determine the rate of photosynthesis?

4. List any factors that you think may affect the rate of photosynthesis. Consider environmental factors that you could manipulate during the lab.

Part 2: Chicago Cyanide Murders- A Case Study in Cellular Respiration

(Adapted from http://www.biologycorner.com/worksheets/case_study_cellular_respiration.html)

Background

In September of 1982, Mary Kellerman gave her 12 year old daughter a painkiller when she awoke during the night complaining of a sore throat. At 7 am the next morning, her daughter was found collapsed on the bathroom floor, and later pronounced dead.


THE DAILY HERALD

5 dead after taking Tylenol capsules filled with cyanide

Adam Janus, a postal worker in another Chicago suburb also died unexpectedly, though originally it was thought he had suffered from a heart attack. While his family gathered to mourn their loss, his brother and sister became ill and later died.

In the days that followed, three more unexplained deaths occurred in nearby Chicago suburbs. Investigators found that all of the victims had taken an extra strength tylenol hours before their death. They suspected that someone had tampered with the medication.

Symptoms exhibited by each of the victims included:

- weakness, dizziness, sleepiness
- flushed, bright red, skin tone
- headache
- shortness of breath and rapid breathing
- vomiting
- confusion and disorientation

1. In your opinion, are the seven deaths connected? What additional information would you need to determine if they are connected?

2. If poison is suspected in the deaths, how would you proceed with the investigation?

Autopsy report

The medical examiner concluded that each of the victims had died of hypoxia. **Hypoxia** means that the person suffered from a lack of oxygen, or they were suffocated. The reason for the hypoxia is not always clear at the first examination.

The medical examiner also showed the tissue samples from the heart, lungs, and liver showed massive cell death. On further investigation, it was shown that the tissues had major mitochondrial damage. Even though the victims died of hypoxia, their level of oxygen in their blood was approximately 110 mm Hg. The normal range is 75-100 mm Hg.

1. Recall your knowledge of the function of organelles. What function of the cells was interrupted in these patients?

2. While poison is the main suspect in the case, what are other ways a person could die of hypoxia?

3. Analyze the oxygen levels of the victims. Were the levels higher or lower than normal? How can you reconcile this observation with the cause of death being hypoxia?

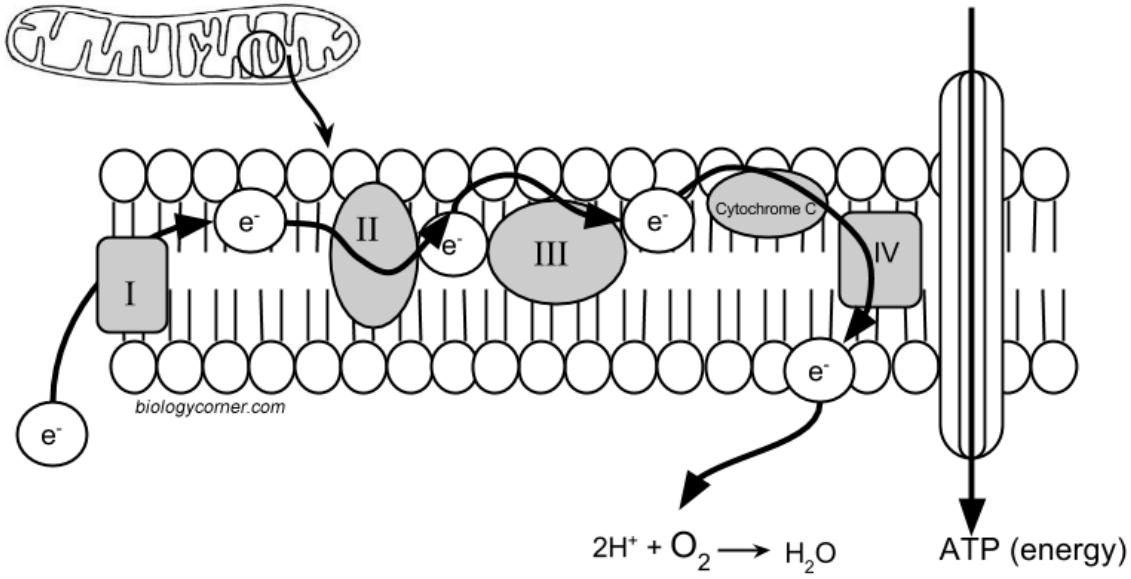
Toxicology reports show that the victims had been poisoned with cyanide. The poison was traced back to extra strength tylenol where the murderer had opened the capsules and replaced acetaminophen (a pain killer) with cyanide. Cyanide acts very quickly, often killing within minutes of ingestion and authorities were slow to identify the cause of the deaths. Once the cause as identified, stores removed tylenol and other drugs from shelves. While there were many suspects, no one was ever charged with the crime and it is still an ongoing investigation. Since the Chicago Tylenol murders, drug companies have drastically changed how medicines are packaged.

Why is cyanide such an effective poison? You might be surprised to learn that it directly interferes with cellular respiration that occurs in the mitochondria.

4. Recall that the mitochondrion is sometimes called the "powerhouse" of the cell. What does this mean? Why is the mitochondrion important?

Why Do We Need Oxygen?

It seems like a simple question, everyone knows you need to breathe to live. Have you ever thought about why oxygen is so important? The victims of the cyanide poisoning all had high levels of oxygen in their blood, but the poison was interfering with how the cells use that oxygen. To understand, we need to take a very close look at the structure of the mitochondrion.



Inside the mitochondrion, there are several layers of membranes. In fact, these membranes resemble the membrane that surrounds the cell. It has a bilayer of phospholipids and embedded **proteins**. On the diagram above, the proteins are labeled I, II, III, IV, and cytochrome C.
The proteins in the membrane pass electrons from one to the other; this is known as the **electron transport chain**. The passing of these electrons allows **ATP** (adenosine triphosphate) to be generated. At the end of the electron transport chain, cytochrome C passes the electron to Complex IV and then to its final acceptor, oxygen. Oxygen then binds with proteins to create water. This process is continuous in cells, with ATP constantly being generated and oxygen being used as the final electron acceptor. Cyanide inhibits cytochrome C, preventing the last protein from doing its job. The electron stops at the end of the chain and cannot be passed to oxygen. The whole chain grinds to a halt and no ATP can be made.

1. On the model of the mitchondrion, highlight the area that is the ELECTRON TRANSPORT CHAIN. Place an X over the protein that is inhibited by cyanide.

   What is the relationship between the ETC and oxygen?

2. Cyanide is an extremely fast acting poison. In fact, it was developed as a suicide pill (called L-pill) during World War II so that British and American spies could avoid being captured alive.

   Given what you know about ATP and cellular respiration, explain why cyanide is so fast acting.

3. Given what you know about cyanide poisoning, do you think that giving a person oxygen would be an effective treatment? Why or why not?

Mitosis and Cytokinesis Lab

By Dr. Leah Howell, Dalton State College

Introduction

All cells come from preexisting cells, and eukaryotic cells must undergo mitosis in order to form new cells. The replication of a cell is part of the overall cell cycle (**Figure 1**) which is composed of interphase and M phase (mitotic phase). M phase, which consists of mitosis and cytokinesis, is the portion of the cell cycle where the cell divides, reproducing itself. Mitosis is the division of the nucleus and its contents. In mitosis, DNA which has been copied in S phase of interphase is separated into two individual copies. Each copy will end up in its own cell at the end of M phase. Mitosis has several steps: prophase, prometaphase, metaphase, anaphase, and telophase (**Figure 2**). The spindle fibers, which are formed by the cell as mitosis progresses, are used to attach to chromosomes, align them down the middle of the cell, and pull chromosomes apart into their identical individual chromatids which will end up in separate cells. As mitosis is nearing its end and the cell is in telophase, the cytoplasm also divides so that both new cells will have their own fluid, organelles, etc. This division of the cytoplasm is called cytokinesis. Mitosis and cytokinesis can be viewed under a microscope.

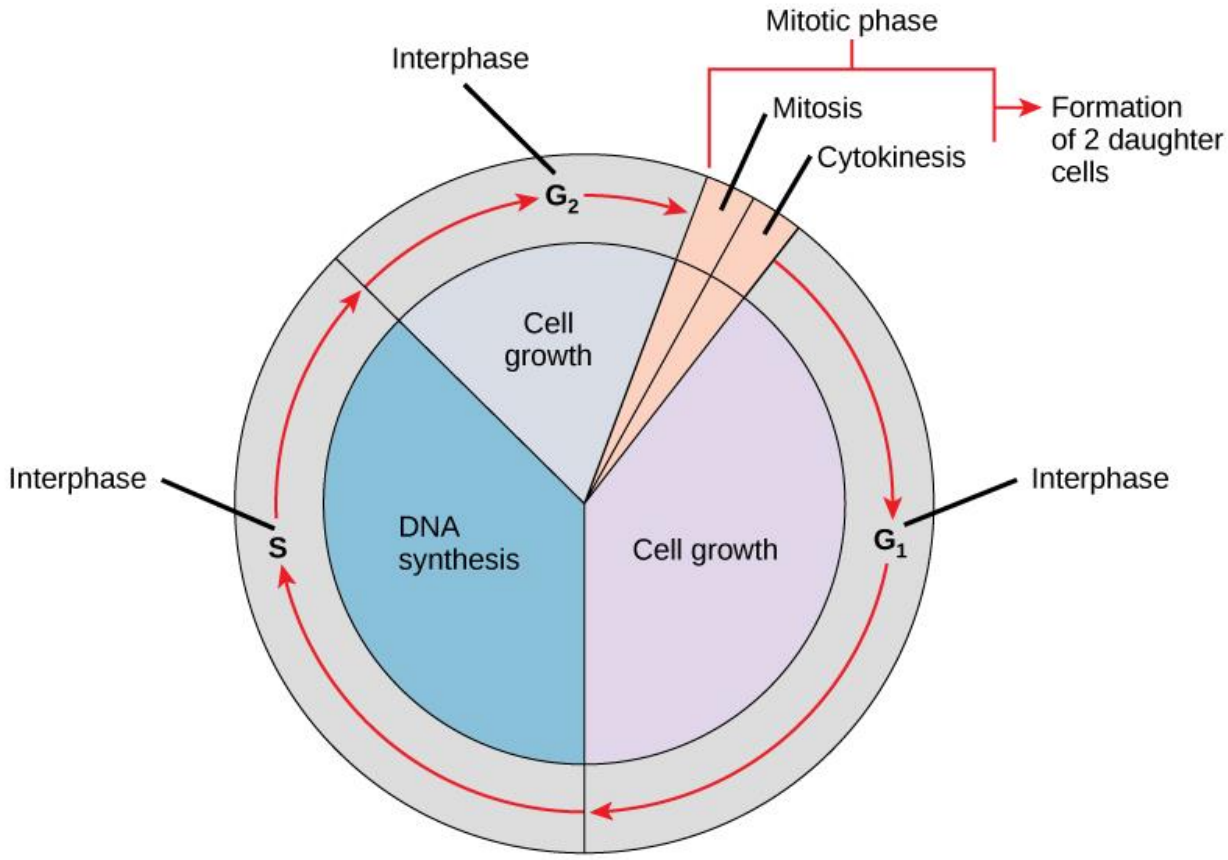


Figure 1- The cell cycle. *Biology. OpenStax College.*

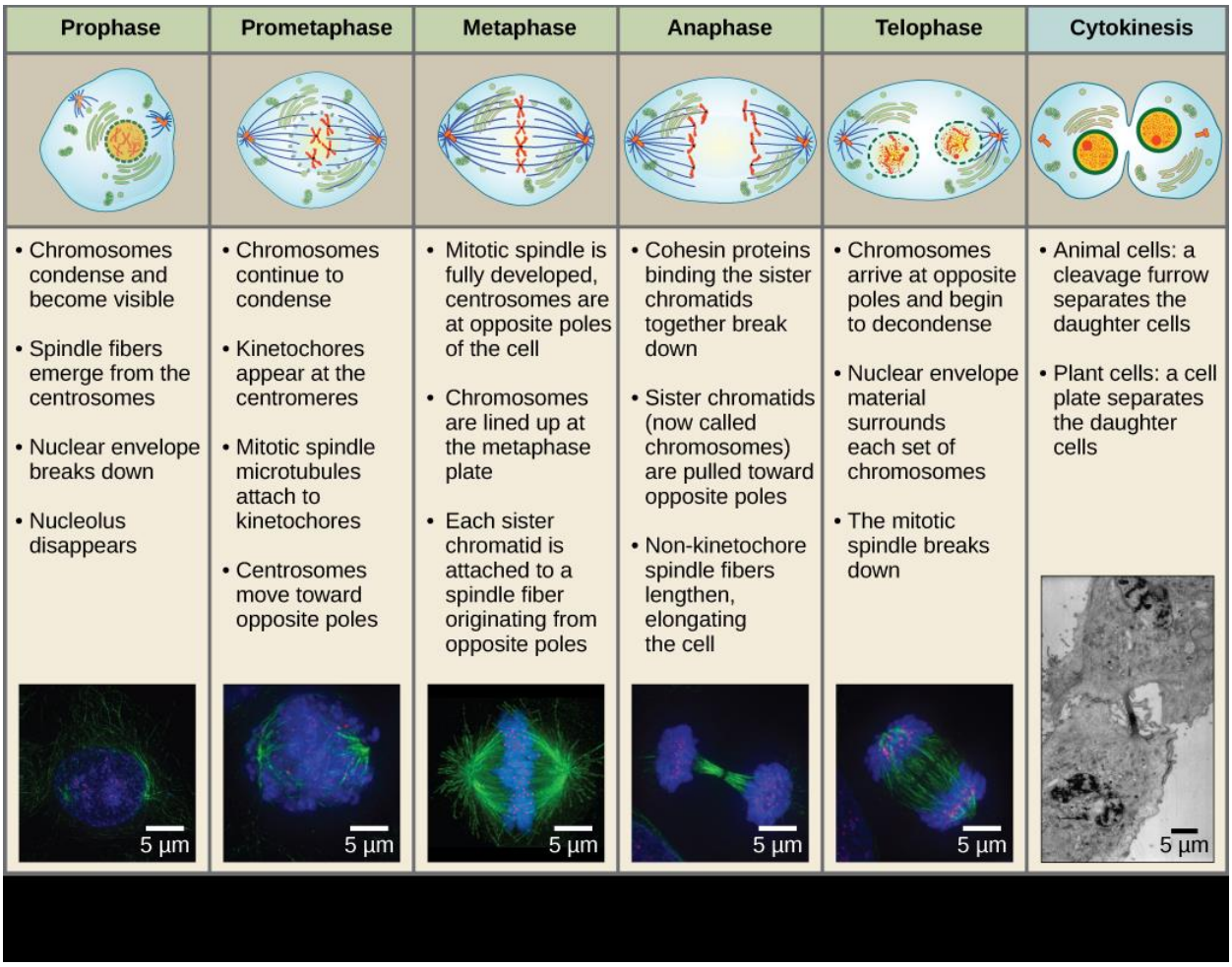


Figure 2- Stages of M phase. *Biology. OpenStax College.*

Exercise 1: Mitosis of onion root tip

(Adapted from Cell Biology Laboratory Manual Online *Dr. William H. Heidcamp, Biology Department, Gustavus Adolphus College, St. Peter, MN 56082 -- cellab@gac.edu*)

Materials

- Prepared slide of onion (allium) root tip
- Microscope

Procedure

1. Obtain a slide of allium root tip for observation of the stages of mitosis in a plant cell.
2. Examine the slide under a microscope.
3. Draw and label all stages of mitosis below. **Figure 3** can be used for help with this.

Interphase

Prophase

Metaphase

Anaphase

Telophase and Cytokinesis

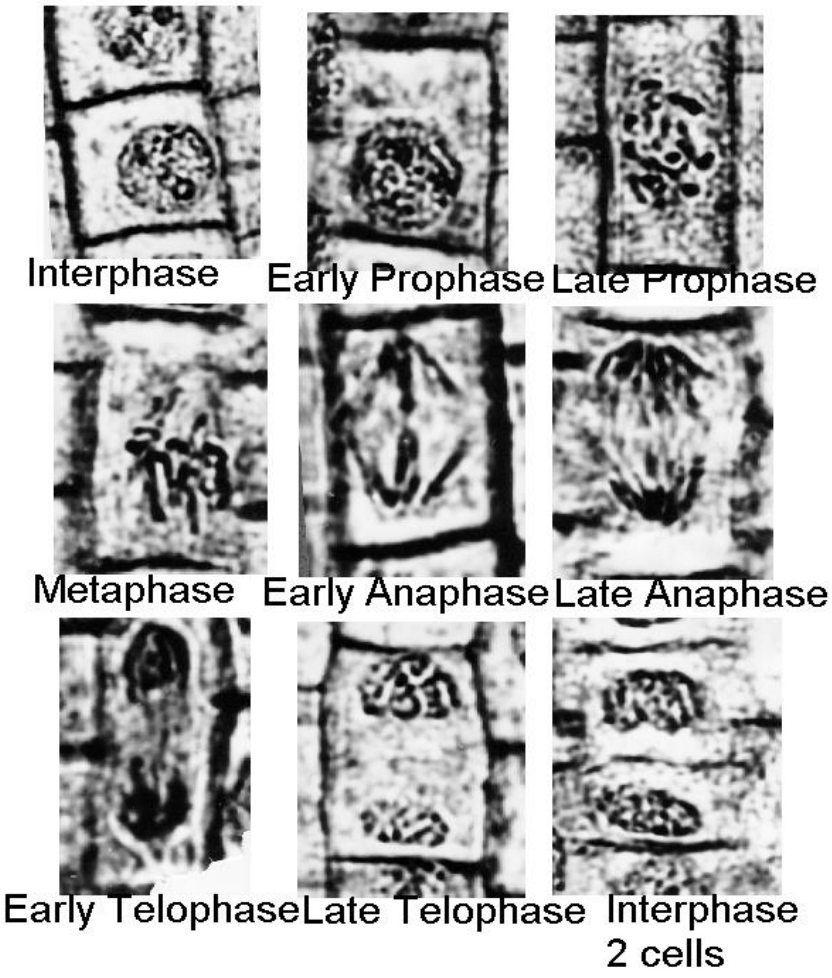


Figure 3- Onion root tip mitosis. *Dr. William H. Heidcamp, Biology Department, Gustavus Adolphus College, St. Peter, MN 56082 -- cellab@gac.edu*).

Exercise 2: Mitosis of whitefish blastula

(Adapted from Cell Biology Laboratory Manual Online *Dr. William H. Heidcamp, Biology Department, Gustavus Adolphus College, St. Peter, MN 56082 -- cellab@gac.edu*)

Materials

- Prepared slide of whitefish blastula
- Microscope

Procedure

1. Obtain a slide of a whitefish blastula for observation of the stages of mitosis in an animal cell. Since early embryogenesis involves rapid cellular division, the whitefish blastula has long served as a model of mitotic division in animals. It also has the advantage of demonstrating clear spindle formation in the cytoplasm.
2. Examine the slide under a microscope.
3. Draw and label all stages of mitosis below.

Interphase

Prophase

Metaphase

Anaphase

Telophase and Cytokinesis

Exercise 3- Simulating mitosis using beads

Materials

- Baggy full of beads and strings

Procedure

1. Organize beads into "chromosomes" as shown in **Figure 4**.
2. Simulate the steps of interphase (specifically S phase) and then M phase using the beads. Hint: The chromosomes in **Figure 4** have not been through S phase yet, so you will eventually need more beads than are shown in **Figure 4**. The strings in the bag are used to simulate spindle fibers.

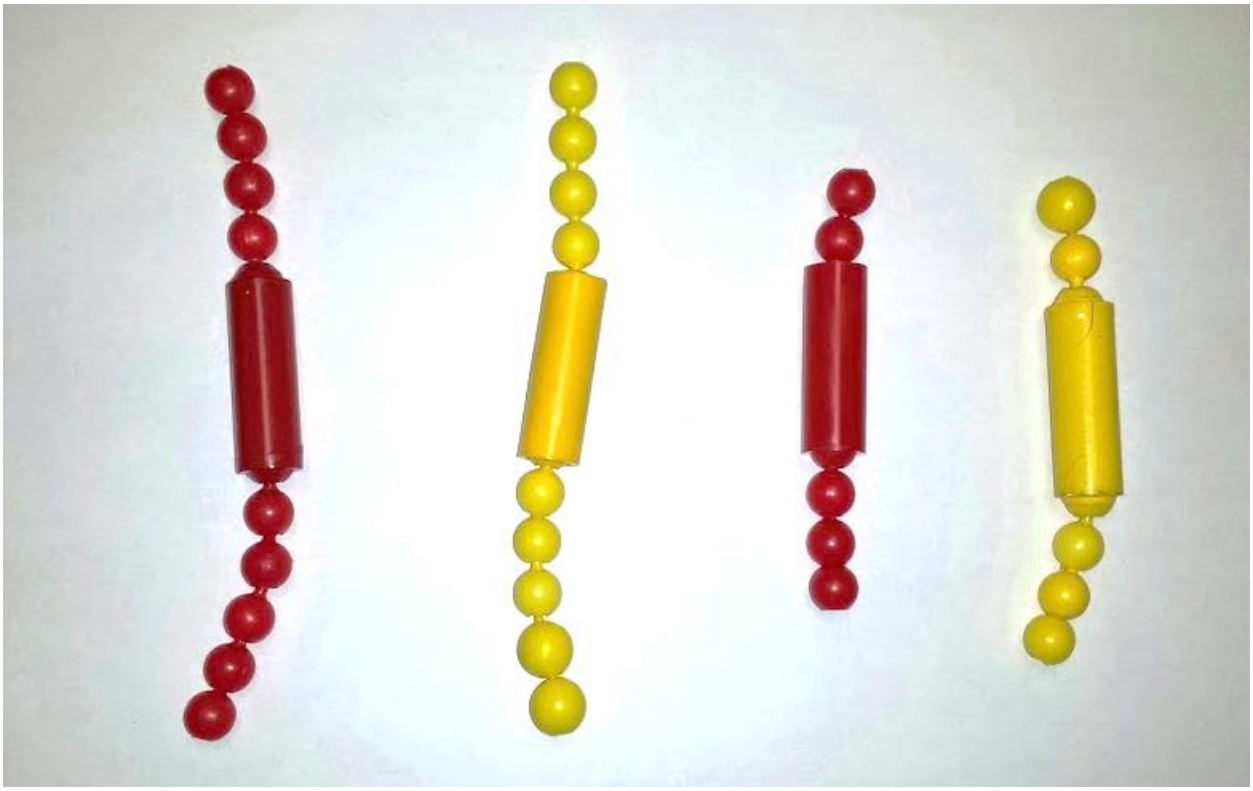
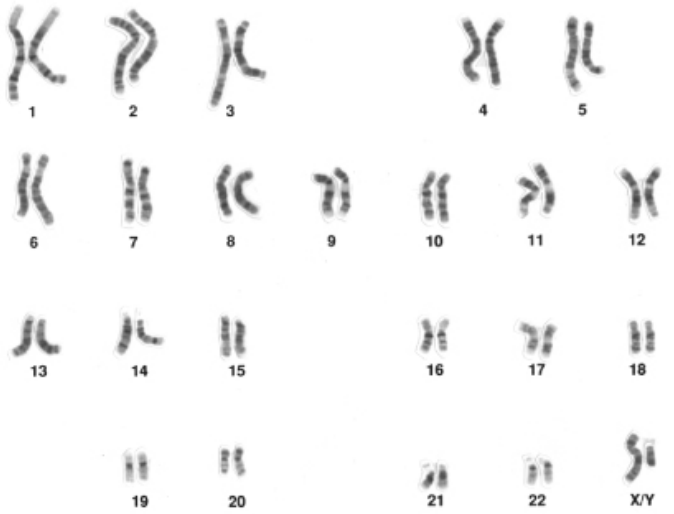


Figure 4- How to set up bead chromosomes to simulate mitosis

Exercise 4: Non-disjunction events during meiosis

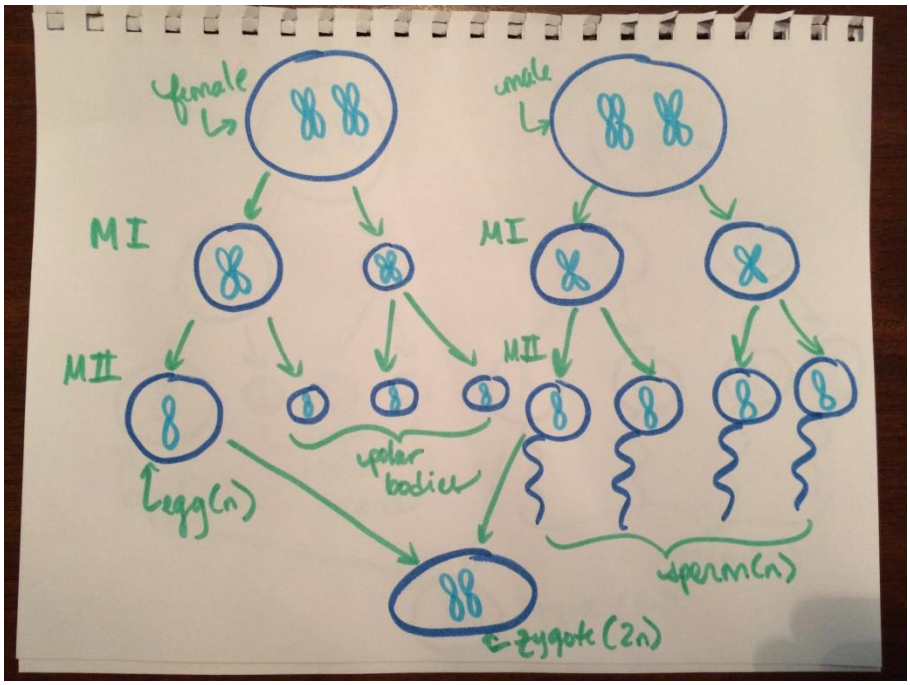
Failure of chromosomes to separate during mitosis or meiosis will result in an incorrect number of chromosomes in daughter cells. This occurrence is known as **non-disjunction**, and it is often triggered by a lapse during a mitotic checkpoint. Should non-disjunction occur during meiosis, the resulting egg or sperm cell will have an incorrect number of chromosomes; if this sex cell is then fertilized, the fetus will have a chromosomal abnormality. The term given for having an incorrect number of chromosomes is **aneuploidy**. A common type of aneuploidy is **trisomy**, which is when there are 3 copies of a particular chromosome instead of 2. Several common chromosomal abnormalities are listed in the table below. The most common trisomy that a human can survive is Down syndrome, which occurs at chromosome 21. To diagnose a chromosomal abnormality, doctors use a map of the chromosomes known as a **karyotype**. Each chromosome pair is laid out side-by-side so it is relatively easy to determine if there are any irregularities. Referring to the karyotype below, it is clear that each chromosome pair is present and of relatively equal length. Note that last chromosome pair (23) is labeled X/Y; these chromosomes are the only 2 that do not exactly match.

Chromosome pair affected	Type	Diagnosis
13	trisomy	Patau Syndrome
18	trisomy	Edwards Syndrome
21	trisomy	Down Syndrome
23 (XO)	monosomy	Turner Syndrome
23 (XXY)	trisomy	Klinefelter syndrome

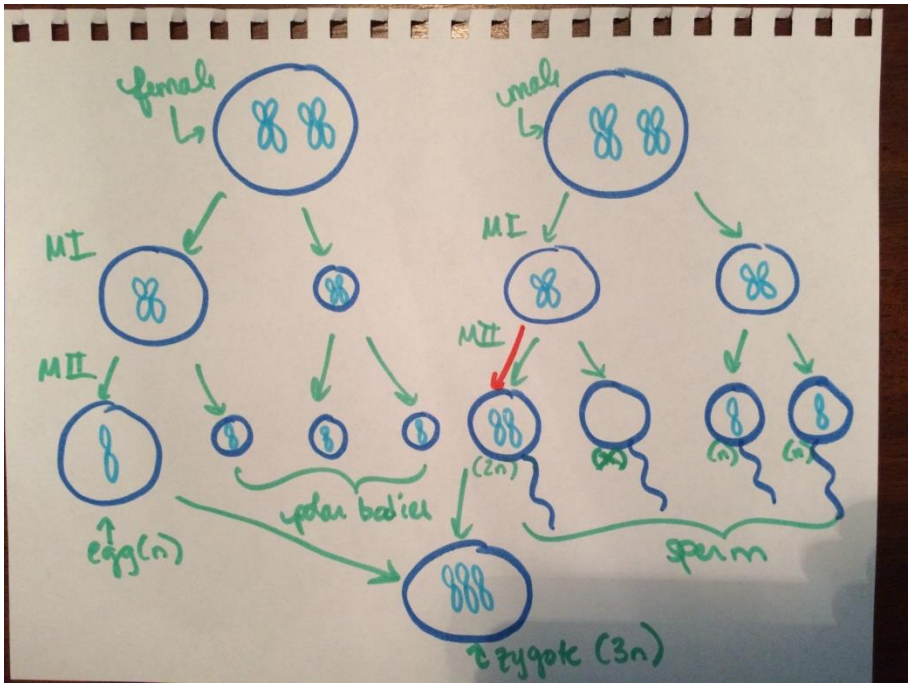


"Human male karyotype" by Courtesy: National Human Genome Research Institute - From w:en:Image:Human male karyotpe.gif, Uploaded by User:Duncharris.. Licensed under Public Domain via Wikimedia Commons

In order for any aneuploidy to occur, there must be an error during meiosis I or II. In the image at right, meiosis occurs without error and the resulting gametes are haploid, leading to a diploid zygote.



In the next image, a non-disjunction event occurs during meiosis II, resulting in trisomy in the zygote.



Questions:

1. Most nerve cells in the adult human central nervous system, as well as heart muscle cells, do not divide. In contrast, cells lining the inside of the small intestine divide frequently. Discuss this difference in terms of why damage to the nervous system and heart muscle cells (think stroke or heart attack) is so dangerous. What do you think might happen to tissues such as the intestinal lining if a disorder blocked mitotic cell division in all cells of the body?

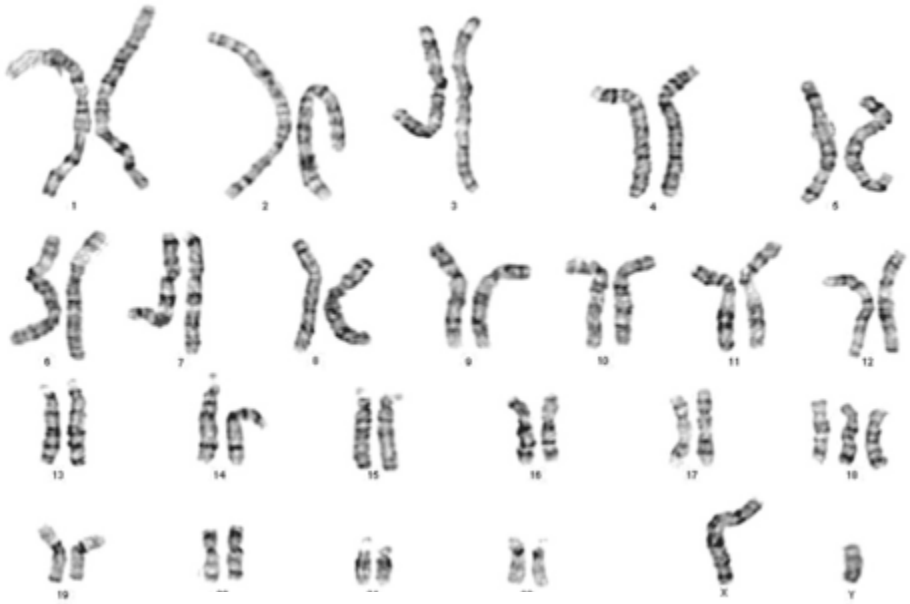
2. How do mitosis and cytokinesis differ?

3. Ultimately, is it the paternal or maternal gamete that determines sex? Explain.

4. In what way are the 23 pairs of human chromosomes “matched” pairs of chromosomes?

5. In order to make a karyotype, cell division is arrested at a point when the chromosomes have condensed and the nuclear envelope has disappeared, but before the sister chromatids separate. Which stage of the cell cycle would be a good point to perform a karyotype?

6. Imagine you are an obstetrician and are performing early genetic testing on a 10 week old fetus. Below is the resulting karyotype. What can you tell about the fetus?



http://genomicdisorders.wikispaces.com/

7. Look up the prognosis for any chromosomal abnormalities you may have detected. What can the parents expect?

8. Use the space below to draw out meiotic divisions that could result in trisomy, assuming that the error occurred during meiosis I.

Inheritance

Introduction
(Adapted from http://www2.le.ac.uk/departments/genetics/vgec/schoolscolleges/topics/inheritancepatterns)

In diploid organisms each body cell (or 'somatic cell') contains two copies of the genome. So each somatic cell contains two copies of each chromosome, and two copies of each gene. The exceptions to this rule are the **sex chromosomes** that determine sex in a given species. For example, in the XY system that is found in most mammals - including human beings - males have one X chromosome and one Y chromosome (XY) and females have two X chromosomes (XX). The paired chromosomes that are not involved in sex determination are called **autosomes**, to distinguish them from the sex chromosomes. Human beings have 46 chromosomes: 22 pairs of autosomes and one pair of sex chromosomes (X and Y).

The different forms of a gene that are found at a specific point (or locus) along a given chromosome are known as **alleles.** Diploid organisms have two alleles for each autosomal gene - one inherited from the mother, one inherited from the father.

Mendelian inheritance patterns

Within a population, there may be a number of alleles for a given gene. Individuals that have two copies of the same allele are referred to as **homozygous** for that allele; individuals that have copies of different alleles are known as **heterozygous** for that allele. The inheritance patterns observed will depend on whether the allele is found on an autosomal chromosome or a sex chromosome, and on whether the allele is **dominant** or **recessive**.

If the phenotype associated with a given version of a gene is observed when an individual has only one copy, the allele is said to be autosomal dominant. The phenotype will be observed whether the individual has one copy of the allele (is heterozygous) or has two copies of the allele (is homozygous).

If the phenotype associated with a given version of a gene is observed only when an individual has two copies, the allele is said to be autosomal recessive. The phenotype will be observed only when the individual is homozygous for the allele concerned. An individual with only one copy of the allele will not show the phenotype, but will be able to pass the allele on to subsequent generations. As a result, an individual heterozygous for an autosomal recessive allele is known as a **carrier**.

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

* It doesn't matter how often you flipped a coin or how many times it's already shown heads, the probability is ALWAYS 50% of heads/tails. For example, if I've tossed a coin 4 times and all four times it came up heads. What is the probability that my next toss will be heads?

Part 1: Simulating a monohybrid cross

(Adapted from http://extension.uga.edu/k12/science-behind-our-food/lesson-plans/MonohybridCrossesPunnettSquare.pdf
Science Behind Our Food, the National Science Foundation and the University of Georgia)

Procedure

1. Each group will pick up 2 paper bags filled with 15 red (R) beans and 15 white (r) beans. This represents 2 heterozygous parents (Rr x Rr).
2. At the same time, each student will reach into their bag and pull out one of the beans. The only possibilities that can be made from this selection are: RR (homozygous red), Rr (heterozygous red), or rr (homozygous white). Mark the resulting genotype and phenotype in the data table.
3. Return the beans back into the bag and conduct the same process 14 more times (15 total trials).

Data Table

Trial	Offspring Genotype	Offspring Phenotype

Questions:

1. What is the dominant trait?
    a. How do you know it is dominant?
2. What is the recessive trait?
3. What are the genotypes of the parents?
4. What are the phenotypes of the parents?
5. Fill in the Punnett Square below using the parents given in the procedure:

Male _____ x Female _____

6. What is the genotypic ratio?

7. What is the phenotypic ratio?

Part 2: Dihybrid Crosses- Crosses that involve 2 traits.

(Adapted from http://www.biologycorner.com/bio2/genetics/notes_dihybrid.html)

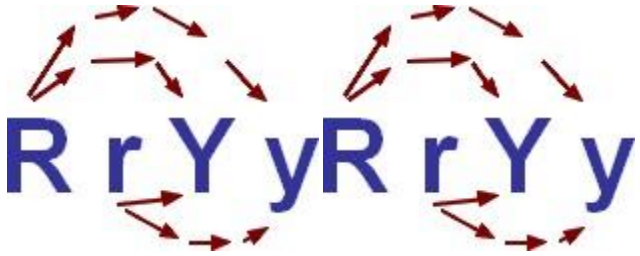
These can be challenging to set up, and the square you create will be 4x4. This simple guide will walk you through the steps of solving a typical dihybrid cross common in genetics. The method can also work for any cross that involves two traits.

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

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

RrYy x RrYy

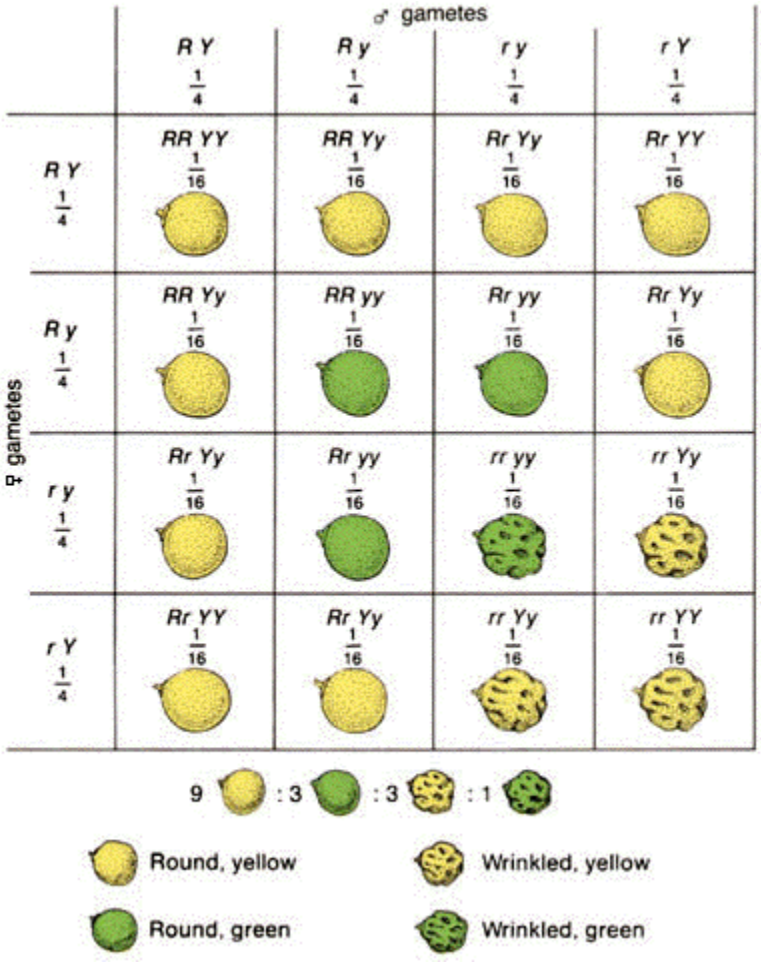
Step 2: Determine the gametes. This might feel a little like the FOIL method you learned in math class. Combine the R's and Ys of each parent to represent sperm and egg. Do this for both parents:



Gametes after "FOIL"
RY, Ry, rY, ry (parent 1) and **RY, Ry, rY, ry** (parent 2)

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

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



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

Another way to determine the ratios is to do it mathematically
3/4 of all the offspring will have round seeds
3/4 of all the offspring will have yellow seeds
3/4 x 3/4 = 9/16 will have round, yellow seeds.

Crosses that Involve 2 Traits
Consider: RrYy x rryy
The square is set up as shown

You might notice that all four rows have the same genotype. In this case, you really only need to fill out the top row, because 1/4 is the same thing as 4/16

	RY	Ry	rY	ry
ry	RrYy round, yellow	Rryy round, green	rrYy wrinkled, yellow	rryy wrinkled, green
ry	RrYy	Rryy	rrYy	rryy
ry	RrYy	Rryy	rrYy	rryy
ry	RrYy	Rryy	rrYy	rryy

1/4 round, yellow
1/4 round, green
1/4 wrinkled, yellow
1/4 wrinkled, green

Additional problems:

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

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

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

1. What are the phenotypes of rabbits that have the following genotypes:

   Ggbb _____ ggBB _____
   ggbb _____ GgBb _____

2. A male rabbit with the genotype GGbb is crossed with a female rabbit with the genotype ggBb The square is set up below. Fill it out and determine the phenotypes and proportions in the offspring.

	Gb	Gb	Gb	Gb
gB				
gB				
gb				
gb				

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

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

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

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

3. A male rabbit has the genotype GgBb. Determine the gametes produced by this rabbit (the sperm would have these combinations of alleles).

4. Use the gametes from #3 to set up a punnet square below. Put the male's gametes on the top and the female's gametes down the side. Then fill out the square and determine what kind of offspring would be produced from this cross and in what proportion.

5. An aquatic arthropod called a Cyclops has antennae that are either smooth or barbed. The allele for barbs is dominant. In the same organism, resistance to pesticides is a recessive trait. Make a "key" to show all the possible genotypes (and phenotypes) of this organism. Use the rabbit key to help you if you're lost.

6. A Cyclops that is resistant to pesticides and has smooth antennae is crossed with one that is heterozygous for both traits. What are the genotypes of the parents?

7. Set up a punnet square for the cross and show the phenotypic ratios.

Part 3: Sex-linked or X-linked inheritance

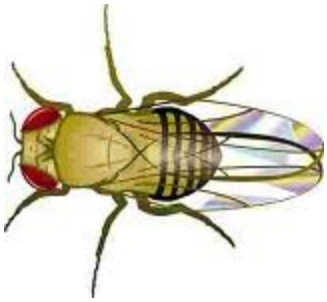
(Adapted from http://www.biologycorner.com/worksheets/genetics_xlinked.html)

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

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

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

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



1. In fruit flies, eye color is a sex linked trait. Red is dominant to white. What are the sexes and eye colors of flies with the following genotypes?

   X^RX^r _____ X^RY _____ X^rX^r _____

   X^RX^R _____ X^rY _____

2. What are the genotypes of these flies:

   white eyed, male _____ red eyed female (heterozygous) _____

   white eyed, female _____ red eyed, male _____

3. Show the cross of a white eyed female X^rX^r with a red-eyed male X^RY.

4. Show a cross between a pure red eyed female and a white eyed male. What are the genotypes of the parents?

   How many of the offspring are:

   white eyed, male ____
   white eyed, female ____
   red eyed, male ____
   red eyed, female ____

5. Show the cross of a red eyed female (heterozygous) and a red eyed male.

   What are the genotypes of the parents?

   How many of the offspring are:

   white eyed, male ____
   white eyed, female ____
   red eyed, male ____
   red eyed, female ____

6. In humans, hemophilia is a sex linked trait. Females can be normal, carriers, or have the disease. Males will either have the disease or not (but they won't ever be carriers)

   $X^H X^H$ = female, non-hemophilic
   $X^H X^h$ = female, carrier
   $X^h X^h$ = female, hemophiliac
   $X^H Y$ = male, non-hemophilic
   $X^h Y$= male, hemophiliac

   Show the cross of a man who has hemophilia with a woman who is a carrier.

   What is the probability that their children will have the disease? _____

7. A woman who is a carrier marries a non-hemophilic man. Show the cross. What is the probability that their children will have hemophilia? What sex will a child in the family with hemophilia be?

8. A woman who has hemophilia marries a non-hemophilic man. How many of their children will have hemophilia, and what is their sex?

9. In cats, the gene for calico (multicolored) cats is both **sex-linked** and **codominant.** Due to a phenomenon known as **dosage compensation**, females that receive a **B** and an **R** gene have black and orange splotches on white coats. Males can only be black or orange, but never calico.

   What would a calico cat's genotype be?

   Show the cross of a female calico cat with a black male.

   What percentage of the kittens will be black and male? _____
   What percentage of the kittens will be calico and male? _____
   What percentage of the kittens will be calico and female? _____

10. Show the cross of a female black cat with a male orange cat.

    What percentage of the kittens will be calico and female?
    What color will all the male cats be?

Part 4: Incomplete Dominance and Codominance

Some heterozygotes express a phenotype that is intermediate between the dominant and recessive phenotype. For example, in 4 o'clock flowers the gene for red pigmentation is dominant and the gene for white pigmentation is recessive. However, heterozygotes are pink. The dominant allele does not completely mask expression of the recessive allele: it is **incompletely dominant.**

1. By observing flower color in 4 o'clock flowers, is it possible to unambiguously determine the genotype? YES/NO

   Is the same true for flower color in snow peas? YES/NO
   Why or why not?

Another inheritance pattern of note is that of **codominance**. Here, both alleles for the same characteristic can be expressed. A common example of this is human A B O blood type. The alleles for the surface antigens are both dominant; the allele for no surface antigen is recessive.

father \ mother	A	B	O
A	AA	AB	AO
B	BA	BB	BO
O	OA	OB	OO

alleles	blood type
A+A	A
A+O	A
A+B	AB
B+B	B
B+O	B
O+O	O

Image via Genetic Science Learning Center, University of Utah, http://learn.genetics.utah.edu

2. If two individuals with blood type AB marry, what are the odds that each of their children will have the AB blood type?

3. A disputed paternity case! Hermione's new baby has a blood type of O. Her blood type is B and Ron Weasley's is A. Harry Potter (blood type AB) insists he is the child's father. CAN THIS BE TRUE???!

DNA Puzzle Pieces Lab

(Adapted from http://www.biologycorner.com/)

Part 1: DNA Structure

In 1953, James Watson and Francis Crick established the structure of **deoxyribonucleic acid (DNA)**. The structure is a double helix, which is like a twisted ladder. The sides of the ladder are made of alternating sugar (deoxyribose) and phosphate molecules.

The rungs of the ladder are pairs of 4 types of nitrogen bases. Two of the bases are **purines** - adenine and guanine. The **pyrimidines** are thymine and cytosine. The bases are known by their coded letters A, G, T, C. These bases always bond in a certain way: adenine will only bond to thymine and guanine will only bond with cytosine. This is known as the base-pair rule. The bases can occur in any order along a strand of DNA. The order of these bases is unique and codes for specific genes.

The combination of a single base, a deoxyribose sugar, and a phosphate make up a **nucleotide**. DNA is actually a molecule of repeating nucleotides.

The two strands of DNA are held together loosely by hydrogen bonds. The strands are also **antiparallel**, meaning that they run parallel but in opposite directions. One strand will go in the 5' → 3' direction and one will go 3' → 5'. The 5' end has a phosphate group attached and the 3' end does not.

Materials:

- DNA Puzzle Kits

**Before beginning this lab, please confirm that your kit contains the correct materials listed in the table below.

Number	Component	Color
24	Phosphate	Yellow
24	Deoxyribose (sugar)	Red
12	Ribose (sugar)	Pale Pink
4	Adenine (base)	Light Green
4	Thymine (base)	Light Blue
8	Guanine (base)	Dark Blue
8	Cytosine (base)	Dark Green
2	Uracil (base)	White
2	Alanine (Amino Acid)	Tan
2	Glycine (Amino Acid)	Brown
2	Alanine activating enzyme	Tan
2	Glycine activating enzyme	Brown
2	Alanine-specific tRNA	Tan
2	Glycine-specific tRNA	Brown
1	Ribosome sheet	White worksheet

Procedure:

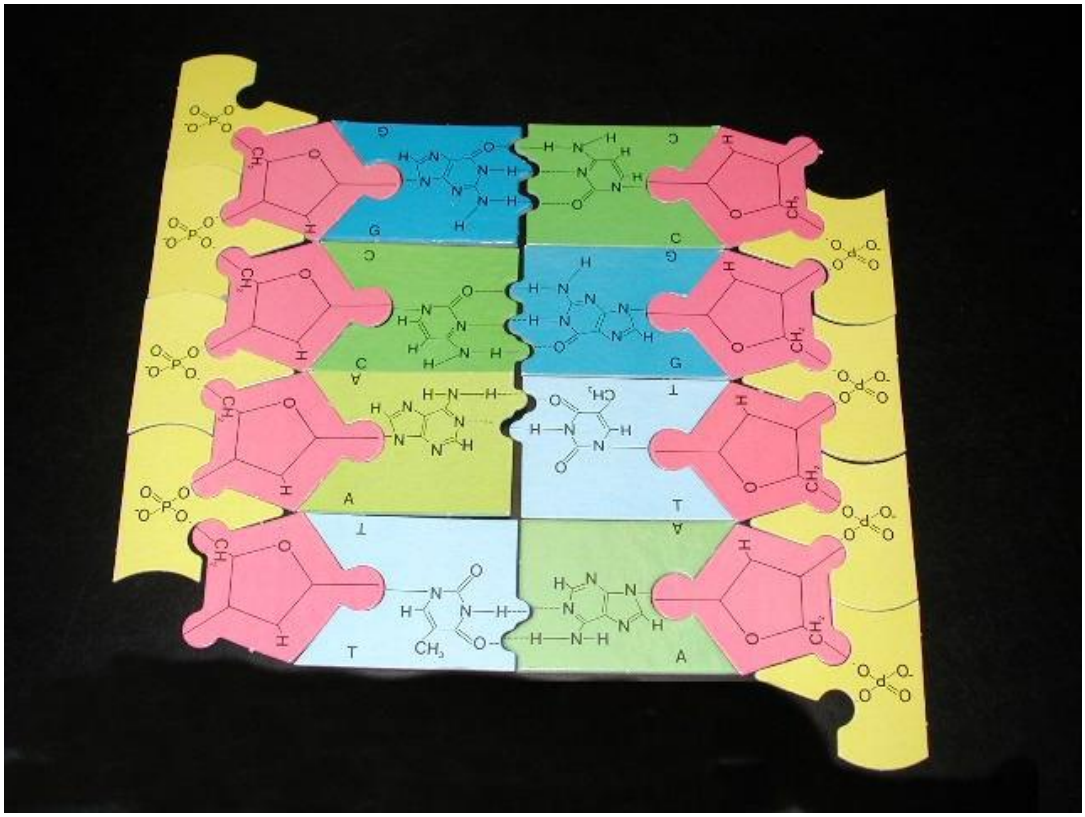
1. Using the materials in your kit, please assemble the longest complete DNA segment you can. In other words, all of the deoxyriboses should be used up! (You may use the photo below as a reference.)

   Keep these factors in mind:
   a. DNA is a double stranded molecule
   b. The double helix is antiparallel
   c. DNA includes deoxyribose and thymine
   d. Base-pairing rules apply

2. Once you have assembled your molecule, record it in the space below:

5’ 3’

3’ 5’

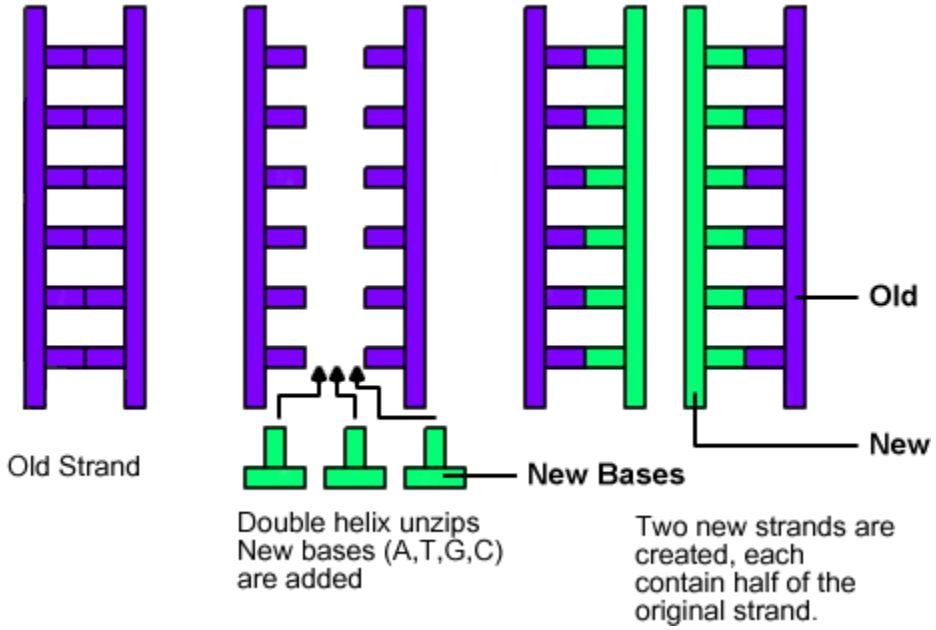


3. Disassemble your DNA molecule.

Part 2: DNA Replication

Replication is the process where DNA makes a copy of itself. Cells divide for an organism to grow or reproduce; every new cell needs a copy of the DNA or instructions to know how to be a cell. DNA replicates right before a cell divides.

DNA replication is **semi-conservative**. That means that when it makes a copy, one half of the old strand is always kept in the new strand. This helps reduce the number of copy errors.



There are a few enzymes involved in DNA replication: **RNA primase** adds a short segment of RNA to start the new strand. **DNA helicase** opens up the double helix by breaking hydrogen bonds that hold complementary strands together. A **DNA polymerase** adds the new nucleotides onto the 3' end of the growing strand. **DNA ligase** connects Okazaki fragments on the lagging strand into a continuous molecule.

For simplicity's sake, an RNA primer will not be used during this activity and the action of the lagging strand will not be modeled.

1. Assemble a new DNA molecule with the following sequence: 5' GCAT 3'
   3' CGTA 5'

   Make sure that your molecule is laid out on the bench exactly as written!!

2. Use your hands to mimic the action of DNA helicase (separate the two strands)

3. Build daughter strands using the original molecule as a template.

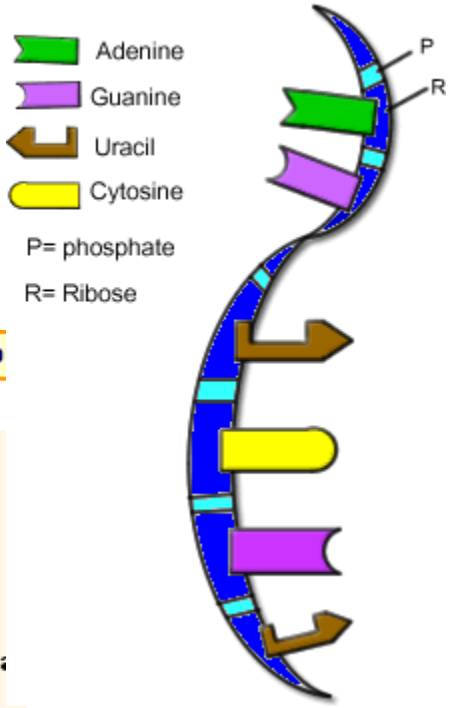
4. Disassemble your DNA molecules

Part 3: Transcription

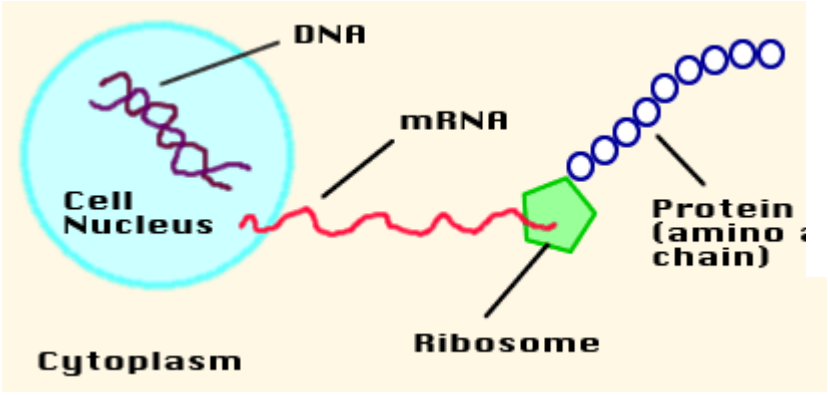
DNA remains in the nucleus, but in order for it to get its instructions translated into proteins, it must send its message to the ribosomes, where proteins are synthesized. The chemical used to carry this message is **Messenger RNA (mRNA)**.

RNA = ribonucleic acid (see figure at right)
RNA is similar to DNA except:

- RNA is usually single-stranded
- uracil replaces thymine
- ribose replaces deoxyribose



During **transcription**, RNA is made from DNA and proteins are made from the message on the RNA via **translation**



Transcription is similar to DNA replication in that the original DNA molecule is used as a template. **RNA polymerase** is an enzyme that is able to open up the double helix and add nucleotides onto the new strand of RNA.

1. Assemble half of a new DNA molecule with the following sequence: 3' CGTCCACGT 5'

   Make sure that your molecule is laid out on the bench exactly as written!!

   (We only need one strand as the template since mRNA is single-stranded)

2. Using this as your template, make an RNA copy of this molecule.
   Remember:
   a. RNA uses ribose
   b. RNA uses uracil

3. Record your mRNA molecule: 5' 3'

4. Leave the complete mRNA molecule alone, it will be used in the next section.

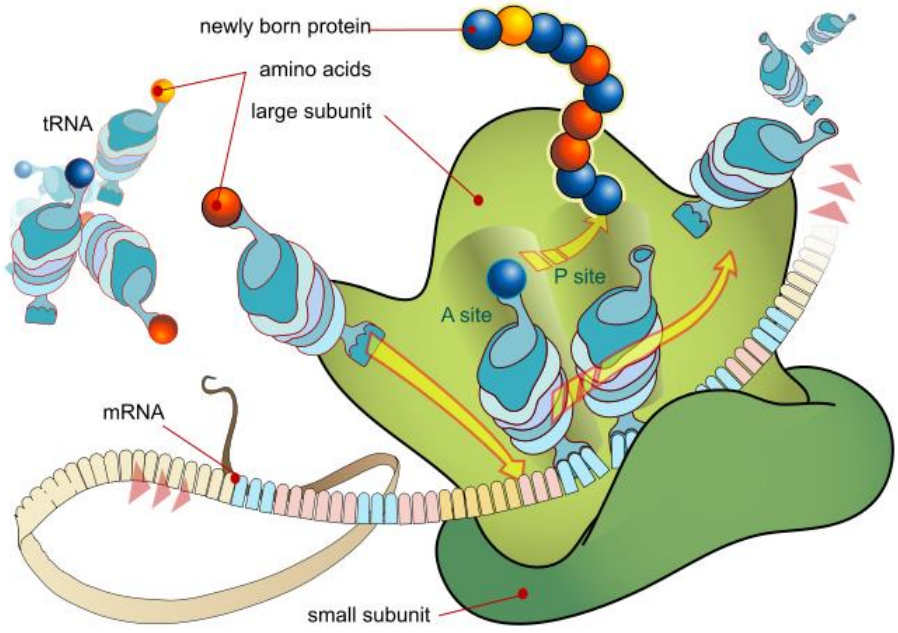
Part 4: Translation

Translation occurs in the cytoplasm, specifically on the ribosomes. The mRNA made in the nucleus travels out to the ribosome to carry the message of the DNA. Here at the ribosome, that message will be translated into an amino acid sequence. The RNA strand threads through the ribsosome like a tape measure and the amino acids are assembled.

Important to the process of translation is another type of RNA called Transfer RNA (tRNA) which carries the amino acids to the site of protein synthesis on the ribosome.

A tRNA molecule has two important areas: the anticodon and the amino acid. The anticodon matches the codon on the RNA strand. Codons are sets of three bases that code for a single amino acid.

Connected to the top of the tRNA molecule is the amino acid. There are twenty amino acids that can combine together to form proteins of all kinds, these are the proteins that are used in life processes. Each tRNA has different amino acids, which are linked together like box cars on a train during the process of translation.
The process of translation: there are 2 tRNA binding sites on the ribosome: P and A. To initiate translation, a tRNA that matches the start codon (AUG) enters the P site. The tRNA that matches the next codon enters into the A site and the amino acid from the tRNA in the P site is attached to the amino acid on the tRNA in the A site. Translocation occurs next: both tRNAs shift over. The tRNA that was in the P site exits the ribosome; the tRNA that was in the A site moves into the P site. Now the A site is available to bind a tRNA that matches the next codon. This process continues until one of the stop codons enters the A site.



1. Place the mRNA molecule from the previous section onto the ribosome sheet.

   For simplicity, we are not going to start at a start codon during this exercise.

2. Align the first codon (5') into the P site of the ribosome and the second codon into the A site.

3. Attach the appropriate tRNA and amino acids.

4. Detach the amino acid from the tRNA in the P site and attach it to the amino acid on the tRNA in the A site.

5. Slide the entire complex (mRNA and attached tRNAs) over and allow the first tRNA to exit.

6. Repeat this process until you reach the end of the mRNA. Record your amino acid sequence below.

Part 5: Coding Practice

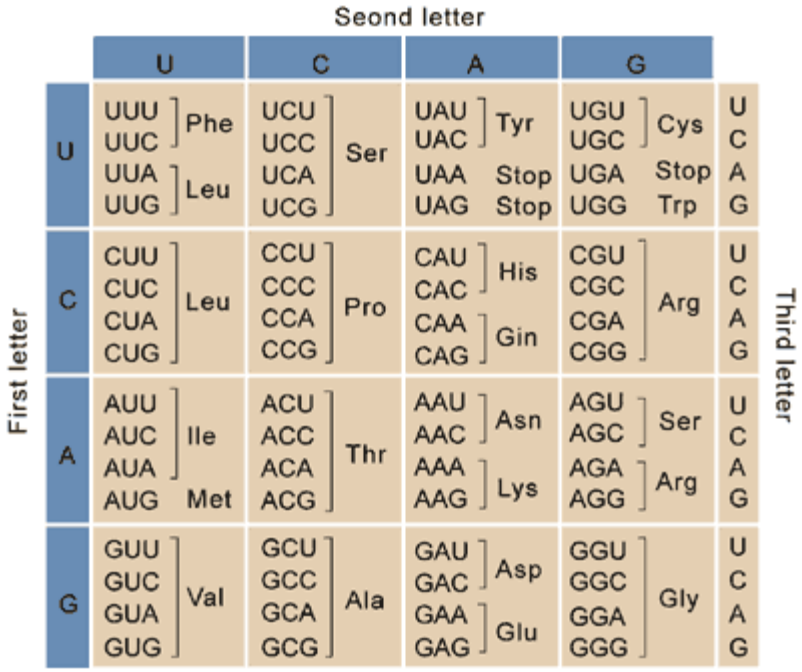
1. Use this sequence of DNA to answer the following former test questions:

   5'-- TTAATGGGACAGCTTGTGTAGAGG --3'

   a. What is the complementary strand of DNA?

   b. Using the complementary strand of DNA (your answer from part a) as the template strand, what is the transcribed mRNA sequence?

   c. What is the amino acid sequence translated from the strand of mRNA synthesized in part b (use the genetic code below)?
   Remember:
      i. Start codon!
      ii. Stop codon!



Additional Images from:
http://www.descrittiva.it/calip/dna/dna03.jpg
http://history.nih.gov/exhibits/nirenberg/HS5_cracked.htm.
https://commons.wikimedia.org/wiki/File:Ribosome_mRNA_translation_en.svg#/media/File:Ribosome_mRNA_translation_en.svg

Biotechnology Lab

Dr. Leah Howell, Dalton State College

Introduction

(Adapted from The Biology Place. Pearson Molecular Biology Lab Bench.)

Gel electrophoresis is a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field. The direction of movement is affected by the charge of the molecules, and the rate of movement is affected by their size. Positively charged molecules will move through the gel toward the negative electrode (black), and negatively charged molecules will move toward the positive electrode (red). Large molecules move slower than small molecules.

DNA is a negatively charged molecule, so DNA molecules will move toward the positive electrode side of the gel when a current is applied. Often times, before DNA is run on a gel, it has first been cut with restriction enzymes. These are enzymes that cut DNA at specific nucleotide sequences. When DNA has been cut by restriction enzymes, the different-sized fragments will migrate at different rates through the gel. DNA that has been exposed to restriction enzymes and then run on a gel will produce multiple bands on the gel. Each band contains DNA of a specific base pair size.

When forensic scientists try to determine the source of DNA found at a crime scene, they can cut the crime scene DNA and DNA from any suspects with the same restriction enzyme. These DNA samples can then be run side by side on a gel and compared to determine if the banding patterns are the same- this is commonly referred to as DNA fingerprinting. Today's lab illustrates the process of gel electrophoresis with dyes instead of with DNA. Molecules of different dyes are different sizes and charges, so they should move different directions and at different rates.

Exercise 1: Gel electrophoresis

Materials

- Gel chamber
- Power supply
- Dyes
- Pre-poured agarose gel
- Pipetman
- Disposable tips
- Running buffer

Procedure:

1. Gently remove the comb and the end caps from the gel. Be careful to avoid damaging the wells.
2. Using the pipetman, add 10 µL of each dye sample to each corresponding lane as listed below. Use a new tip for each sample.

Lane 1	Bromophenol blue
Lane 2	Xylene cyanol
Lane 3	Methyl orange
Lane 4	Ponceau G
Lane 5	Explorer I
Lane 6	Explorer II

3. Place the gel in the center of the gel chamber
4. Add buffer to one end of the chamber until the liquid is almost even with the top of the gel.
5. Add buffer to the other side of the chamber. Keep filling until gel is completely submerged.
   - Be careful to avoid washing the samples out of the wells.
6. Put lid on chamber. Be sure outside surface is dry.
7. Plug cords into power supply. Be sure that red goes to red, and black to black.
   - *Check with me before turning on power supply*.
8. Turn on power supply.
   - Look for tiny bubbles coming off the electrodes.
9. Notice how the dye migrates as the current runs through the gel.
10. Run until the first dye nears the bottom of the gel, then turn off the power supply and disconnect the cords.
11. Examine the results to answer the questions.

Questions:

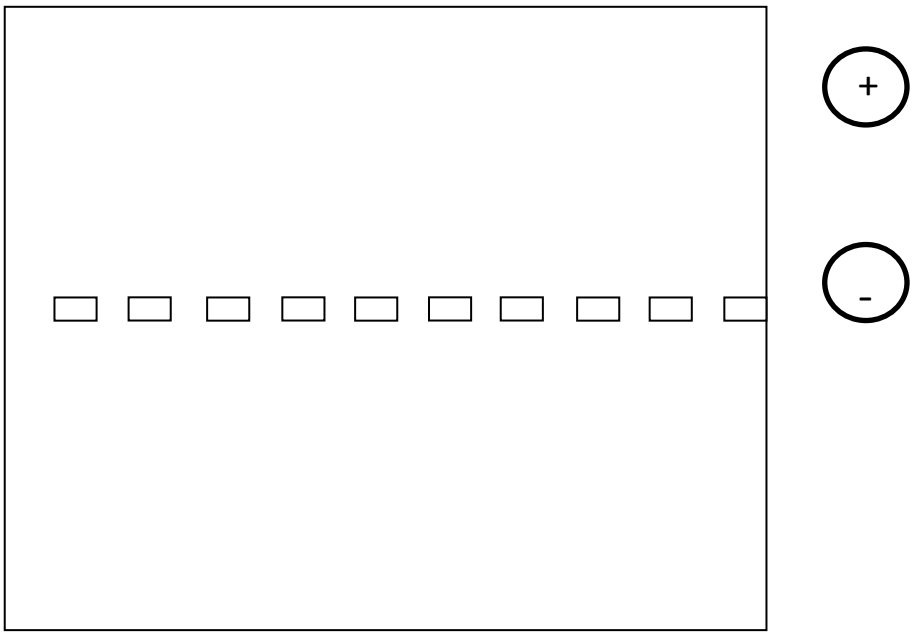
1. What is the purpose of electrophoresis?

2. What purpose does the buffer serve?

3. Why do dye molecules migrate toward the anode (positive electrode)?

4. Why do smaller molecules migrate faster than larger molecules?

Draw your finished gel in the space below. Be sure to label which band is which dye on you figure.



6. Which dye molecule is the smallest? Which is the largest? List dyes by name, not appearance.

7. Which dyes are in the Explorer I mix? List dyes by name, not appearance.

8. Which dyes are in the Explorer II mix? List dyes by name, not appearance.

Exercise 2: Who Ate the Cheese?!

(Adapted from: http://www.biologycorner.com/worksheets/who_ate_the_cheese.html)

Objectives: In this simulation you will examine crime scene evidence to determine who is responsible for eating the Queen's special imported Lindbergher Cheese. You will model the process of electrophoresis and DNA fingerprinting.

ROYAL GUARD INCIDENT REPORT

Incident Data

Incident Type:	Theft	Complaint Status	Pending DNA results
Processed by:	Chief Wiggam	Other Officers:	Officer Li Gase

Property

Property Code:	Rare cheese	Owner's Name	Queen Elizabeth
Name:	Lindbergher	Value:	$12,000

Burglary Data

Method of Entry: Unknown, no evidence of force on doors or windows.

Narrative: The cheese was allegedly stolen from the Queen's sitting room the night before the grand ball. The cheese was listed as a gift from the Manchurian diplomat. Officer Li Gase dusted for fingerprints and found none on the table or doors, the maid claimed that they had been wiped clean earlier. The wheel of cheese was on a platform in the sitting room, and half of it had been eaten. We took pictures of the half eaten cheese and sent it to the lab for further tests. Edna N. Zime, the lab technician said that saliva samples could be taken from the teeth imprints of the cheese that was left behind.

Suspect Data

Suspect Number: 1
Name: Princess Dubbah Elix
Description of Suspicion: The princess was seen entering the sitting room earlier in the evening. She is well known for her love of cheese.

Suspect Number 2
Name: Electra Foresis
Description of Suspicion: Electra was recently involved in a relationship with the Manchurian diplomat that sources say ended badly. Her motive may have been to sabotage the diplomat's gift to the Queen.

Suspect Number 3
Name: Ada Nine
Description of Suspicion: Ada was the maid in charge of cleaning the sitting room. She had access to the cheese.

Suspect Number 4

Name: Gene Tics
Description of Suspicion: Gene is the leader of the local Cheese-Makers Guild, he may not have wished for Queen Elizabeth to have cheese from anywhere but his own guild.

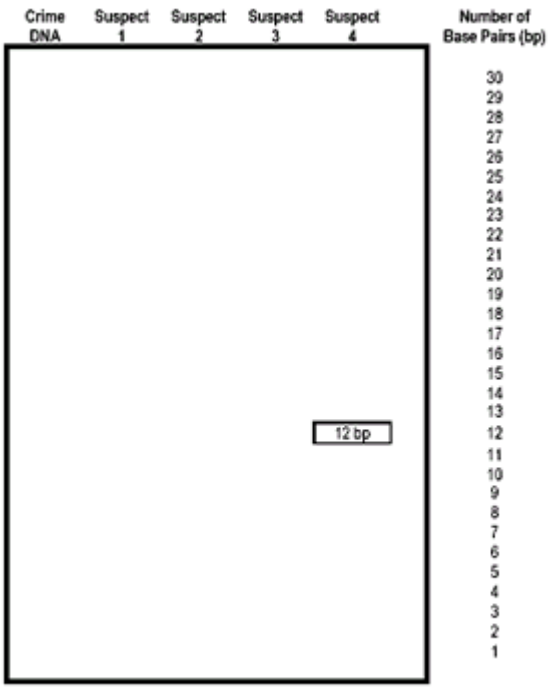
Crime Lab Data

Crime Lab Investigator	R. Renee	Lab Technician	Edna N. Zime
List of Evidence Received	Plastic bag with cheese crumbs	List of Procedures Used	DNA extraction Polymerase Chain Reaction DNA restriction Analysis

Narrative: After receiving the package with the plastic bag marked Crime Scene, the DNA was extracted. Because the sample was so small, the DNA was amplified using the polymerase chain reaction. We isolated the DNA from the four suspects and compared them to the crime scene DNA using DNA restriction analysis.

Results: See attached DNA Results

DNA Evidence Evaluation



1. On the following page you will find several DNA sequences. Cut each out and turn the resulting strips right side up. The restriction enzyme cuts at every point it finds **C C G G**, always cutting between the C and the G. Use scissors to cut the DNA sequence at the C C G G points. Label the back of the slips with the suspect number so that you don't get them confused after cutting.

2. Count the number of base pairs (bp) in each piece of DNA that you created. Record the base pair number on the back side of the DNA fragment.

3. Make an enlarged chart like the one shown at left. Use a ruler to ensure that the lengths are uniform.

4. Tape your DNA fragments to the chart, using the base pair numbers as a guideline for fragment placement.

5. Compare the crime scene DNA to the suspects and indicate on your chart which suspect is guilty of eating the cheese.

Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA

GTCGACCGGTGACCGTGCGTACACAGTGCTCCGGATAGCTGATAGCTCCGGTG

CAGCTGGCCACTGGCACGCATGTGTCACGAGGCCTATCGACTATCGAGGCCAC

Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA

GTCCCAGCCGGACCGTACCGGTAGATCAGCCGGTAGATTGATAGCGTGATGTG

CAGGGTCGGCCTGGCATGGCCATCTAGTCGGCCATCTAACTATCGCACTACAC

Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA

GTCTACGTAATCGTAGCCATCCGGACAGTGTGCACGATCGTACATGCTACGTG

CAGATGCATTAGCATCGGTAGGCCTGTCACACGTGCTAGCATGTACGATGCAC

Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA

GTCGACCGGTGACCGTGCGTACACAGTGCTCCGGATAGCTGATAGCTCCGGTG

CAGCTGGCCACTGGCACGCATGTGTCACGAGGCCTATCGACTATCGAGGCCAC

Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA

GTCTCCATCCGGACTACCATACATCTGGTGTACCCGGTGATATCGTCCGGGTG

CAGAGGTAGGCCTGATGGTATGTAGACCACATGGGCCACTATAGCAGGCCCAC

6. For each of the following tasks performed in the activity, describe what they are actually simulating.

Cutting the DNA into fragments: _____
Taping the DNA onto the large paper: _____

7. Describe how each technique below relates to DNA Fingerprinting:

Polymerase Chain Reaction
Gel Electrophoresis
Restriction Enzyme