LABORATORY MANUAL

Foundations of Biology – BIOL1010K

LABORATORY SAFETY PROTOCOLS

You are expected to read the appropriate sections of this manual before coming to lab. You are also expected to follow instructions provided in each exercise. Inform your instructor if you do not understand a procedure.

1. Notify your instructor if you are pregnant, allergic to any chemicals (such as latex), or have another medical condition that requires precautionary measures in the laboratory.
2. Place book bags, large purses, etc., under your lab bench or where instructed. The only things on the lab bench should be what you need for lab that day.
3. Do not eat, drink, handle contact lenses, or apply cosmetics in the lab.
4. Confine long hair, loose clothing, and dangling jewelry.
5. Wear close-toed shoes and be aware that some chemicals can stain clothing.
6. Cover cuts, scrapes or other wounds with a bandage.
7. Assume all chemical and reagents are poisonous and act accordingly.
8. Never pipet chemicals by mouth. Use pipettes, or other devices as directed, to measure and transfer chemicals.
9. Do not pour chemicals, or other materials, back into "stock bottles" unless told to do so.
10. Put the right cap back on reagent bottles.
11. Dispose of reagents, or equipment, as instructed.
12. Keep all chemicals away from edge of lab bench to avoid spills.
13. Wash skin immediately and thoroughly if contaminated by chemicals or microorganisms.
14. Report all spills, no matter how minor, to your instructor immediately.
15. Do not leave heat sources unattended.
16. Use appropriate apparatus when handling hot glassware.
17. Never point a test tube that is being heated in the direction of someone else.

18. Report accidents immediately. Do not attempt to clean up glassware that is dropped and shatters.

19. Other broken glassware, and glass slides, should be disposed of in "Sharps" containers.

20. Be particularly careful; when handling scalpels, razor blades, scissors, etc.

21. Know the location of the fire extinguisher, eye wash station, first aid kit, glass disposal boxes and clean-up materials for spills.

22. Wipe off your bench at the end of lab. Wash, dry and replace all the materials and equipment you used.

23. Wash your hands with soap and water when you leave the lab.

EXERCISE 1: SCIENTIFIC METHOD

EFFECTS OF ETHANOL AND CAFFEINE ON HEART RATE OF WATER FLEAS

1.1 The Scientific Method: What is it?

The scientific method is the method of science. It is a process by which the natural world can be studied and understood. While the scientific method is often presented as a set of necessary steps, it is important to realize that the actual process of science…science as practiced on a day-to-day basis by real-world scientists… is much more casually pursued. Yes, scientists do formulate hypotheses. Yes, scientists do conduct experiments and collect data. Yes, scientists analyze that data to determine if their hypothesis is verified or not. But scientists do not go through life with a clipboard and a checklist of what they should do and in what order they should do it. The scientific method is more a pattern of thought…a habit of mind.

Scientists think a certain way in their approach to the natural world. You might be surprised to realize that this particular habit of mind is employed by everyone at one time or another. The woman who has received multiple tickets for traveling 55 in a 35 mph zone will probably opt to slow down. The man who makes coffee in the office knows the exact number of tablespoons of ground coffee to brew the perfect cup. The toddler avoids playing with the cat. "These are people making decisions on the basis of experience. What does this have to do with the scientific method?" Experience leads to knowledge. Each and every experiential event can result in some sort of conclusion. A conclusion is knowledge. "If I exceed the speed limit, I stand a good chance of getting a ticket." If you are a scientist, there is a good chance that you are interested in gaining knowledge about some aspect of the natural world. So how do you get to that knowledge?

1.2 Starting Out: What do I know and what do I want to know?

I am a scientist. I am curious about ethanol and caffeine. These are two substances that are commonly used…and abused… by people I am told that ethanol is a depressant and caffeine is a stimulant. I suspect that these two substances might have an effect on heart rate. How can I test this suspicion of mine? I know that water fleas have a heart that is visible and easily assessed in terms of heart rate. Hmmmm…I could introduce different concentrations of

ethanol and caffeine into the immediate environment of a water flea to determine effects on heart rate. We are going to pursue this very idea in our laboratory today.

1.3 Starting Out: How do I formulate a hypothesis?

The hypothesis is a predictive statement about the natural world….it is testable. While hypotheses are written in many different formats, the simplest way to express a hypothesis is to do so in an "if … then" format. "If" is the part of the hypothesis that establishes the conditions of the natural world. "Then" is the prediction. Here is an example: "If a plant is deprived of water, then the plant will wilt." I realize that sounds a bit too simple or unscientific. How about: "If a plant is deprived of water, then the turgor pressure in its leaf cells will decrease." Bring it up a notch: "As soil moisture decreases, turgor pressure in leaf cells will decrease proportionally."

Why don't you write a couple of hypotheses given our interest in ethanol and caffeine and heart rate in water fleas (information found in section 1.2 above)? Use the IF/THEN format that we just covered in the last paragraph. Write your hypothesis statements in the spaces provided below.

Hypothesis 1 [ethanol]:

_____
_____
_____

Hypothesis 2 [caffeine]:

_____
_____
_____

1.4 Figuring It Out: How do I test the hypothesis?

A hypothesis is a testable statement. An experiment is a controlled test of a hypothesis. In testing the hypothesis, we should probably come up with some kind of design that will best determine if our prediction is valid or not. In the case of the ethanol, we will be subjecting our water flea to different concentrations of

ethanol in an effort to determine its effect on heart rate. We will do the same thing with caffeine. Notice that we are manipulating some aspect of the natural world in order to arrive at a conclusion. Something that changes in an experiment is a variable. If the researcher (or researchers) intentionally manipulate some aspect of an experiment, it is called an independent variable. There are two independent variables in our proposed experiment. What are they?

Independent Variables: _____ _____

Manipulation of the independent variable should result in some measureable change in our experiment. The dependent variable is a feature of the experiment that changes in response to changes in the independent variable. Put another way, the dependent variable is the thing that is measured in an experiment. So what is the dependent variable in our proposed experiment?

Dependent Variable: _____

1.5 Figuring It Out: How do I know that the independent variable is influencing the dependent variable?

One unavoidable fact of any experiment is the uncertainty concerning your results: How do you know if the changes in your dependent variable are due to the independent variable? Scientists cope with this question by creating a control in the experiment. Simply put, a control is the absence of a variable. If we were to assess heart rate in a water flea that is not being subjected to ethanol or caffeine, we might expect there to be no change in heart rate. Put another way, a control is the standard for absence of a result. So, in our experiment, we will need a control to make sure that the water flea's heart rate is changing due to the ethanol or caffeine only. In our experiment, we will have a series of treatments consisting of water only. The water flea lives in water; we would not expect water to have an impact on the water flea's heart rate. We have two hypotheses (see p. 2). We can write a third hypothesis to express our prediction of water's effect on heart rate. Write this third hypothesis below:

Hypothesis 3 [water]:

_____

_____
_____

1.6 Figuring It Out: Consistency

In any experiment…in design and in execution, the researcher should be very careful to eliminate any sources of variation that might compromise his or her results. All samples in an experiment should be treated exactly the same. All measurements should be taken in exactly the same manner. In our experiment, we will be sure to follow a standard procedure in changing the conditions of the water flea's environment. We will also follow a standard procedure in measuring heart rate.

1.7 Wrapping It Up: Data

Experiments generate results, or data. Data can be quantitative data (as numeric measurements) or data can be qualitative (descriptive). In either case, the data generated will allow us to make some sense from our initial hypothesis statement: it is right or is it wrong?

Quantitative data should be expressed (displayed) in some way as to make it easier for the researcher to determine the relationship between the independent variable and the dependent variable. At which point, the researcher will be able to render a conclusion about his or her experiment. Data can be arranged in a tabular form, or as a graph. We will be displaying our data in both of these forms today.

1.8 Wrapping It Up: Conclusion

So, the experiment is done. What do we do now? The data mentioned in 1.7 needs to be assessed in terms of the proposed hypothesis or hypotheses; a conclusion needs to be established. A conclusion is the summarized result of an experiment; it is a statement that establishes if the experiment verified the hypothesis or not. In a formal laboratory report (see section 1.9 below), the conclusion is established in the Discussion section of the lab report. Additional

commentary about the conclusion and its ramifications are also included in this section of the lab report.

1.9 Communicating Science: Writing a lab report

A lab report is a written documentation of a scientific study. It communicates the basic purpose of the research and the hypothesis or hypotheses under investigation. The lab report documents the procedure in sufficient detail such that anyone in the future could replicate the experiment exactly. The lab report presents the data (in tabular or graphic form or both [Some scientific studies are better supported by the inclusion of photographs – a form of qualitative data.] Finally, the researcher reports the conclusion and often adds additional commentary to more fully explore the specific features of the experiment, its hypotheses, and its conclusion.

A typical lab report would have the following sections / content:

Title. A brief, but descriptive title that leaves no question as to the content of the report.

Abstract. A very brief synopsis of the purpose, hypotheses, method, and results (strive for no more than 6 sentences). The abstract saves the reader time in that it gives more information than the title, but does not require a complete reading of the entire document.

Introduction. A descriptive narrative that establishes the scientific background for the research. The purpose and hypothesis statement (s) are documented in this section.

Materials and Methods. A narrative that completely and accurately describes the procedure used in the research. Names and sources of study organisms are documented here. Specific brand name(s) of instrumentation (if relevant) are mentioned here as well. The ultimate purpose of the Materials and Methods section is to make it possible for some other researcher to completely replicate the experiment as it was carried out.

Results. A display of the data generated from the research; tables and graphs (quantitative data) or illustrations and photographs (qualitative data). Any

statistics generated in the assessment of quantitative data will be found here as well.

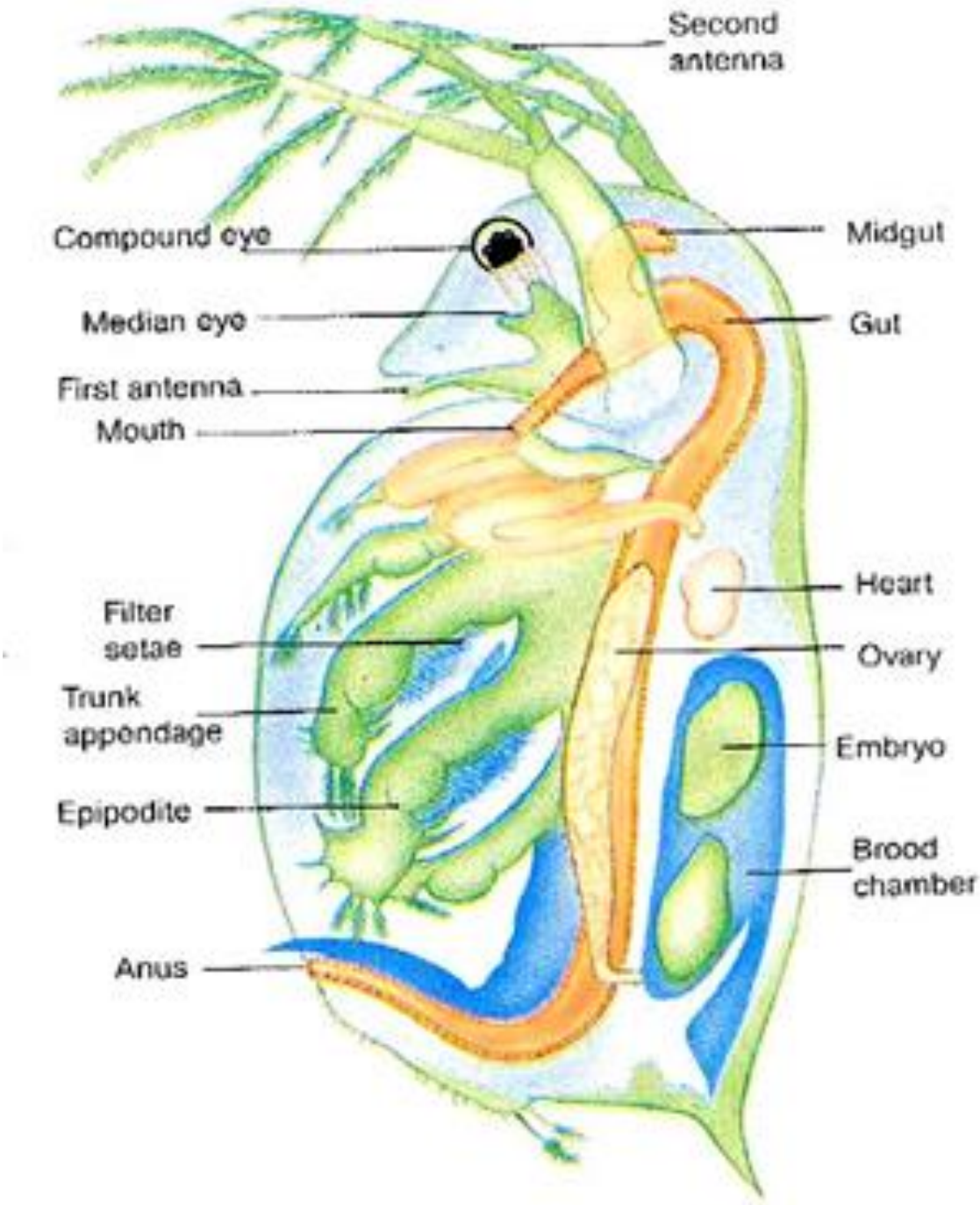
Conclusions (Discussion). The results are assessed with respect to the initial purpose of the research and the hypothesis (hypotheses) that were documented in the Introduction section. Any ramifications of the conclusion(s) or other commentary on the experiment's purpose, hypothesis (hypotheses), procedure, or results can be mentioned here.

2.0 PROCEDURE

1. Our study organism is the common water flea (Daphnia magna). You will want to be sure of the origin of our water flea cultures so you can document this in your lab report.
2. We will be using dissecting microscopes to assess heart rate in the water flea. There are two modes of illumination for these instruments: T (transmission) is "bottom-up" illumination, and I (incidental) is top down illumination. You will want to use the Incidental setting for most of your work in locating and focusing on your water flea. The T setting is most effective for viewing the beating heart, but it also generates heat that can cause your water flea problems; you will want to limit your use of the T setting to those times where you are recording heart rate.
3. You will obtain a shallow, circular curved dish (a "watch glass") and, using a wooden applicator stick, place a tiny dab of petroleum jelly in the middle of the dish. This dab of petroleum jelly will immobilize your water flea, making it possible for you to record its heart rate.
4. Using a disposable pipette, obtain an individual water flea and put it (with the culture water) in the center of your watch glass. You will want to be sure that the area occupied by the culture water is no bigger than the size of a dime (preferably smaller). The water flea should get "hung up" on the petroleum jelly; it will not be able to swim about. If your water flea should continue to swim freely, you can often "nudge" it towards the petroleum jelly with a wooden applicator stick.
5. View your water flea under the dissecting microscope. You will want to (1) become familiar with the controls of the dissecting microscope in magnifying and viewing your water flea. You will want to (2) find the heart of the water flea (diagrams will be found on your lab tables) and practice

recording heart rate. You will also want to (3) be sure that your water flea is correctly positioned. The correct position is on its side. If the water flea is on its ventral side, or on its back, you will not be able to see the heart. You will also want to be sure that the water flea is not "gummed" up with petroleum jelly, in which case your water flea could suffocate and die.

6. You will be assessing the effects of 8 sequential treatments of culture water (control), 5 sequential treatments of ethanol (2%, 4%, 6%, 8%, and 10%), and 3 sequential treatments of caffeine (1%, 2%, and 3%). Following the introduction of a new treatment, you will wait for one full minute – this gives your water flea a chance to adjust to the new environment. You will then record heart rate for an interval of 15 seconds (multiplying that number by 4 will give you the number of beats per minute). After the heart rate has been recorded, a transition in treatment will require that one of your lab group members will use a piece of paper towel to absorb the old treatment away from the water flea, while another lab group member will simultaneously add the new treatment to the water flea. Your goal is to try to maintain the same amount of liquid surrounding your water flea. Your goal is to not touch or disrupt your water flea in any way.
7. You will record your group's data in the table provided below. Be sure to record your data as beats per minute. If your water flea should die during the experiment, you will still need to record heart rate (0 beats per minute). If you water flea should die during the course of the first 10 treatments (water through 4% ethanol), you will need to get another organism and start over.
8. Once all data has been collected, you will need to sacrifice your water flea and clean / return all glassware.

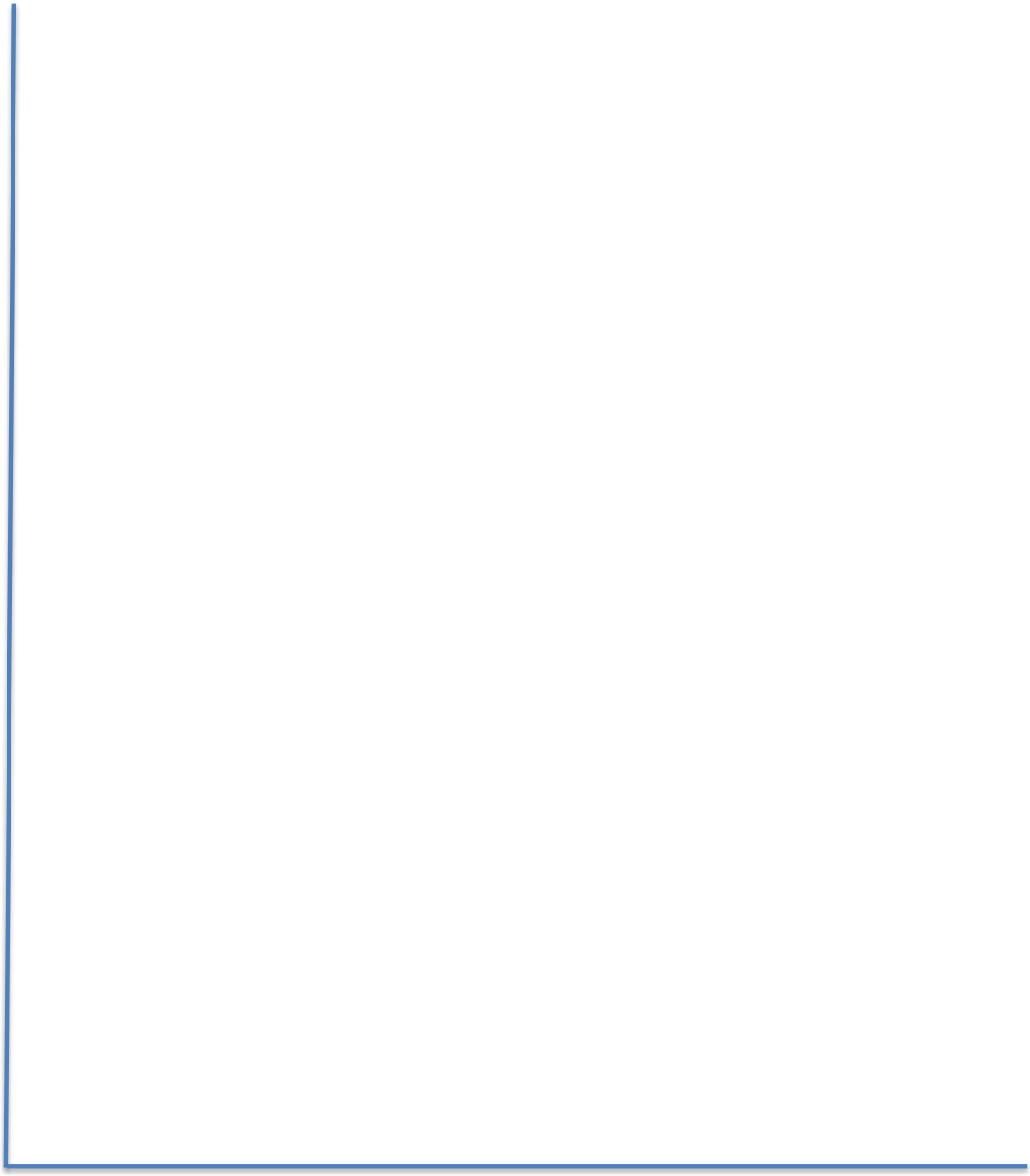


Daphnia magna

INDIVIDUAL DATA (Table)

Treatment	Heart Rate (beats per minute)
water	
water	
water	
water	
water	
water	
water	
water	
2% EtOH	
4% EtOH	
6% EtOH	
8% EtOH	
10% EtOH	
1% Caffeine	
2% Caffeine	
3% Caffeine	

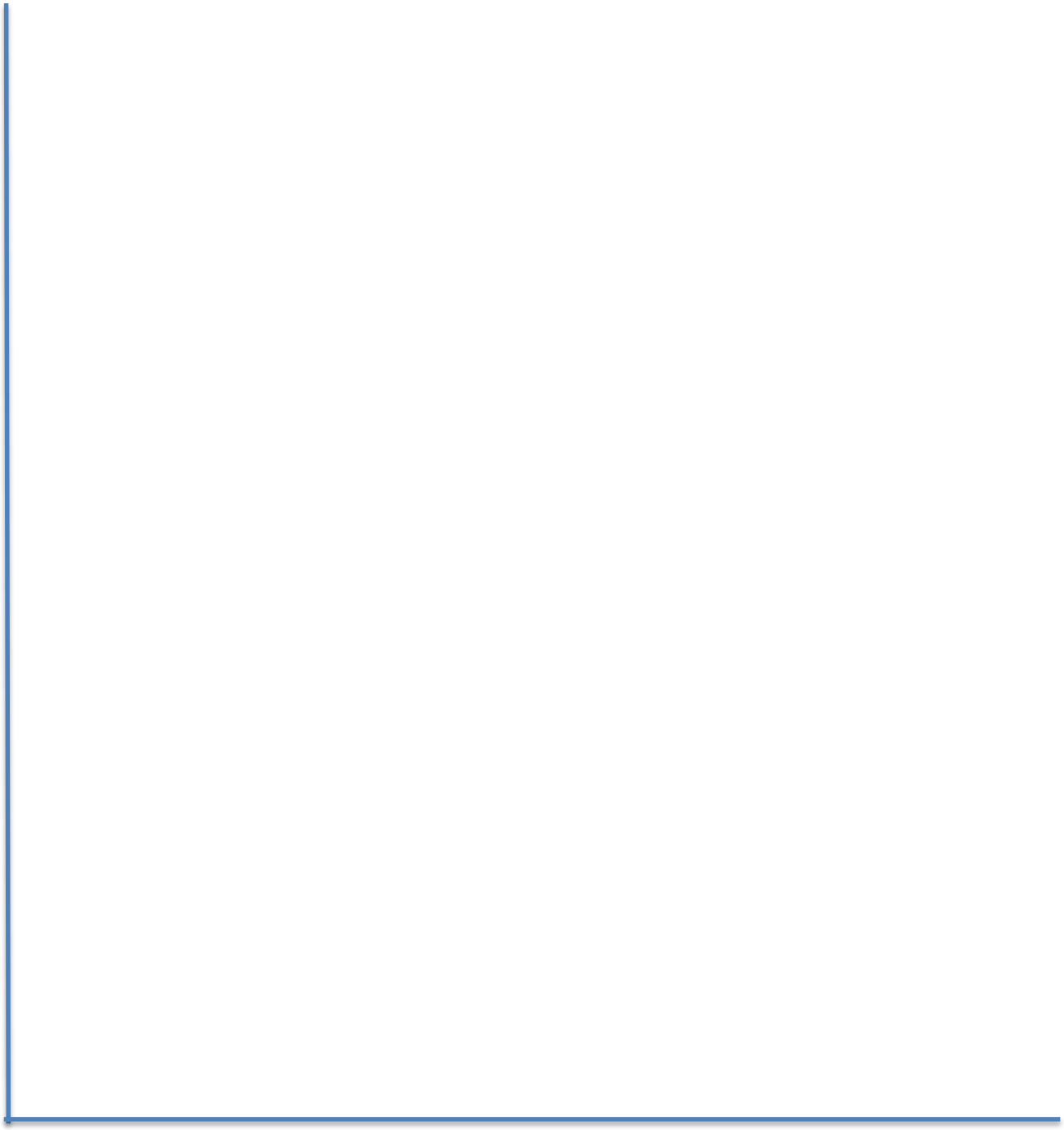
INDIVIDUAL DATA (Graph)



CLASS DATA (Table)

Drug Percent	Group #1	Group #2	Group #3	Group #4	Group #5	Group #6	Group #7	Group #8	Class Avg.
Water Treatment									
Water Treatment									
Water Treatment									
Water Treatment									
Water Treatment									
Water Treatment									
Water Treatment									
Water Treatment									
2% EtOH									
4% EtOH									
6% EtOH									
8% EtOH									
10% EtOH									
1% caffeine									
2% caffeine									
3% caffeine									

CLASS DATA (Graph)



EXERCISE 2: ORGANIC MOLECULES

Have you ever heard the expression "you are what you eat"? As living organisms, we all need to eat to stay alive, but what happens to the food we eat? It depends on what kind of food it is: some foods are used mainly for energy, while others are used to make new structures in our bodies. The reason for this is that foods contain different types and/or amounts of **biomolecules** in them. Biomolecules are organic molecules that are made by a living organism, such as proteins, lipids/fats, carbohydrates and nucleic acids.

Carbohydrates – (sugars, starches) Carbohydrates can be found in almost all food sources. Rice, cereal, potatoes, fruits, pasta, vegetables, etc., have some kind of carbohydrate in them. Carbohydrates can be compounds that are as simple as a single glucose (sugar), to strings of sugars (starches). Starches are broken down into sugars, and sugars are the main energy source for the body. There are some carbohydrates that cannot be digested by humans (cellulose). This type of carbohydrate is also called fiber.

Lipids – (fat, oil, lard, butter) Depending on the state of the lipid it is classified as a saturated fat or a non-saturated fat. Saturated fats are solid at room temperature, while non-saturated fats are liquids at room temperature. Fat is a necessary biomolecule because it is used to maintain the membranes of your cells, to make certain hormones, and to help your neurons function.

Proteins – (beans, meat, dairy, nuts) We need proteins to put together almost everything in our bodies. Proteins are broken down into amino acids, which are then used to make new proteins: everything from membrane proteins and antibodies, hormones, hemoglobin, muscle, hair and nails.

Nucleic acids – the genetic material. We consume the cells of an organism.
Therefore we are also eating its DNA/RNA. These molecules are broken down into nucleotides which can then be reused by our cells.

How do you know what your food is made of? When you eat meat, you are eating the biomolecules that the animal tissue is made of (mainly protein and fat). When you eat a plant, you are eating the biomolecules that the plant tissue is made of (mainly carbohydrate). Foods that contain protein or fat are used to make new proteins and fats in your cells, while carbohydrate-rich foods are used for energy. If you eat "extra" food (more than your body needs), it will be stored in your cells as fat.

In this laboratory exercise, you will test food sources and determine what types of biomolecules they contain: lipid, protein, sugar or starch. Because each group of biomolecule is unique, we will use different methods to detect its presence in food samples. Before beginning, select 5 foods from the front of the room that you want to test and list them on your **data table**. Formulate a hypothesis regarding the presence of each nutrient sample within that particular

food, and write your hypotheses in the space provided below your data table. Now you are ready to test! The following procedures explain how to detect different biomolecules. They can be performed in any order, but the fat test should be performed **first**.

Materials

- Test Tube/ Test Tube Rack
- Graduated cylinder (10 or 25mL)
- Plastic Knife
- Weigh boats or petri dishes
- Disposable pipettes
- Spot Plate
- Boiling Water Bath
- Paper towels
- Iodine, Biuret reagent, Benedict's Solution, NaOH
- Control solutions: protein, sugar, starch, vegetable oil
- Food items (crackers, cheese, potato chips, grapes, apples, meat, butter,etc.)
- Wax pencil or marker for labeling samples

I. Prepare food samples:

Crush or mash your food samples in a weigh boat or petri dish. Add water and continue mashing your foods into a runny paste. Your samples should be thin enough to draw up into a pipette. Label the samples and set them aside.

II. To test for Fat:

Fats are a type of lipid common in many foods. They are insoluble in water and are therefore **hydrophobic**. Fats are difficult to remove from many substances, because they do not evaporate (as water does).

1. Add 15 drops of water to one test tube
2. Add 15 drops of oil to one test tube
3. Add 15 drops of food sample to the one test tube
4. Now add 10 drops of Sudan IV to each test tube
5. Mix well
6. Let sit for 2-4 minutes
7. Record where the red is located

III. To test for Proteins:

Buiret reagent is a copper sulfate solution used to detect the presence of protein. The reagent changes color in the presence of proteins or peptides because it chemically reacts with them. This chemical reaction only occurs at elevated pH: therefore, you will add a strong base (NaOH) to the biuret regent during this exercise.

1. Add 5 drops of water to one well on a spot plate.
2. Add 3 drops of Biuret reagent to the same well.
3. Add 1-2 drops NaOH to the same well.
4. Mix thoroughly by pipetting the solution up and down a few times.

What color is the solution in the well? _____
Record this observation in your data table.

Now you will perform a positive control, which shows what happens when biuret reagent reacts with a protein.

5. Add 5 drops of protein solution (albumin) to one well on a spot plate.
6. Add 3 drops of Biuret reagent to the same well.
7. Add 1-2 drops of NaOH to the same well
8. Mix thoroughly by pipetting the solution up and down a few times.

What color is the solution in the well? _____
Record this observation in your data table.

What can you conclude from the previous 6 steps?

9. Conduct a protein test on your food samples by adding 10 drops of each to separate wells of a spot plate, followed by 6 drops of biuret reagent and 2-3 drop NaOH. Mix thoroughly by pipetting the solution in each well up and down a few times. Based on the color of the solution in each well, you should be able to determine the presence/absence of protein. Record your results in the data table.

IV. To test for Starch:

Iodine interacts with starch. When this occurs, the structure of the iodine molecules changes, as well as the way that light is reflected from the solution.

1. Add 6 drops of iodine to one well on a spot plate.

What color is iodine? _____

2. Add 10 drops of water to the same well. Mix thoroughly by pipetting the solution up and down a few times.

What color is the solution in the well? _____
Record this observation in your data table.

Now you will perform a positive control, which shows what happens when iodine reacts with starch.

3. Add 10 drops of starch solution (or potato juice) to one well on a spot plate.
4. Add 6 drops of iodine to the same well. Mix thoroughly.

What color is the solution in the well? _____
Record this observation in your data table.

What can you conclude from the previous 4 steps?

5. Conduct a starch test on your food samples by adding 10 drops of each to separate wells of a spot plate, followed by 6 drops of iodine. Mix thoroughly, and record your results in the data table.

V. To test for Sugar:

Benedict's reagent reacts with monosaccharides and disaccharides, causing a color change. A greater color change indicates a larger concentration of these simple sugars in a solution. This reaction occurs best when Benedict's reagent is heated.

1. Add 10 drops of Benedict's reagent to a test tube.

What color is Benedict's reagent? _____

2. Add 15 drops of water to the same tube. Mix well. Place the tube in a boiling water bath for 3 minutes.

What color is the solution in the tube? _____
Record this observation in your data table.

Now you will perform a positive control, which shows what happens when Benedict's reagent reacts with sugar.

3. Add 15 drops of glucose or sucrose solution to a test tube.
4. Add 10 drops of Benedict's reagent to the same tube. Mix well. Place the tube in a boiling water bath for 3 minutes.

What color is the solution in the tube? _____
Record this observation in your data table. What can you conclude from the previous 4 steps?

5. Conduct a sugar test on your food samples by adding 15 drops of each sample to separate test tubes, followed by 10 drops of Benedict's reagent. Mix well, heat the tubes for 3 minutes, and record your results in the data table.

***BIOMOLECULES DATA TABLE*: use +/– to indicate the presence/absence of each biomolecule**							
Test performed →	**Buiret**		**Iodine**		**Benedict**		**Sudan IV**
	Protein (+ or -)	**Color in well**	**Starch (+ or -)**	**Color in well**	**Sugar (+ or -)**	**Color in well**	**Fat (+ or -)**
Water							
Protein solution (Albumin)							
Starch solution (or potato juice)							
Sugar solution (glucose or sucrose)							
Oil							
Food#1:							
Food#2:							
Food#3:							
Food#4:							
Food#5:							

Hypotheses:

Food#1:

Food #2:

Food #3:

Food #4:

Food #5:

EXERCISE 3: METRIC MEASUREMENT

In this laboratory exercise, you will learn to make measurements using the metric system using the metric ruler, an electric balance, a graduated cylinder, and a thermometer.

Often the standardized unit for each kind of measurement is too large (or small) to measure the intended object, so we use units of measure that are fractions or multiples of the standard units. For example, if we measure an object using the English System it might measure 18 inches, or 1½ feet. Instead of using fractions, the Metric System allows us to measure objects using different units that are based on multiples of 10. If we were to measure the same object using a metric ruler, it would measure 0.457 meters (m), which is the same as 45.7 centimeters (cm). The **meter** is the standard unit used for measuring length, while **grams** (g), **liters** (L), and **Celsius** (^{o}C) are the standard units used to measure mass, volume and temperature, respectively. The table below lists commonly used prefixes (in bold) and their relationship to the standardized unit of measure.

Table 1: Metric equivalents

Unit	Equivalent
kilometer (km)	1000 meters
hectometer (h)	100 meters
decameter (da)	10 meters
Meter	1 meter
decimeter (dm)	1/10 meter
centimeter (cm)	1/100 meter
millimeters (mm)	1/1,000 meter
micrometer (μm)	1/1,000,000 meter
nanometers (nm)	1/1,000,000,000 meter

In addition to using the metric system to make measurements, you will also demonstrate your ability to convert the original measurements to lower and higher values by moving the decimal point the correct number of places in the proper direction.

Converting

Conversions within the metric system can be made easily using a metric staircase. Each step of the staircase represents a ten-fold change in the value of the measure or a shift of the decimal point one place. Therefore, each step you move down the staircase represents multiplication by ten or a movement of the decimal one place to the right. Each step up the staircase represents a division by ten or the movement of the decimal

point one place to the left. Two steps up or down the staircase represents a movement of the decimal point two places to the left or right and three steps up or down the staircase represents a movement of the decimal point three places to the left or right.

Conversions from the standard system (inches, feet, ounces, etc…) to metric can be a bit more challenging but some of the common examples are listed below.

Measurement	**Unit**	**Metric to Standard**	**Example**
Length	Meter (m)	1m = 39.3701 inches	1m = About the width of a doorway
Mass	Gram (g)	1g = 0.035 oz	1g = the weight of a dollar bill
Volume	Liter (L)	1L = ~1 quart	Many alcoholic beverages are measured in liters
Temperature	Celsius (C)	$0^oC = 32^oF$ (freezing point of water) $T_{(°F)} = T_{(°C)} \times 9/5 + 32$ or $T_{(°F)} = T_{(°C)} \times 1.8 + 32$ $T_{(°C)} = T_{(°F)} \times 5/9 - 32$	Ice ~ 0^oC Boiling water ~100^0C

Based on the information above convert the following.

1l = _____ml 5kg = _____ mg

222.7g =_____kg 98.6^{o}F = _____^{o}C

100mm = _____m 1000 µm = _____nm

15cm = _____km 1,000,000 nm = _____m

-200^{0}C = _____ ^{o}F 15g = _____ oz

20oz = _____ g 2l = _____ quarts

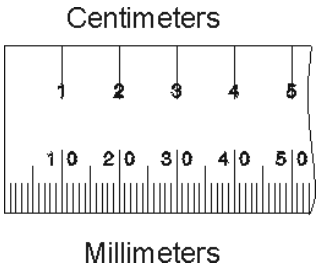
12 inches = _____cm 200mm = _____inches

1 inch = _____ nm 4 quarts = _____ kL

Materials:

- Metric stick, ruler
- Glassware: graduated cylinders, beaker, small & large test tubes
- Coins
- Book
- Electronic balance
- Thermometer

Procedures and Data:



1. Linear Measure

Use the meter stick or ruler to measure the items listed below (Table 2). Above each column write the name of the unit that is abbreviated below it. Circle the unit you used to **directly** measure each item. You will need to convert for the other units.

Table 2: Linear measurements				
Diameter of Penny	**_____M.**	**_____cm.**	**_____mm.**	**_____km.**
Height of lab counter	**_____M.**	**_____cm.**	**_____mm.**	**_____km.**
Width of a Textbook	**_____M.**	**_____cm.**	**_____mm.**	**_____km.**
Length of the Room	**_____M.**	**_____cm**	**_____mm.**	**_____km.**

1a. Did you use the same unit to measure each item? Explain why you selected the units you did.

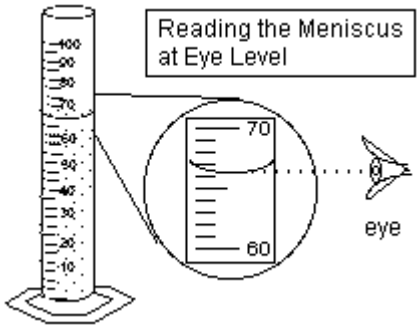
2. Mass/Weight

Use the scale to measure the mass of the following materials and record the values below (Table 3). Above each column write the name of the unit that is abbreviated below it.

Table 3: Mass measurements				
Mass of coin	**_____g.**	**_____cg.**	**_____mg.**	**_____kg.**
Mass of empty graduated cylinder	**_____g.**	**_____cg.**	**_____mg.**	**_____kg.**
Mass of graduated cylinder with 10 ml water	**_____g.**	**_____cg.**	**_____mg.**	**_____kg.**

2a. Calculate the mass of 10ml water. **Show your work!**

3. Volume (of liquids)



Find the volume of the items listed below. To do this, you should first fill the item completely with water. Once the item is completely full, empty the water into a graduated cylinder. Record the volume of water by reading the **meniscus** of the water. The meniscus is the curve in the surface of a liquid, and the bottom of the curve should always be read at eye level (see figure). Once you have measured volumes using a graduated cylinder, repeat the procedure using a beaker. Place your measurements in table 4, below. Above each column write the name of the unit that is abbreviated below it.

Table 4: Volume measurements			
Volume of small test tube using a graduated cylinder	**_____L.**	**_____cl.**	**_____ml.**
Volume of large test tube using a graduated cylinder	**_____L.**	**_____cl.**	**_____ml.**
Volume of small test tube using a BEAKER	**_____L.**	**_____cl.**	**_____ml.**

Volume of large test tube using a BEAKER	**_____L.**	**_____cl.**	**_____ml.**

3a. Does all glassware measure with the same degree of accuracy? Explain

3b.When should you use a graduated cylinder (rather than a beaker) to measure volume?

3c. When would you use a beaker (rather than a graduated cylinder) to measure volume?

4. Temperature

Use a thermometer to measure the temperature of the following items. The thermometer has both the Fahrenheit and the Celsius scales. Record your values in table 5.

Table 5: Temperature measurements		
Ice water	˚F	˚C
Boiling water	˚F	˚C
Room temperature	˚F	˚C

4a. How do the two temperature scales compare?

4b. If the temperature of the room is 72˚C, what is the temperature in ˚F?

Summary:

1. What is the standard metric unit used for measuring length? _____.
2. The metric prefix denoting $^1/_{1000}$ is _____.
3. How many times larger is a centigram then a milligram? _____.
4. If you are changing M to cm. what direction do you move the decimal point? _____

5. To convert from a large value to a small value, we move the decimal point to the _____. To convert from a small value to a large value, we move the decimal point to the _____.

6. What is the standard metric unit for mass? _____.

7. What is the standard metric unit for volume ? _____.

EXERCISE 4: MICROSCOPY

Many biological subjects are too small to see with the naked eye. We need magnification to study the specimens and processes that occur on this level, so an important skill in any biology lab is proper care and operation of the microscope.
Two basic types of microscopes are used in biology: the **compound light microscope** and the **electron microscope**. Compound light microscopes use light to magnify objects, while electron microscopes use a stream of electrons. An electron microscope has greater resolving power than a light microscope and can reveal the structure of smaller objects. For example, the electron microscope can magnify structures up to about 10 million times, while most light microscopes are limited to about 2000 times. You will use the compound light microscope in this laboratory to observe various specimens.

Proper microscope practice is as follows:

1. **Carry the microscope upright with one hand supporting the base.** Care should be taken not to bump your microscope on objects such as chairs, tables, or walls. **Gently** place your microscope on your laboratory table and remove the protective plastic cover.
2. **Only clean the lenses with lens paper.** The lenses on the microscope scratch easily. If you need to clean them, use ONLY lens paper (found in station drawers).
3. **Do not push or slide the microscope across the table.** This causes vibrations that can loosen screws or misalign microscope parts.
4. **Always begin viewing a slide using the scannin (4X) objective**. Never begin an observation with the higher powered (10X, 40X) objectives. Doing so could result in broken slides or scratched lenses.
5. **Never use course focus adjustment at high magnification.** Once a specimen is brought into focus using the lowest power, you can rotate to a higher powered objective to increase the magnification. You should need to focus slightly, as most light microscopes are **parfocal**, meaning that the image remains nearly in focus as you change lenses.
6. **Replace microscope properly.** When you are finished using the microscope, turn off the light, remove the last slide from the stage, and wipe any water or other materials from the stage. Lower the stage and move the lowest power objective into position. Wrap the electrical cord securely (not around the arm of the microscope!) and place the plastic cover over the microscope before returning it to the storage cabinet.

Materials:

- Prepared slides: letter “e”, colored threads, human epithelial cells, onion epithelial cells
- Clear plastic ruler
- Compound light microscope

1. Parts of the Microscope

In this exercise, you will identify and learn the function of several microscope parts, practice focusing the microscope, and learn to use the microscope as a measuring tool. Use the figure provided to identify and learn the parts listed in the following table.

Table 1: Microscope parts and functions

Microscope Part	Function
Arm	Supports the body tube and lenses. Use the arm and base to carry your microscope.
Base	Supports the entire microscope.
Ocular or Eyepiece	The lens in the upper part of the microscope. **Monocular** microscopes have one ocular, while **binocular** microscopes have two oculars.
Body Tube or Turret	Holds the ocular at one end and the nosepiece at the other. Conducts light rays.
Revolving Nose Piece	Located at the lower end of the body tube. A revolving device that holds the objectives.
Objective Lenses	Located on the revolving nosepiece. Each lens has a different magnifying power. The smallest objective is the lowest power, also called **scanning power**. Only one objective may be used at a time. The selected lens is rotated into position by turning the nosepiece.
Stage	The horizontal platform upon which the slide rests.
Condenser	Lens beneath the stage that concentrates light before it passes through the specimen to be viewed.
Diaphragm Lever	Small lever beneath the condenser. Controls the amount of light passing through the specimen.
Light Source	Directs a beam of light through the specimen.
Mechanical Stage	Moveable stage controlled by circular knobs adjacent to or below the stage. Allows the observer to move the stage forward/backward or laterally.
Coarse Adjustment Knob	Located on either side of the arm. Moves the stage to bring object into focus. **This knob should only be used when using the scanning objective.**
Fine Adjustment Knob	Located within the coarse adjustment knob. Allows fine focus of the specimen.

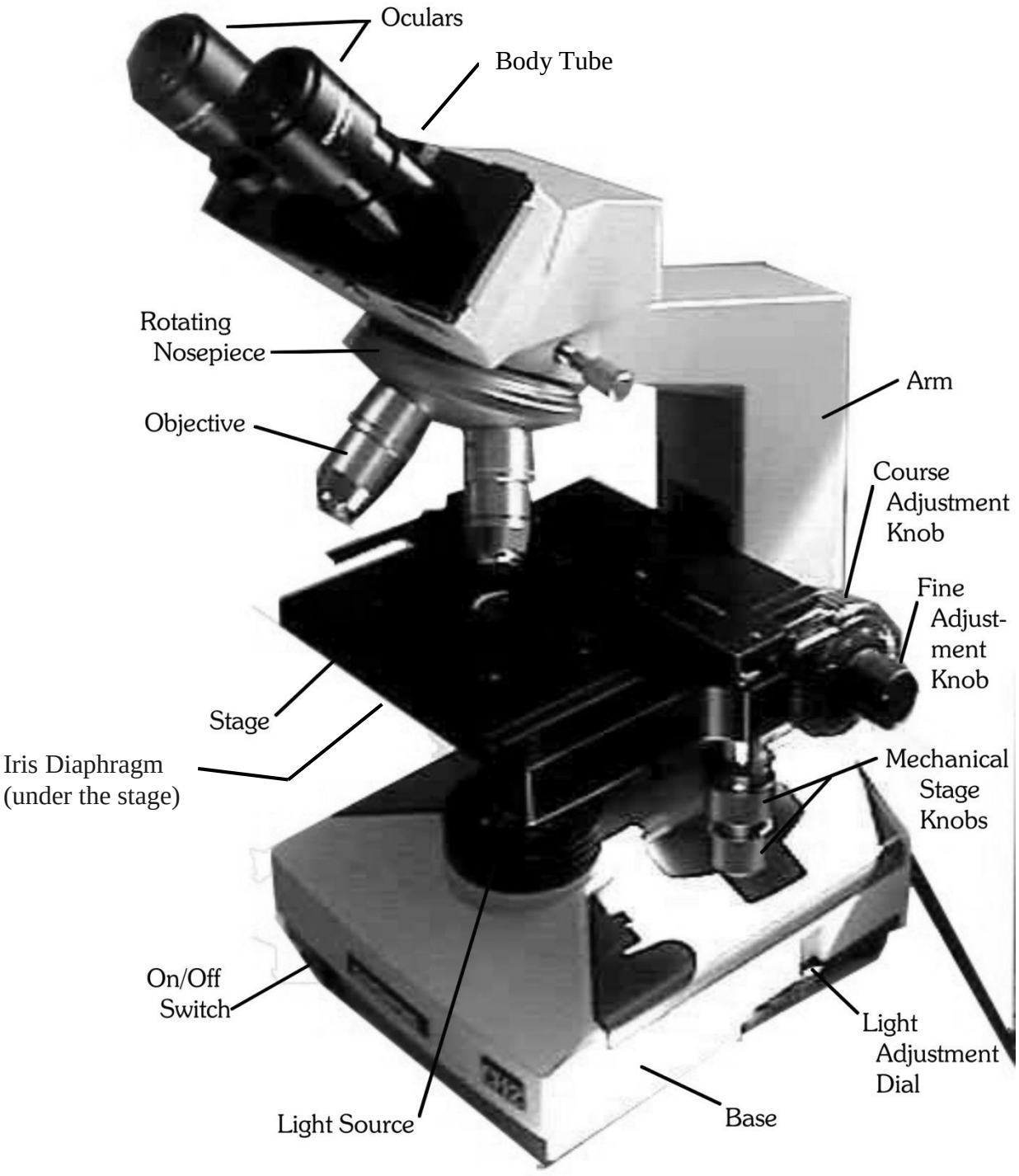


Figure 1: The binocular, compound, light microscope.

2. Calculating Magnification

When using a compound light microscope, we are using two lenses to magnify an image: the **ocular** lens and the **objective** lens. Therefore, to calculate the **total magnification** of an image, we must include both. Total magnification can be calculated by using a simple formula:

Total Magnification = Ocular lens power x Objective lens power

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

Table 2: Total Magnification calculations

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

3. Focusing & Inversion

a) Obtain a slide of the letter "e" and place it on the microscope stage. Using your mechanical stage, center the letter "e" over the condenser lens. Make sure that your scanning objective is clicked into place.
b) While looking into the ocular, use the coarse adjustment knob to bring the letter "e" into focus. Use the fine adjustment knob to "fine tune" the image. Try adjusting the light with the iris diaphragm lever. How does this change the image?

c) Observe the position of the letter "e" as it appears in the **field of view** (the circular area that can be seen when looking through the ocular. **Draw the letter as it appears**. Now observe the letter "e" as it actually is on the slide. Note differences. Does the microscope flip the "e" upside down, backwards, or both?

d) When you view the letter "e" using the scanning objective, how many times has it been magnified (what is the total magnification)? _____

e) Center the letter "e" in the field. Look at your microscope from the side (not through the oculars) and rotate the revolving nosepiece so the low power (10x) objective clicks into place.

f) Focus the letter using the fine adjustment knob (use of the coarse adjustment knob should **not** be necessary).

g) Under low power, how many times has the letter "e" been magnified? _____

h) Compared to scanning magnification, does the letter appear larger? _____

i) Compared to scanning, can you see more detail in the letter or the paper it is printed on? _____

4. <u>Diameter of field</u>

Since the scanning objective is 4X and the low power objective is 10X, images will be magnified **more** with low power than with scanning power. Because objects will appear larger, the low power field of view will be **smaller** than the scanning power field.

a) Lower the stage, and bring the scanning objective back to the center position. Leaving the letter "e" slide in place, position a clear plastic ruler across the stage so that the edge is visible in the field of view (see figure 2).

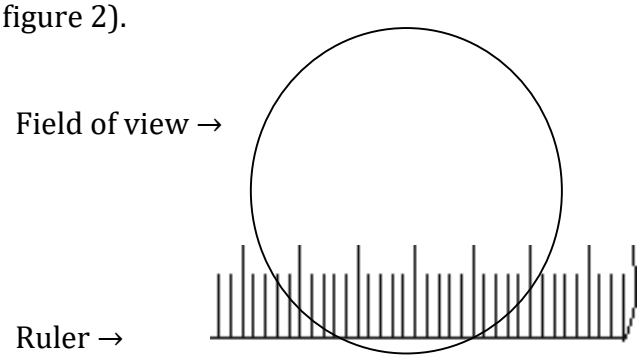


Figure 2: Measuring field diameter

b) Estimate the number of millimeters that you see in the field of view _____mm.

c) Leaving the ruler in place, rotate the low power objective into position.

d) Use the fine focus if necessary to bring the ruler into focus.

e) Estimate the number of millimeters that you see in the field of view _____mm.

f) What happens to the field of view when you increase magnification?

g) Do you see MORE or LESS of an object if you increase magnification? Explain.

5. Depth of field

Obtain a slide with colored threads mounted together. Place the slide on the stage and focus using scanning magnification. The center of your field should be at the point where the three fibers cross each other.

a) Focus up and down using your fine focus knob. Under scanning magnification, are all three fibers in focus at the same time?

b) Can you easily tell which fiber is on top and which is on the bottom?

c) Rotate the nosepiece so that the low powered objective clicks into place
d) Focus up and down using your fine focus knob. Under low magnification, are all three fibers in focus at the same time?

e) Can you easily tell which fiber is on top and which is on the bottom?

f) At which magnification is there a greater **depth of field**? Depth of field is the area (top to bottom) of an object that comes into focus while slowly moving the fine adjustment knob up and down.

Use the iris diaphragm to change the amount of light passing across the fibers. Note how changing the position of the diaphragm helps to increase (or decrease) your ability to view objects.

6. Microscopic Observations

Obtain a slide of human epithelial cells and observe under the microscope. Locate the nucleus, cytoplasm and cell membrane. Can you estimate the size of one cell?

Obtain a sample of either a live organism or preform your own cheek smear (see instructor for specific instructions) and observe what you see and draw below.

EXERCISE 5: CELL STRUCTURE & FUNCTION

Introduction

The **cell theory** states that all living things are composed of cells and that cells come only from other cells. Some cells are fairly simple, while others are extremely complex. For example, some organisms are unicellular—they exist as a single cell, while multicellular organisms are composed of many cells that form tissues and organs. In either case, all cells share some common properties: the presence of DNA, intracellular proteins that enable the cell to perform its functions, and a plasma membrane. Some cells also contain membrane bound organelles that allow a more complex level of functioning. In this exercise, you will examine the properties of the plasma membrane. The membrane controls what enters and exits the cell, and therefore serves a very important cellular function.

Materials:

- Potassium permanganate
- Clear plastic ruler
- Petri dishes containing 1.5% gelatin or agar
- Stopwatch/timer
- Glass petri dish
- Dialysis tubing
- String
- 250 mL beakers
- Electronic balance
- Sucrose solutions (15% and 30%)
- Graduated cylinder
- Wax pencil
- Potato strips
- NaCl solutions (10%, 0.9%)
- Microscope slides, coverslips
- Elodea

Potassium permanganate ($KMnO_4$) is a strong oxidizer. If spillage occurs, wash all surfaces thoroughly. $KMnO_4$ will also stain clothing.

I. Diffusion

Diffusion is the movement of molecules from high concentration to low concentration. Molecules move down a **concentration gradient** until they are equally distributed, or **equilibrium** is reached (Fig 4.1). At equilibrium, there is no concentration gradient. Molecules still move once equilibrium is reached, but there is no **net** movement in any one direction.

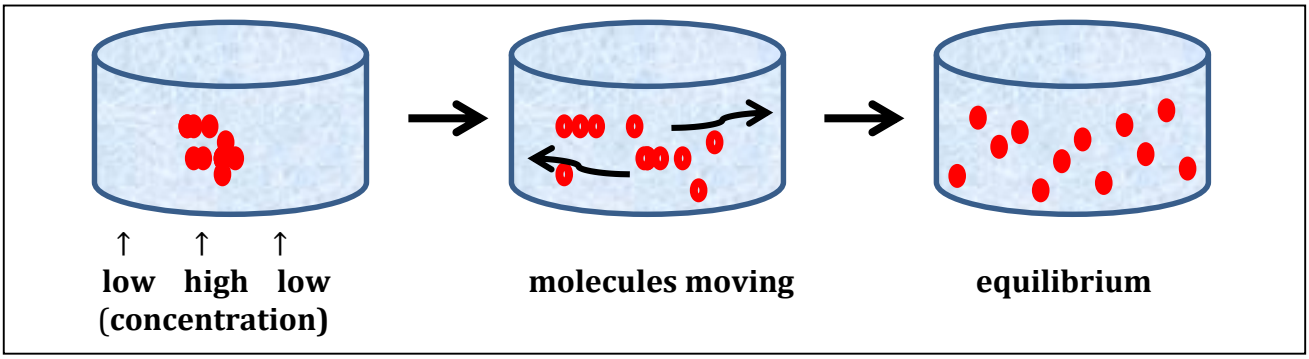


Figure 4.1 Diffusion of molecules from an area of high concentration to low concentration. Equilibrium is reached when the molecules are equally distributed.

There are many examples of diffusion that occur in our daily lives. For example, if you spray air freshener in a room, the fragrance molecules will at first be very concentrated where you spray. However, as the molecules diffuse, they will move throughout the room until they are equally distributed. The speed of diffusion depends on factors such as temperature, the size of the molecule, and the type of medium (air versus liquid, for example).

Diffusion through Air

1. Working in groups, have one group member stand 3 meters away from another student. One student should hold a can of air freshener, while the other student should have a timing device ready.
2. With the timer set to zero, one student will spray the air freshener straight upward. The other student should begin timing as soon as the spray is released.
3. When the student with the timer detects the fragrance (smells it), he/she should stop the timer.
4. Record the diffusion time in the first row in Table 4.1. Note: *if the time is in minutes or seconds, you will need to convert it to HOURS.*
5. What distance did the fragrance travel in the time recorded? _____(m)
6. Convert the distance from m to mm and record in Table 4.1.
7. Calculate the speed of diffusion (mm/hr) Record in Table 4.1.

Diffusion through a Liquid

1. Add enough water to cover the bottom of a glass petri dish.
2. Place the petri dish over a thin, flat ruler.
3. With tweezers, add a crystal of potassium permanganate ($KMnO_4$) directly over a millimeter measurement line.

4. Set a timer and wait 10 minutes for the color to diffuse. DO NOT disturb (bump, shake) the dish during this time!
5. After 10 minutes, note the distance the color has moved. Record this value in Table 4.1 (convert to mm if necessary).
6. Record the diffusion time (in hours) in Table 4.1.
7. Calculate the speed of diffusion (mm/hr) and record in Table 4.1.

Diffusion through a Semisolid

1. Observe a petri dish containing 1.5% gelatin (or agar) to which potassium permanganate ($KMnO_4$) was added in the center depression at the beginning of the lab.
2. Obtain *time zero* from your instructor, and calculate how much time has passed since time zero. Convert this to hours if necessary, and record in Table 4.1.
3. Using a ruler placed over the petri dish, measure (in mm) the distance from the center of the depression (where the crystal was placed) outward in **one** direction: _____ mm.
4. Calculate the speed of diffusion: _____ mm/hr.
5. Record all data in Table 4.1.

Table 4.1 Speed of Diffusion

Medium	Diffusion Time (hr)	Distance Moved (mm)	Speed of Diffusion (mm/hr)
Air			
Liquid			
Semisolid			

Conclusions

- In which experiment was diffusion the fastest? _____
- What accounts for the difference in speed?

  _____

1. Osmosis

In living organisms most substances are transported as **solutes**, dissolved in water, a **solvent**. For example, if we dissolve salt in a beaker of water, salt is the solute and water is the solvent. If a solute diffuses across a membrane, we refer to this type of diffusion as **dialysis**. If a solvent (water) diffuses across a membrane, the process is referred to as **osmosis**. In either case, the plasma membrane will either inhibit or facilitate the process of diffusion: some molecules can easily diffuse across a plasma membrane and some cannot. For example, small, nonpolar molecules (such as CO_2 and O_2) can cross a membrane by simple diffusion. Large molecules or

polar molecules, however, cannot easily diffuse across a membrane. Cells must have specialized membrane-bound proteins that function to transport such substances across the membrane.

In this experiment, you will learn about osmosis using dialysis membrane, a selectively permeable sheet of cellulose that permits the passage of water, but does not allow larger molecules to diffuse across. This is because the membrane has microscopic pores that only allow small molecules through; anything larger than the size of the pores is prevented from crossing.

Procedure:

1. Cut 4 pieces of dialysis membrane approximately 10 cm long. Soak the pieces in tap water until they are soft and pliable (3-5 minutes).
2. Cut 8 pieces of string approximately 6 cm long. Set aside.
3. Obtain 4 beakers and label them #1-4. Fill each beaker with 150 mL of solution as follows:
    - Beaker #1 – H_2O
    - Beaker #2 – H_2O
    - Beaker #3 – H_2O
    - Beaker #4 – 30% sucrose solution
4. Set beakers aside.
5. Remove one piece of dialysis membrane from the soaking water and open it, forming a tube. Close one end of the tube by tying with a piece of string. (Figure 4.2).

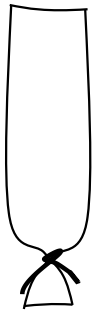
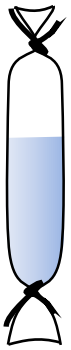


Figure 4.2 Dialysis tube

6. Fill the tube with 10 mL of H2O. Remove excess air, and close the other end of the tube with a piece of string (Figure 4.3). Set aside on a paper towel.



7. Repeat steps 5-6 for the 3 remaining dialysis tubes, filling them with 10 mL of solution as follows:
    - Tube #2 – 15% sucrose
    - Tube #3 – 30% sucrose
    - Tube #4 – H_2O
8. Rinse off the outside of the bags with water and carefully blot dry.
9. Weigh each bag to the nearest 0.5g. Record the weights in Table 4.2, in the column labeled “0 min.”
10. Place each bag in the corresponding beaker (Bag #1 in Beaker #1, etc.).
11. Set a timer for 5 minutes.
12. At the end of 5 minutes, remove each bag from its beaker, blot excess fluid, and record the mass in Table 4.2.
13. Return the bags to the appropriate beaker, and wait another 5 minutes.

14. Repeat steps 12-13 every 5 minutes and record the weights in Table 4.2.

Table 4.2 Osmosis - Mass over time for dialysis bags					
			Mass (g)		
Time (min):	**0**	**5**	**10**	**15**	**20**
Bag #1					
Bag #2					
Bag #3					
Bag #4					

Table 4.3 Rate of Osmosis			
	Weight change (g)	**Time (min)**	**Rate (g/min)**
Bag #1			
Bag #2			
Bag #3			
Bag #4			

Calculate the total weight change (weight change = final weight – initial weight) for each bag. Record the values in Table 4.3. Calculate the **rate** (g/min) of osmosis for each bag by dividing the weight change by the time change. Since all 4 bags were recorded for a total of 20 minutes, the time change for all 4 bags is 20 minutes. Record the rate of osmosis for all 4 bags in Table 4.3.

Did the weight of each bag change significantly over 20 minutes? _____
In which bag(s) was there a net movement of water? _____
Explain what is meant by "net movement".
_____

Why wouldn't sugar molecules be able to move across the membrane?
_____

In terms of solvent concentration, water moved from the area of _____ concentration to the area of _____ concentration across a selectively permeable membrane.

2. Tonicity

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

- An **isotonic solution** has the same concentration of solute (and therefore of water) as the cell. When cells are placed in an isotonic solution, there is no net movement of water.
- A **hypertonic solution** has a higher solute (therefore, lower water) concentration than the cell. When cells are placed in a hypertonic solution, water moves out of the cell into the solution.
- A **hypotonic solution** has a lower solute (therefore, higher water) concentration than the cell. When cells are placed in a hypotonic solution, water moves from the solution into the cell.

Potato Strips. Your instructor will set up two test tubes. In one test tube, a potato strip in soaking in water. In another tube, a potato strip is soaking in 10% sodium chloride (NaCl). Observe each strip for limpness (water loss) or stiffness (water gain). Which tube has the limp potato strip? _____

Why? _____

Which tube has the stiff potato strip? _____

Why? _____

EXERCISE 5: ENZYME FUNCTION

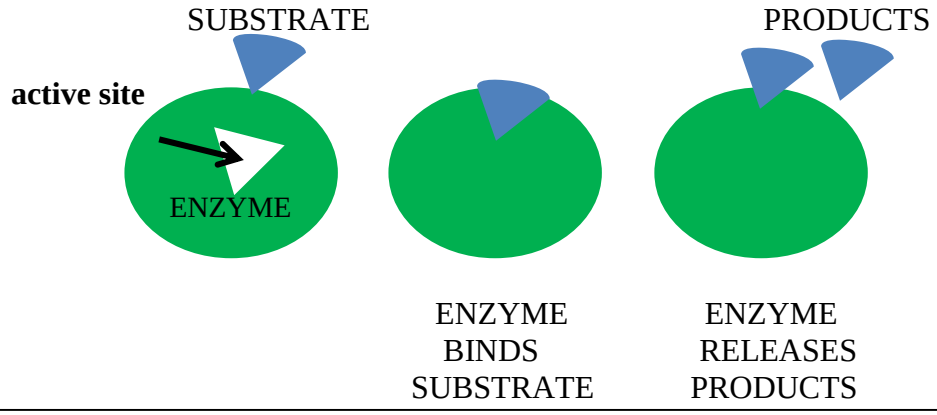
In every living cell, many chemical reactions are performed. In chemical reactions, the **reactants** are molecules that undergo a change, which results in the **products** (Figure 5.1). The arrow stands for the change that produced the product(s). As you can see in the figure below, the number of reactants and products can vary, but the number of atoms is the same on both sides of the arrow. During synthesis reactions (Example 1), the substrates are joined to form a product, while during degradation reactions, the substrate is broken down to the products (Example 2). With replacement reactions, one element replaces another in a compound (Example 3).

Example 1:	A + B	→	AB	$2\ H_2 + O_2$	→	$2\ H_2O$
Example 2:	AB	→	A + B	$2\ H_2O$	→	$2\ H_2 + O_2$
Example 3:	A + BC	→	AC + B	$2\ Na + 2\ H_2O$	→	$2\ NaOH + H_2$

Figure 5.1: In a chemical reaction, reactants are converted to products

Enzymes are organic molecules that speed chemical reactions. They work by binding to the reactants, and converting them to a different molecule (the product). Enzymes are specific and speed only one type of reaction, because they have a shape that only permits binding to one type of reactant, much like a key fits a lock (Figure 5.2). The reactants in an enzymatic chemical reaction are called **substrate(s)**. Notice how the shape of the enzyme fits is substrate. The location where the enzyme binds a substrate is called the **active site** because the reaction occurs here. At the end of the reaction, the product is released and the enzyme can then bind to more substrate. Thus, an enzyme can perform the reaction over and over again, as long as the substrate is present. In general, an increase in the amount of substrate will correspond to an increase in the speed of a chemical reaction.

Figure 5.2 Enzymes bind a specific substrate and convert it to the product(s). The product(s) are released, and the enzyme is unchanged.



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

Materials:

- Catalase
- Hydrogen peroxide
- Small test tubes
- Ruler
- Wax Pencil
- Ice, boiling water bath, refrigerator, warm incubator
- HCl, NaOH, pH paper

I. Catalase Activity

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

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

hydrogen peroxide → water + oxygen gas

What is the reactant in this reaction? _____

What is the substrate for catalase? _____

What are the products in this reaction? _____

Bubbling occurs as the reaction proceeds. Why? _____

1. With a wax pencil, label three test tubes (#1, #2, #3) and mark each tube at the 1cm and 5cm levels.
2. Fill tube #1 the first mark with catalase. Fill to the second mark with hydrogen peroxide. Swirl well to mix, and wait at least 20 seconds for bubbling to develop.
3. Measure the height of the bubble column (in millimeters), and record your results in table 5.1, below.
4. Fill tube #2 to the first mark with water. Fill to second mark with hydrogen peroxide. Swirl well to mix, and wait at least 20 seconds.
5. Measure the height of the bubble column (in millimeters), and record your results in the table below.

6. Fill tube #3 to the first mark with catalase. Fill to the second mark with sucrose solution. Swirl well to mix; wait 20 seconds.
7. Measure the height of the bubble column and record your results in table below.

Table 5.1: Catalase activity

Tube	Contents	Bubble Column Height (mm)
1	Catalase, Hydrogen peroxide	
2	Water, Hydrogen peroxide	
3	Catalase, Sucrose solution	

Which tube showed the most bubbling? _____

Why? _____

Which tube was a negative control? _____

Which tube was a positive control? _____

Why didn't you observe bubbles in tube #3? _____

_____

II. Effect of Temperature on Enzyme Activity

In general, cold temperatures slow chemical reactions, and warm temperatures speed chemical reactions. Every enzyme, however, has an **optimal temperature** at which it works best. Some enzymes prefer cooler temperatures, and others prefer warm temperatures. In any case, boiling an enzyme will denature it, making it inactive.
In the following exercise, you will test enzyme function at four different temperatures (on ice, in a refrigerator, in a warm incubator, and in a boiling water bath). Before setting up this experiment, formulate a hypothesis regarding the effect of temperature on enzyme function. For example, do you think the enzyme will perform better at warmer, cooler, one ice or boiling? State your hypothesis:

_____

_____

1. With a wax pencil, label four test tubes (#1, #2, #3, #4) and mark each at the 1cm and 5 cm levels.
2. Fill each tube to the first mark with catalase.
3. Place tube #1 in on ice, tube #2 in a refrigerator, tube #3 in an incubator, and tube #4 in a boiling water bath. Wait 15 minutes.
4. While you are waiting, use a thermometer to measure the temperature of the environment for all four tubes. Record the values in table 5.2, below.

5. After 15 minutes, fill each tube to the second mark with hydrogen peroxide.
6. Swirl well to mix, and wait 20 seconds.
7. Measure the height of the bubbles column (in mm) for each tube, and record your results in table 5.2, below.

Table 5.2: Effect of temperature on enzyme function

Tube	Temperature ^{0}C	Bubble Column Height (mm)
1 - On Ice		
2 - Refrigerator		
3 - Incubator		
4 - Boiling water		

Which tube showed the most enzyme activity? _____

What is the optimal temperature for catalase? _____

Was your hypothesis supported? _____

What is your conclusion concerning the effect of temperature on enzyme activity?

_____

What is the optimal temperature for enzymes in the human body? _____

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

_____

III. Effect of Concentration on Enzyme Activity

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

1. With a wax pencil, label three test tubes (#1, #2, #3).
2. Mark tube #1 at the 1cm and 5 cm levels.
3. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
4. Swirl well to mix, and wait 10 seconds.
5. Measure the height of the bubble column (in mm), and record your results in table 5.3.
6. Mark tube #2 at the 2cm and 6cm levels.
7. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
8. Swirl well to mix, and wait 10 sec.
9. Measure the height of the bubble column (in mm), and record your results in table 5.3.
10. Mark tube #3 at the 3cm and 7cm levels.

11. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
12. Swirl well to mix, and wait 10 seconds.
13. Measure the height of the bubble column (in mm), and record your results in Table 5.3

Table 5.3: Effect of concentration on enzyme activity

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

The amount of bubbling corresponds to the degree of enzyme activity. Which tube showed the most activity? _____
If we waited for an unlimited amount of time, would the results be the same in all tubes? Explain.

_____

_____

In this experiment, was the amount of substrate the same in all three tubes?
Would you expect the same results if the substrate concentration were varied in the same manner as the enzyme concentration? Explain.

_____

_____

IV. Effect of pH on Enzyme Activity

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

1. With a wax pencil, label three test tubes (#1, #2, #3) and mark at the 1cm, 2cm, and 6cm levels. Fill each tube to the 1cm level with catalase.
2. Fill tube #1 to the second mark with water adjusted to pH 3.
3. Fill tube #2 to the second mark with water adjusted to pH 7.
4. Fill tube #3 to the second mark with water adjusted to pH 11.
5. Carefully swirl to mix, and wait for 20 seconds.
6. Fill all three tubes to the third mark with hydrogen peroxide.
7. Carefully swirl to mix, and wait for 20 seconds.
8. Measure the height of the bubble column (in mm) for each tube, and record your results in table 5.4, below.

Table 5.4: Effect of pH on enzyme function		
Tube	**pH**	**Bubble Column Height (mm)**
1	3	
2	7	
3	11	

Which tube showed the most activity? _____
What is the optimal pH for catalase? _____
If an enzyme functions well at a pH of 4, would you expect it to also work well at a pH of 7? Explain. _____
_____

The average pH of human blood is 7.4. Many enzymes in the blood speed up chemical reactions.

What do you think the optimal pH for these enzymes is? _____

What would happen to enzyme function if the pH of your blood became acidic or basic?
_____

EXERCISE 7: CELLULAR RESPIRATION

Cell respiration refers to the process of converting the chemical energy of organic molecules into a form immediately usable by organisms. Glucose may be oxidized completely if sufficient oxygen is available according to the following equation:

$$C_6H_{12}O_6 + 6O_2\,(g) \rightarrow 6\,H_2O + 6\,CO_2\,(g)$$

All organisms, including plants and animals, oxidize glucose for energy. Often, this energy is used to convert ADP and phosphate into ATP. Peas undergo cell respiration during germination, since they require energy to grow. Do you think peas undergo cell respiration before germination? **State your hypothesis here**:

_____

Today you will collect data to test your hypothesis, and answer additional questions regarding respiration and non-germinating peas.

Using a CO_2 Gas Sensor and O_2 Gas Sensor, you will monitor the carbon dioxide produced and the oxygen consumed by peas during cellular respiration. Both germinating and non-germinating peas will be tested. Additionally, cell respiration of germinating peas at two different temperatures will be investigated.

In today's laboratory exercise, you will

- Use an O_2 Gas Sensor to measure concentrations of oxygen gas.
- Use a CO_2 Gas Sensor to measure concentrations of carbon dioxide gas.
- Study the effect of temperature on cell respiration.
- Determine whether germinating peas and non-germinating peas respire.
- Compare the rates of cell respiration in germinating and non-germinating peas.

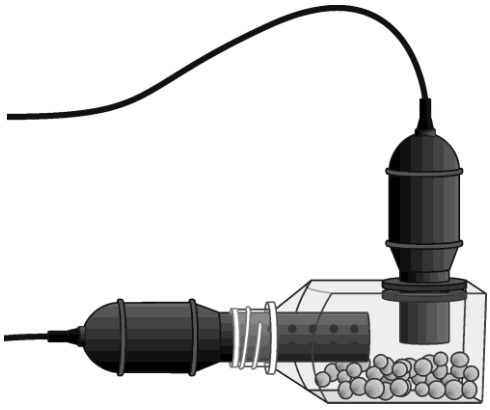


Figure 6.1

Materials:

- LabQuest App
- Vernier CO2 Gas Sensor
- Vernier O2 Gas Sensor
- Vernier BioChamber 250
- Two 100 mL beakers
- Thermometer
- 25 germinated peas
- 25 non-germinated peas
- Ice

I. Germinating vs. non-germinating respiration rate

In this part of the experiment, you will compare respiration rates of germinating peas with non-germinating peas.

1. If your CO_2 Gas Sensor has a switch, set it to the Low (0–10,000 ppm) setting. Connect the O_2 Gas Sensor and the CO_2 Gas Sensor to LabQuest.
2. Choose **New** from the **File** menu.
3. Measure the room temperature using a thermometer and record the temperature in Table 6.1.
4. Obtain 25 germinated peas and blot them dry between two pieces of paper towel.
5. Place the germinated peas into the respiration chamber.
6. Place the O_2 Gas Sensor into the respiration chamber as shown in Figure 6.1. Insert the sensor snugly. **The O_2 Gas Sensor should remain vertical throughout the experiment**. Place the CO_2 Gas Sensor into the neck of the respiration chamber.
7. After 2 minutes, start data collection.
8. When data collection has finished, remove the sensors from the respiration chamber. Place the peas in a 100 mL beaker filled with cold water and an ice cube.
9. Fill the respiration chamber with water and then empty it. Thoroughly dry the inside of the respiration chamber with a paper towel.
10. Perform a linear regression to calculate the **rate** of respiration as follows:
    - Choose **Curve Fit** from the **Analyze** menu and select CO_2 Gas.
    - Select **Linear** as the Fit Equation. The linear-regression statistics for these two data columns are displayed for the equation in the form

$$y = mx + b$$

    - Enter the value of the slope (m) as the rate of respiration for the CO_2 Gas Sensor in Table 6.2.
    - Select OK.

11. Next, calculate the rate of respiration for the O_2 Gas Sensor:
    - Choose **Curve Fit** from the **Analyze** menu and select O_2 Gas.
    - Select **Linear** as the Fit Equation.
    - Enter the value of the slope (*m*) as the rate of respiration for the O_2 Gas Sensor in Table 6.2 and select OK.
12. Repeat Steps 5–11 **substituting the germinated peas with non-germinated peas**. In Step 8 place the non-germinated peas on a paper towel and **not** in the ice bath. Record your results in Table 6.2.

II. Germinating respiration rate at cooler temperatures

You will now examine the effect of temperature on cellular respiration. Do you think peas will have higher or lower respiration rates in colder temperatures? **State your hypothesis here**:

_____

1. Remove the peas from the cold water you previously placed them in. Gently blot them dry between two paper towels.
2. Place the cold, germinated peas into the respiration chamber.
3. Place the O_2 Gas Sensor into the respiration chamber as shown in Figure 6.1. Place the CO_2 Gas Sensor into the neck of the respiration chamber.
4. After 2 minutes, start data collection.
5. When data collection has finished, remove the sensors from the respiration chamber. Place the peas on a paper towel.
6. Perform a linear regression to calculate the **rate** of **cold** respiration as follows:
    - Choose **Curve Fit** from the **Analyze** menu and select CO_2 Gas.
    - Select **Linear** as the Fit Equation.
    - Enter the value of the slope (*m*) as the rate of respiration for the CO_2 Gas Sensor in Table 6.2.
    - Select OK.
7. Next, calculate the rate of cold respiration for the O_2 Gas Sensor:
    - Choose **Curve Fit** from the **Analyze** menu and select O_2 Gas.
    - Select **Linear** as the Fit Equation.
    - Enter the value of the slope (*m*) as the rate of respiration for the O_2 Gas Sensor in Table 6.2.
    - Select OK.

Table 6.1: Experimental conditions	
Room Temperature (°C)	

Table 6.2: Respiration rates		
	O_2 Rate of respiration (ppt/s)	CO_2 Rate of respiration (ppt/s)
Germinating, room temperature		
Non-germinating, room temperature		
Germinating, cold temperature		

1. Do you have evidence that cellular respiration occurred in peas? Explain.

2. What is the effect of germination on the rate of cell respiration in peas?

3. What is the effect of temperature on the rate of cell respiration in peas?

4. Why do germinating peas undergo cell respiration?

EXERCISE 8: PHOTOSYNTHESIS

Plants make sugar, storing the energy of the sun into chemical energy, by the process of photosynthesis. When they require energy, they can tap the stored energy in sugar by a process called cellular respiration.

The process of photosynthesis involves the use of light energy to convert carbon dioxide and water into sugar, oxygen, and other organic compounds. This process is often summarized by the following reaction:

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

Cellular respiration refers to the process of converting the chemical energy of organic molecules into a form immediately usable by organisms. Glucose may be oxidized completely if sufficient oxygen is available by the following equation:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2 + \text{energy}$$

All organisms, including plants and animals, oxidize glucose for energy. Often, this energy is used to convert ADP and phosphate into ATP.

OBJECTIVES

In this experiment, you will

- Use an O_2 Gas Sensor to measure the amount of oxygen gas consumed or produced by a plant during respiration and photosynthesis.
- Use a CO_2 Gas Sensor to measure the amount of carbon dioxide consumed or produced by a plant during respiration and photosynthesis.
- Determine the rate of respiration and photosynthesis of a plant.

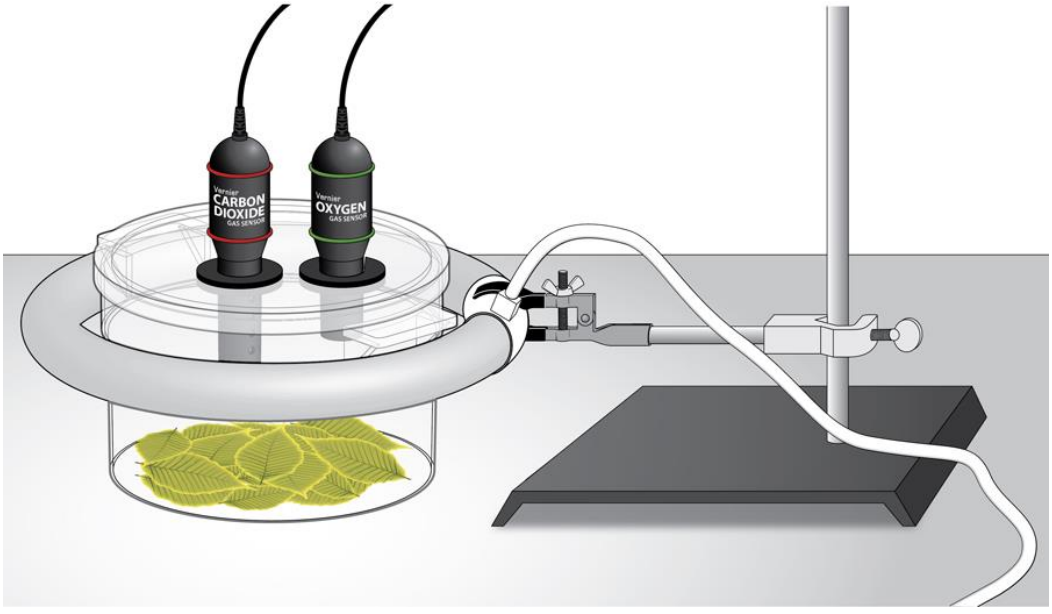


Figure 7.1

Materials:

- LabQuest App
- Vernier CO2 Gas Sensor
- Vernier O2 Gas Sensor
- Vernier BioChamber 250
- Aluminum foil
- Ringstand
- Utility clamp
- 12-inch ring fluorescent lamp setup
- Elodia

PROCEDURE

1. Wrap the BioChamber with aluminum foil so that no light will reach the leaves.
   a. Wrap the outside of the chamber with foil.
   b. Cover the lid with foil, poking the holes open to insert the sensors.
2. Cover the bottom of the chamber with a layer of water and a few Elodia plants.
3. If your CO_2 Gas Sensor has a switch, set it to the Low (0–10,000 ppm) setting. Connect the CO_2 Gas Sensor to Channel 1 and the O_2 Gas to Channel 2 of the Vernier computer interface.
5. Secure the lid on the chamber and insert the sensors into the holes.
6. Wait five minutes for the sensors to equilibrate, then click Collect to begin the 15-minute data collection.
7. When data collection is complete, determine the rate of respiration.
   a. Click anywhere on the CO_2 graph. Autoscale the data by clicking the Autoscale button on the toolbar.
   b. Use the stylus to highlight a region of increase or decrease by dragging over it.
   c. Click on the Linear Fit button, , to perform a linear regression. A box will appear with the formula for a best fit line.
   d. Record the slope of the line, *m*, as the rate of respiration in Table 1.
   e. Close the linear regression box.
   f. Repeat Steps 7a–e for the O_2 graph, selecting the region of decreasing or increasing O_2 concentration.

8. Store your data by choosing Store Latest Run from the Experiment menu.
9. Assemble the lamp as shown in Figure 1. **Important:** Do not turn the lamp on until instructed to do so.
10. Remove the aluminum foil from the respiration chamber and invert the chamber to remove the leaves and accumulated gases.
11. Line the bottom of the chamber with fresh elodia.
12. Secure the lid on the chamber and insert the sensors into the holes.
13. Place the chamber inside the bulb and turn on the lamp.
14. Repeat Steps 6–7 to collect and analyze data.
15. Clean and dry the chamber.

DATA

Table 7.1

Leaves	CO_2 rate of production/consumption (ppt/min)	O_2 rate of production/consumption (ppt/min)
In the dark		
In the light		

QUESTIONS

1. Was either of the rate values for CO_2 a positive number? If so, what is the biological significance of this?

2. Was either of the rate values for O_2 a negative number? If so, what is the biological significance of this?

3. Do you have evidence that cellular respiration occurred in leaves? Explain.

4. Do you have evidence that photosynthesis occurred in leaves? Explain.

5. List five factors that might influence the rate of oxygen production or consumption in leaves. Explain how you think each will affect the rate?

EXTENSIONS

1. Design and perform an experiment to test one of the factors that might influence the rate of oxygen production or consumption in Question 5.

2. Compare the rates of photosynthesis and respiration among various types of plants.

EXERCISE 9: MITOSIS & MEIOSIS

Eukaryotic cells exist in a **cell cycle**, which consists of separate phases. The first phase, **interphase**, is the period of time during which the cell performs its normal functions. Cells spend most of their time in this phase. **Chromosomes** are not fully condensed, and appear as a tangled mass of threads. The nuclear membrane is present, as are one or more **nucleoli**. Toward the end of this phase, many cells prepare to divide. In this case, cells will grow, make proteins, and replicate the DNA. Interphase is followed by a second phase, either **mitosis or meiosis**, in which the nucleus and cytoplasm are divided. This laboratory exercise examines both mitotic and meiotic cell division.

Materials:

- Whitefish blastula slide
- Compound light microscope
- Pop beads

III. Mitosis

Mitosis is nuclear division that results in two new nuclei with the same number of chromosomes as the original call. Most human cells (skin, muscle, bone, etc.) divide via mitosis. This process is necessary for normal growth and repair. At the end of mitosis, two **daughter cells** are formed, and they are identical to the original (parent) cell. Mitosis is a form of **asexual reproduction**.

The process is complex and highly regulated, and occurs in 4 separate phases:

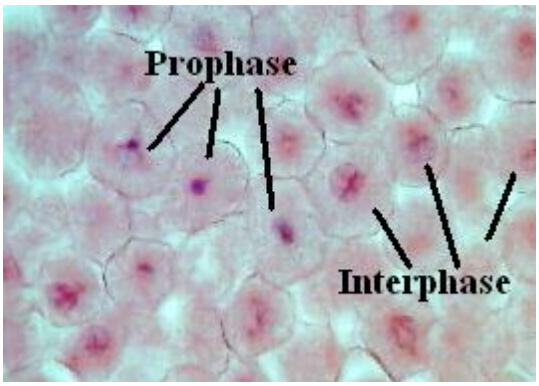
Prophase: During prophase, cells prepare for division by coiling and condensing the chromosomes. By late prophase, individual chromosomes can be seen, each consisting of two **chromatids** joined at a **centromere. Spindle fibers** begin to form, while the **nucleoli** and the **nuclear membrane** are degraded.

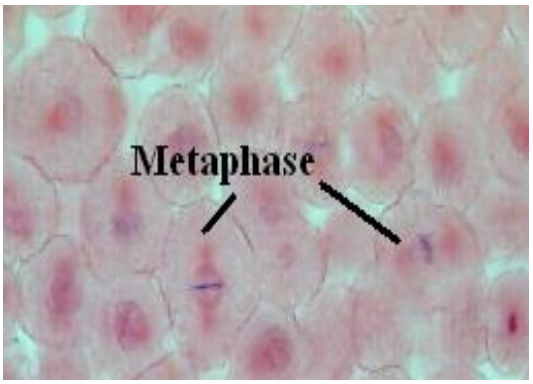
Metaphase: During this phase, chromosomes become attached by their centromeres to the spindle fibers. The fibers pull the chromosomes and align them on a plane that passes through the center of the cell (the equatorial plane).

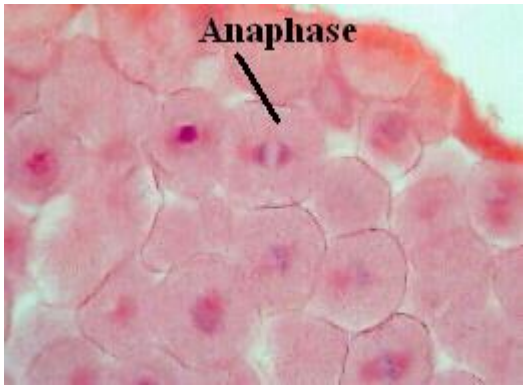
Anaphase: Chromosomes are equally distributed to opposite sides of the cell during anaphase. Each centromere divides, and the two identical chromatids are pulled apart (toward opposite poles of the cell) by the spindle fibers.

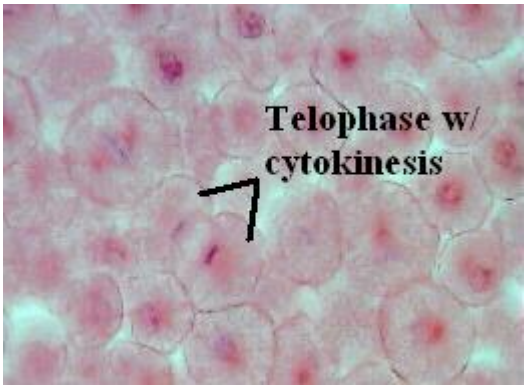
Telophase: During the final phase of mitosis, chromosomes uncoil and become difficult to see individually. The spindle fibers are degraded, and a new nuclear membrane is formed around each set of chromosomes. Nucleoli reform within each of the new nuclei. **Cytokinesis** (division of the cytoplasm) occurs by the formation of a cleavage furrow (or cell plate in plants).

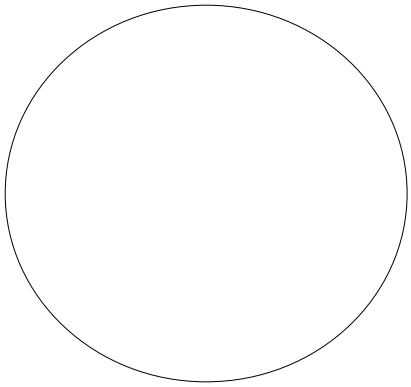
1. Examine slides of whitefish blastula, and draw each stage of the cell cycle:



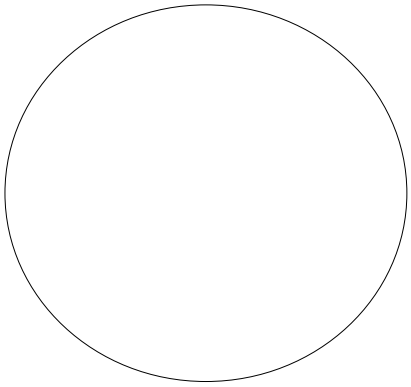




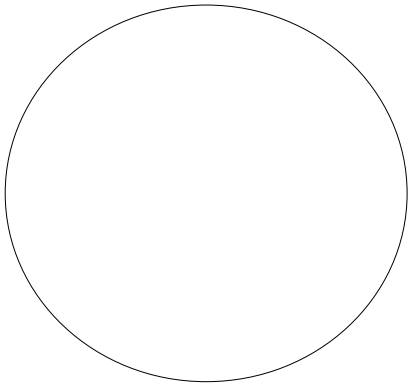




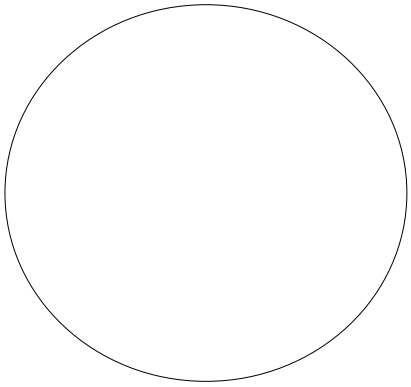
interphase



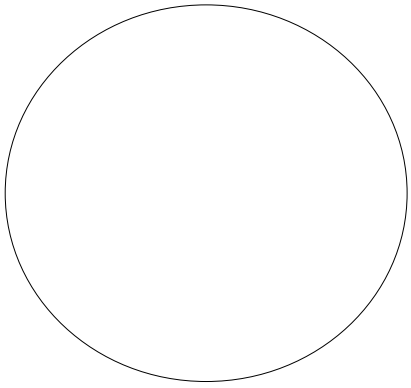
prophase



metaphase



anaphase



telophase

For the next part of this activity, you will be using pop beads to represent DNA.

1. To familiarize yourselves with the pop beads, construct two pair of homologous chromosomes. The yellow beads will represent maternal DNA, and the red beads will represent paternal DNA. Thus, for one pair of homologous chromosomes, one should be yellow and one should be red.
2. Once you have constructed your chromosomes, make an identical copy of each, attaching the chromatids at the (magnetic) centromere.
3. Recall that when the chromosomes are fully condensed, and consist of two chromatids joined at the centromere, they are in prophase. Place the chromosomes on your desk to represent their position in the nucleus of the cell. Use string to represent the nuclear membrane and the cell membrane.
4. Now, remove the nuclear membrane and align the chromosomes as they would appear in metaphase. Separate the chromatids, as they would be separated in anaphase. Use string to represent new nuclear membranes forming in telophase.

IV. Meiosis

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

Cells that divide via meiosis prepare for division (during interphase) much like every other cell. Meiosis also progresses through the same phases as mitosis (prophase, anaphase, metaphase, telophase). Unlike mitosis, meiosis involves two divisions (meiosis I and meiosis II), which reduces the chromosomal number.

Prophase I: During prophase of meiosis I, the chromosomes join in homologous pairs. **Homologous chromosomes** are the same length, and carry genetic information (**genes**) for the same traits, but perhaps different versions (**alleles**) of the gene. [For example, human chromosome #19 contains a gene for eye color. One allele codes for blue eyes, and another codes for green. Since every human inherits two of chromosome 19 (one from mom, one from dad), a person could have 2 blue alleles, 2 green alleles, or one of each.] Once homologous chromosomes pair up, they are in a stage called **synapsis**. During synapsis, equivalent pieces of homologous chromatids are exchanged between the chromosomes. This is called **crossing-over**, and can occur several times along the length of the chromosomes. Similar to mitotic division, prophase of meiosis I also involves the degradation of the nuclear membrane and formation of the spindle fibers.

Metaphase I: Metaphase of meiosis I occurs when the joined homologous chromosomes are pulled to the center of the cell by the spindle fibers. The spindle fibers arrange the pairs so that homologs are on opposite sides of the equatorial plane. **Anaphase I** follows, as homologs are pulled apart, toward opposite ends of the cell. [*Note: this is significantly different from the separation of **chromatids**, which occurs during mitosis]. **Telophase I** marks the end of meiosis I, as new nuclei form and cytokinesis separates the cytoplasm.

At the end of meiosis I, the two daughter cells have half the number of chromosomes as the parent cell. Thus, the cells have been reduced from **diploid** (2n) to **haploid** (n). [n refers to the number of chromosomes for a species. Humans have 23, so a diploid human call would have 2(23), or 46].

Meiosis II follows meiosis I, which proceeds very much like mitosis. Chromosomes line up on the equators of each cell, the centromeres are separated apart, and sister chromatids are pulled to each pole of the cell. Telophase II completes the process of gamete formation, which results in four haploid cells. These cells (egg or sperm, in humans) will either unite (via fertilization) or die. They do not divide further on their own.

To fully understand the mechanism involved in the production of gametes, we will manipulate models of chromosomes carrying specific genes.

1. Make 4 chromosome models that have already completed DNA replication (and therefore are composed of 2 identical chromatids). See Figure 1, or ask your instructor.
2. Place the 4 chromosomes on your desk, or large sheet of paper (use string to represent the cell and nuclear membranes).
3. Note the genes on the short pair of homologous chromosomes. At one point (**locus**) on each, there is a gene for insulin production. At a different locus is a gene for hair color. The various versions of a gene are alleles.
4. Some alleles are indicated with a capital letter, and others with a lowercase letter. **Dominant** alleles (those that always express themselves) are in capital, while **recessive** (only expressed when no dominant allele is present) are lowercase.
5. Recall that the red beads are paternal (from the father) and yellow beads are materal (from the mother) DNA. The “cell” you are dealing with has 2 sets of genetic information (red and yellow) and is a diploid cell. In Table 7.1, list the **genotype** and **phenotype** of your organism.

Figure 7.1: Homologous chromosome set up

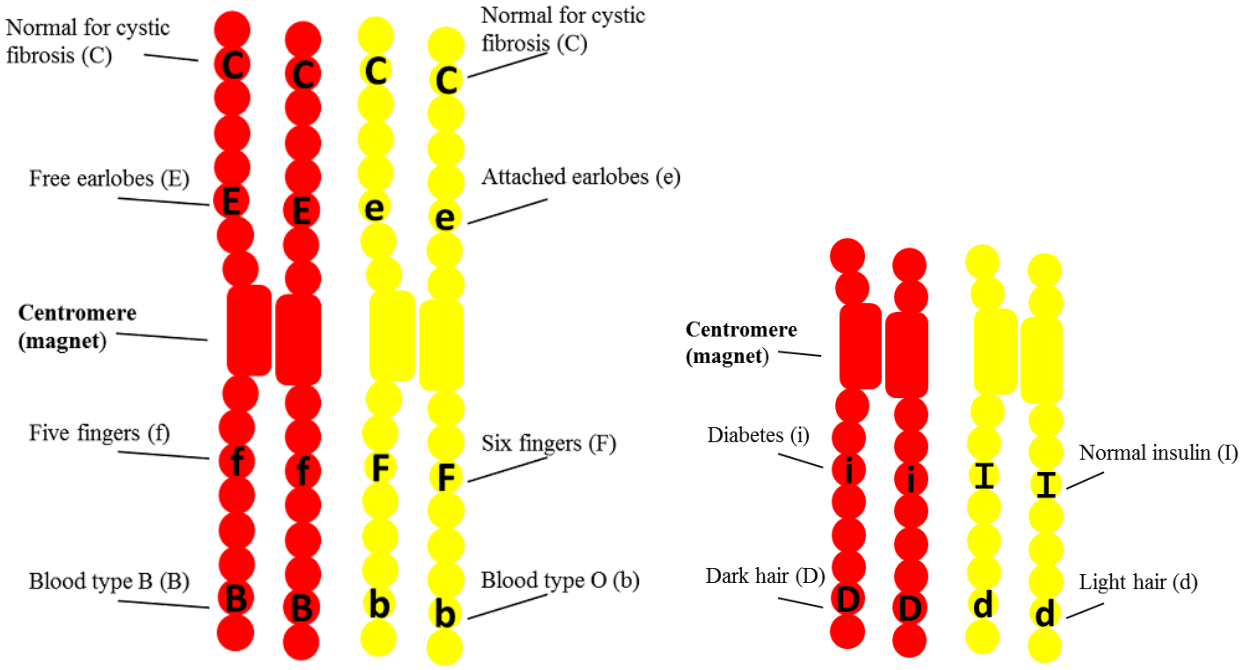


Table 7.1: List of characteristics.	Genotype of Organism		Phenotype of Organism
TRAIT	allele from mom	allele from dad	
Cystic fibrosis			
Ear Shape			
Finger number			
Blood type			
Insulin production ability			
Hair color			

PROPHASE I – synapsis and crossing over

1. Put homologous chromosomes next to each other on your desk to simulate synapsis, the state of the homologs in which they exchange equal pieces of DNA.
2. Detach, exchange, and reattach equal sections of chromatids to simulate crossing over. Make a minimum of 1 crossover for each pair of homologous chromosomes.
3. You should now have chromosomes that contain both paternal AND maternal alleles (i.e., red and yellow). If you do not, reassemble the chromosomes to match Figure 1, and try again (ask your instructor for help if you need it)! The "recombined" chromosomes are the ones that you will continue to use for this activity.
4. Compare your chromosome models with other groups in the class. Are they all identical? Why or why not?

   _____

   _____

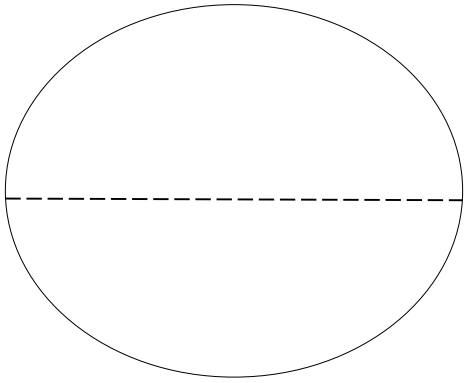
   _____

If you do not understand the process of crossing over, ask your instructor before continuing to the next procedure.

METAPHASE I – independent assortment

During this phase, homologous chromosomes are pulled to the center of the cell. For each pair of chromosomes, its homologs are arranged on opposite sides of the equatorial plane. One pair of chromosomes is arranged independent of another; thus, there are several possible arrangements you can have. (This is significantly different from what happens during mitosis).

1. Arrange your chromosomes on your desk as they would appear in metaphase I (use string for the equatorial plane).
2. Using 2 colors, draw your chromosomes below, where the dashed line represents the equatorial plane.



3. How does the arrangement of chromosomes in metaphase I differ from their arrangement in metaphase of mitosis?

_____

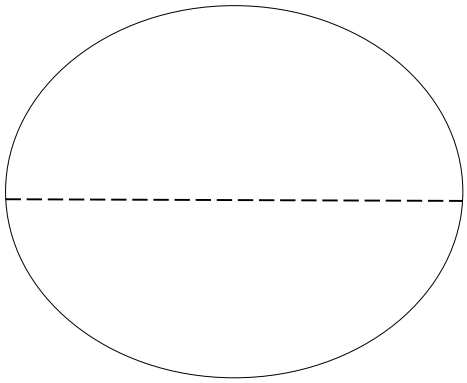
_____

_____

ANAPHASE I – segregation

During this phase, homologous chromosomes are separated. This is called segregation.

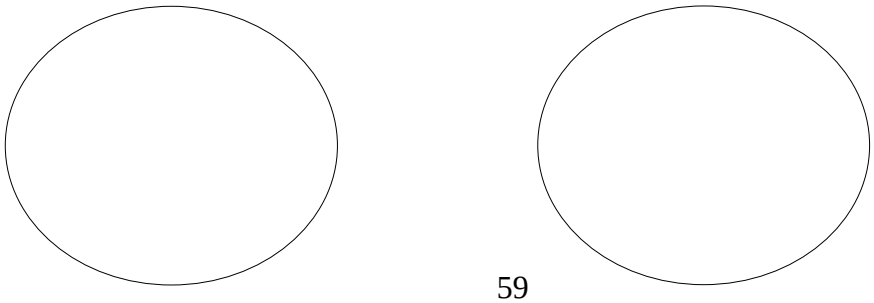
1. Segregate the 2 homologs of each pair of chromosomes by moving them to opposite ends of your "cell".
2. Draw the arrangement of the chromosomes below:



TELOPHASE I – cytokenesis

During this phase, the cytoplasm is divided and 2 daughter cells are formed.

1. Once the chromosomes reach opposite poles of the cell, a **cleavage furrow** (cell plate in plants) forms, dividing the cytoplasm. A nuclear membrane forms around each set of chromosomes (at the poles). Use string to represent new membranes, and construct 2 daughter cells on your desk. Draw the chromosomes in each of the daughter cells.



Compare the genetic information in each of your daughter cells. Are they identical? In Table 7.2, list the genes present in of each of your daughter cells.

Table 7.2				
Genotype-daughter #1	LONG CHROMOSOME		SHORT CHROMOSOME	
	Chromatid #1	**Chromatid #2**	**Chromatid #1**	**Chromatid #2**
Cystic fibrosis				
Ear Shape				
Finger #				
Blood type				
Insulin				
Hair color				
Genotype-daughter #2	LONG CHROMOSOME		SHORT CHROMOSOME	
	Chromatid #1	**Chromatid #2**	**Chromatid #1**	**Chromatid #2**
Cystic fibrosis				
Ear Shape				
Finger #				
Blood type				
Insulin				
Hair color				

List TWO events that occur during meiosis I that contribute to differences among daughter cells.

_____

_____

PROPHASE II – cells prepare for the second division

DNA replication does not occur after telophase I, and each daughter cell enters prophase II. This phase is much like prophase of mitosis. The chromosomes fully condense, the nuclear membrane is degraded, and new spindle fibers form.

1. Simulate this phase using the daughter cells on your desk.
2. How does prophase II differ from prophase I?

   _____

   _____

   _____

METAPHASE II– chromosomes are aligned at the equator

Chromosomes, still composed of 2 chromatids, are lined up on the equatorial plane.

1. Move your chromosomes in each daughter cell as they would appear during metaphase II.
2. The original number of chromosomes in your diploid cell was _____. The number of chromosomes in each daughter cell is _____.
3. Are the daughter cells diploid or haploid? _____

ANAPHASE II– chromatids separate

Centromeres split and the chromatids are pulled toward opposite poles of the cell by the spindle fibers. Once separated, the chromatids are referred to as daughter chromosomes.

1. Separate your chromatids and move them toward opposite poles in each daughter cell.
2. How does anaphase II differ from anaphase I?

   _____

   _____

TELOPHASE II– gametes are formed

During telophase II, cytokinesis occurs in each cell, resulting in four haploid cells called gametes. New membranes are formed around each set of chromosomes, and meiosis is complete.

1. Simulate telophase II in your daughter cells.

2. In table 7.3, list the alleles found in each of your four gametes.
3. Are your gametes different from one another? _____
4. Review meiosis I&II and identify the processes that contribute to the different allele combinations in your gametes. List them.

Table 7.3: Alleles present in gametes

	GAMETE #1	**long chromosome**	**short chromosome**
	Cystic fibrosis		
	Ear Shape		
	Finger #		
	Blood type		
	Insulin		
	Hair color		
	GAMETE #2	**long chromosome**	**short chromosome**
	Cystic fibrosis		
	Ear Shape		
	Finger #		
	Blood type		
	Insulin		
	Hair color		
	GAMETE #3	**long chromosome**	**short chromosome**
	Cystic fibrosis		
	Ear Shape		
	Finger #		
	Blood type		
	Insulin		
	Hair color		
	GAMETE #4	**long chromosome**	**short chromosome**
	Cystic fibrosis		
	Ear Shape		
	Finger #		
	Blood type		
	Insulin		
	Hair color		

FERTILIZATION – joining gametes to form a zygote

Your instructor will assign each group in the class as being a male or female.

1. A male group randomly selects one of its 4 sperm and delivers it to a female group. A female group similarly selects one gamete (randomly).

2. One male group and one female group then unite these two cells to simulate fertilization.
3. Record the genotype and phenotype of your zygote in table 7.4. List the alleles it received from each parent (genotype) and the phenotype (trait) that would be observed.
4. Compare the phenotype of your offspring with that of both parents. Are they the same?

   Explain.

Table 7.4: Genotype & phenotype of offspring

TRAIT	allele from mom	allele from dad	PHENOTYPE
Cystic fibrosis			
Ear Shape			
Finger #			
Blood type			
Insulin			
Hair color			

EXERCISE 10: MENDELIAN GENETICS

Family traits are passed down from generation to generation: we receive one set of chromosomes from our fathers, and one set from our mothers. The set of genes that we inherit is referred to as our **genotype**. A **homozygous** genotype means having two of the same alleles (you inherit the same allele from both parents). A **heterozygous** genotype means that a person has two different alleles (you inherited one allele from mom, and a different allele from dad). A **phenotype** is the outward expression of genes (i.e., eye color, hair color, blood type).

In this exercise, you will be using playing cards to represent alleles for genes (Table 8.1). You will create organisms with specific genotypes and phenotypes and then make offspring.

Materials:

- Playing cards

V. Gamete production

You will first need to come up with a name for the fictitious animal you will be working with today. Table 8.1 below lists the phenotypic possibilities for these animals. After looking through the characteristics, choose a name for this animal:
_____.

Table 8.1 Alleles Each characteristic corresponds to one suit. The dominant alleles are represented by the higher cards (K, Q, J, 10, 9, 8) and the recessive alleles are represented by the lower cards (7 - 2).

Fur color- **HEARTS**	Hair- **CLUBS**	Eyes- **DIAMONDS**	Tail- **SPADES**
Red is dominant (F)	Straight is dominant (S)	2 eyes is dominant (E)	Tail present is dominant (T)
Pink is recessive (f)	Curly is recessive (s)	4 eyes is recessive (e)	Tail absent is recessive (t)

1. Separate a deck of cards into 4 piles by suit (hearts, clubs, spades, diamonds). Remove the aces and jokers.
2. Using your 4 piles of cards, create an animal that is **homozygous dominant** for all 4 traits. List your cards: _____

3. Next, create an animal that is **heterozygous** for all 4 traits. List your cards: _____.

4. Finally, create an animal that is **homozygous recessive** for all 4 traits. List your cards: _____

5. What are the **genotypes** of the 3 animals you just created (use Table 8.1)?
   a. homozygous dominant _____
   b. heterozygous _____
   c. homozygous recessive _____

Now you are ready to create "parents". Return the cards to the correct pile, so that you again have 4. Shuffle each pile, then deal 2 cards from each pile to a NEW pile, which will represent a female.

female cards: _____

female genotype: _____

female phenotype: _____

Create a male by dealing 2 cards from each suit to a NEW pile.

male cards: _____

male genotype: _____

male phenotype:_____

Now create gametes from your parents by RANDOMLY splitting each set of alleles into 2 piles. Remember that gametes only contain **one** allele for each trait.

female gamete #1: cards _____

female gamete #1: genotype_____

female gamete #2: cards _____

female gamete #2: genotype_____

male gamete #1: cards _____

male gamete #1: genotype_____

male gamete #2: cards _____

male gamete #2: genotype_____

Combine one gamete from each parent to create an offspring.

female gamete: genotype_____

male gamete: genotype_____

offspring: genotype_____

offspring: phenotype_____

VI. Crossing single traits

Next you will use a **punnett square** to determine the probability of offspring inheriting a given trait. For example, if you cross two red animals, can you predict what color their offspring will be?

If mom is homozygous for red, her genotype is FF. If dad is heterozygous for red, his genotype is Ff. The mother can pass either of her dominant alleles to her offspring, so we'll list those on one side of a punnett square. The father can pass either F or f, so we'll list those on the other side of the punnett square. The four boxes in the center represent all of the possible allele combinations that these 2 parents can create. By bringing the alleles down or across, we can determine the 4 possibilities.

Table 8.2 Punnett square for monohybrid cross

	F	**F**
F	FF	FF
f	Ff	Ff

In this case, 2 of the 4 possible offspring would be FF, and the other 2 would be Ff. Thus, the genotypic ratio for the offspring is 50%: 50% (which is the same as 1:1). The phenotypic ratio is 1: 0 (100% red, 0% pink).

Now try crossing traits using your cards. Put the cards back into their original 4 piles, according to suit.

1. Deal 2 clubs to represent one animal's hair type.

   Record the cards _____

2. Deal 2 clubs to represent a second animal's hair type.

   Record the cards _____

3. Cross the animals in the punnett square below

4. Record the genotypic ratio _____
5. Record the phenotypic ratio_____
6. Deal 2 diamonds to represent one animal's eyes.

   Record the cards _____

7. Deal 2 diamonds to represent a second animal's eyes

   Record the cards _____

8. Cross the animals in the punnett square below

9. Record the genotypic ratio _____
10. Record the phenotypic ratio _____

VII. Crossing two traits

If we were to cross an animal with a tail and 4 eyes (Ttee) with an animal that has a tail and 2 eyes (TtEe), what would the offspring express? When we consider two different traits, the number of possible allele combinations increases, as shown in the following table.

Table 8.3 Dihybrid cross

	Te	**te**
TE	TTEe	TtEe
Te	TTee	Ttee
tE	TtEe	ttEe
te	Ttee	ttee

Genotypic ratio 1:2:1:2:1:1

Phenotypic ratio 3:3:1:1

Now try crossing traits using your cards. Put the cards back into their original 4 piles, according to suit.

1. Deal 2 hearts and 2 spades (parent #1).
   Record the cards _____
2. Deal 2 hearts and 2 spades (parent #2).
   Record the cards _____
3. List the parental gametes below, and record genotypic and phenotypic ratios for the offspring. YOU MAY NOT NEED TO USE ALL OF THE BOXES!

Genotypic ratio _____

Phenotypic ratio _____

VIII. Other types of inheritance

Practice using punnett squares to determine the genotypic and phenotypic ratios for other types of traits. **Sex linked** traits, for example, display a different pattern of inheritance than those we have looked at throughout this activity. Some forms of color blindness, hemophilia and muscular dystrophy are sex linked disorders resulting from genes found on the X chromosome. In the case of color blindness, a recessive sex linked disorder, males are much more likely to exhibit the recessive condition, because they only carry one X chromosome. Try crossing a female with normal color vision who is a carrier for the disorder (meaning she has the recessive allele) with a male with normal vision. Alleles: XN=normal vision
Xn =color blind

1. Record the male and female genotypes

   Male genotype _____

   Female genotype _____

2. Cross the parents below. Remember that the mother will pass either of her X chromosomes, while the father will pass his X or his Y.
3. Cross the parents, and record genotypic and phenotypic ratios.

   Genotypic ratio _____

   Phenotypic ratio _____

4. What is the probability that the offspring will be color blind?

   _____

5. How could 2 parents have a female child that is color blind? Show your work

Some traits exhibit **codominance**, when there are 2 dominant alleles. Human blood types are one example. The 3 alleles for blood type are A, B (both dominant) and o (recessive). If a person inherits both the A and B alleles, he/she will express them both equally (and therefore have "type AB blood").

1. What is the probability that a woman with type O blood and a man with type AB blood will have a child with type O? Show your work.

Many human traits are **polygenic**, meaning that multiple genes contribute to the phenotype. Hair color, eye color and skin color are examples of polygenic traits, which are more difficult to predict due to the greater number of possible allele combinations. Be sure to ask your instructor if you do not understand the differences among the various types of inheritance.

IX. Summing up

1. Why do gametes contain half the alleles of the parent cell?

2. Is it possible to have offspring with a recessive phenotype if both parents show the dominant phenotype? Explain.

3. Is it possible to have offspring with a dominant phenotype if both parents show the recessive phenotype? Explain.

4. Why are sex linked disorders more common in males?

LAB 11: Gel Electrophoresis, Restriction Enzymes & DNA Fingerprinting (rev. 1/10/2017)

Purpose of Exercise: Gain a basic understanding behind concepts used in DNA fingerprinting. It should be noted that this exercise greatly simplifies the actual process of DNA fingerprinting.

In this exercise, **gel electrophoresis** (Fig. 10.1) will be used to analyze (aka "profile") the DNA of two individuals who are suspects in a crime from which human DNA samples (such as skin cells or hair) were recovered. Your goal is to match the DNA (in reality, this would be **DNA *fragments*** generated by **restriction enzymes**, explained below) from one of these two people (Suspect 1 and Suspect 2) to the DNA (also analyzed as fragments) found at the crime scene.

If the DNA sample from a suspect matches the DNA at a crime scene, then that signifies that the suspect in question *was present at* the crime scene (although he or she may not have actually committed the crime). If the DNA profiles from the crime scene do not match either suspect, then it can be concluded that neither of those individuals was at the crime scene.

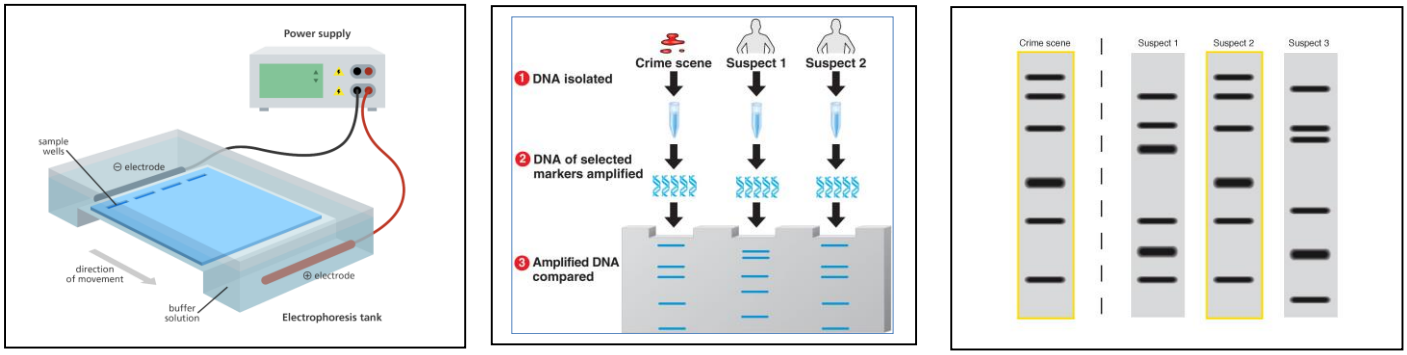


Fig. 10.1. Gel electrophoresis apparatus (left), (stylized) example of methodology used in this technique (center), and (stylized) example of gel from crime scene and three potential suspects (right). The point is to match characteristics of the DNA found at a crime scene with the DNA from potential suspects in the crime. In the right-most figure above, DNA from the crime scene matches DNA from Suspect 2.

Background

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

DNA Finger-printing (aka *DNA typing* or *DNA profiling*) in humans

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

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

Repeats are referred to by a variety of terms – sometimes confusing -- depending on their size. For example, sequence repeats of 9 to 80 bp are called **minisatellites** or **variable number tandem repeats** (VNTR). **Microsatellites**, also known as **short tandem repeats (STR)** contain repeated units of 1 to 6 bp.
Regardless of their size (number of base pairs) or names, DNA repeats show greater variation from one person to another than do other parts of our respective genomes.

The number of times a given repeat unit (for example CTTG indicated above) occurs in any individual's DNA is a function of the DNA that person received from his or her mother and father at conception. For example, three individuals (Mary, Jake and Sue; Fig. 10.2) could exhibit the following variation in the length of a particular repeat sequence on the chromosomes they received from their parents:

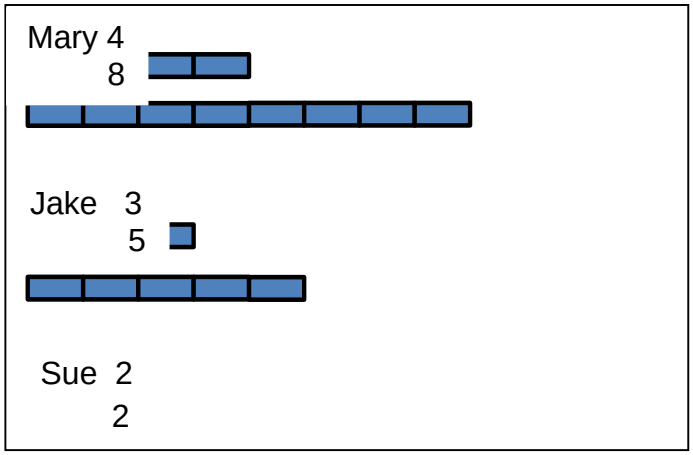


Fig. 10.2. Hypothetical variation in number of STRs in the DNA of 3 individuals. The blue rectangles represent DNA repeats on holomologous chromosomes

The process of DNA profiling uses molecular "scissors" called **restriction enzymes** to cut specific **nucleotide sequences** – in this case repeating sequences of bases -- from our DNA. In this example, such enzymes would recognize particular nucleotide bases at the beginning and end of the repeating string of nucleotides. Consequently, one segment produced in this manner might be CTTGCTTG (2

repeats long) while another might be CTTGCTTGCTTGCTTGCTTGCTTG (6 repeats long). (The DNA segments used in forensic investigations are, of course, much longer than this.)

These DNA pieces of various lengths are separated using gel electrophoresis (see Fig. 10.3 and text below).

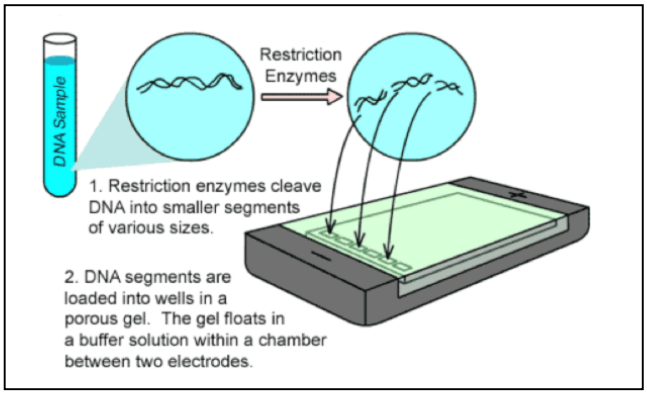


Fig. 10.3. How restriction enzymes are used in DNA fingerprinting.

Restriction Enzymes

First discovered in the 1970s, the **restriction enzymes** used in DNA profiling were developed from the 3000 or more restriction enzymes (aka **restriction endonucleases**) that have been identified from bacteria and that are a defense against the DNA of invading viruses. Specific bacterial restriction enzymes cut double stranded viral DNA -- at specific locations (**base pair sequences**) -- into smaller non-infectious fragments (Fig. 10.4).

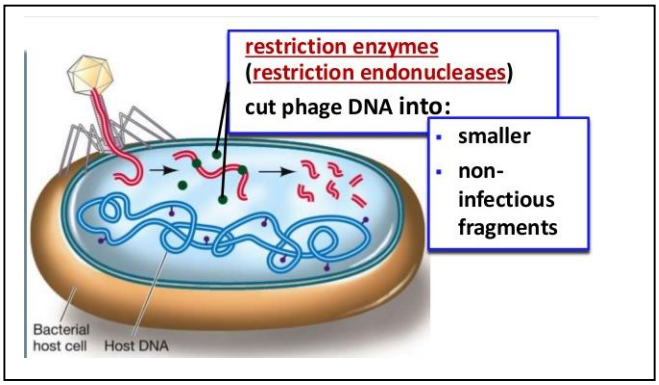


Fig. 10.4. How restriction enzymes function in bacteria.

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

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

*Eco*RI was the first restriction enzyme isolated from the RY13 strain of the bacterium *Escherichia coli*.

In the example below, the enzyme *Eco*RI has cleaved DNA between the G and neighboring A in the GAATTC **recognition site** (Fig. 10.5, top).

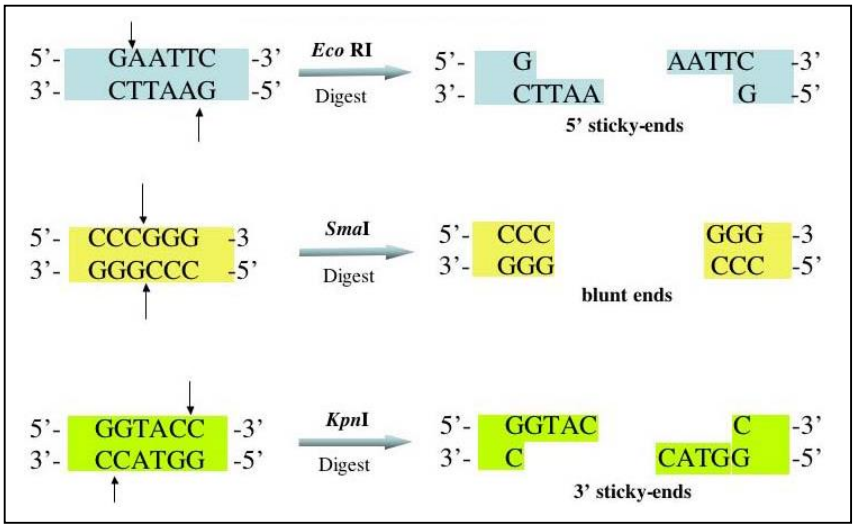


Fig. 10.5. Examples of how restriction enzymes can cut DNA.

It is important to note that the ends of the cleavage (cut) produced by *Eco*RI are staggered, so that the resulting fragments project short overhangs of single-stranded DNA with complementary sequences. Such overhangs are referred to as **"sticky ends"** because the single strands produced can interact with – or stick to – other overhangs of single-stranded DNA with complementary sequences. Digestion of the same piece of DNA using different enzymes can produce sticky ends of different lengths and strand orientations -- 5' vs 3' as occurred with the *Kpn*I enzyme; (Fig. 10.5 bottom).

In contrast, other restriction enzymes (*Sma*I, above, middle) cut both DNA strands at the same position, which generates fragments without an overhang. These so-called **"blunt" ends** can be joined with any other blunt-ended DNA without regard for complementarity.

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

What is Gel electrophoresis?

Gel electrophoresis is a laboratory technique that allows macromolecules, such as DNA or RNA fragments or proteins, in a mixture to be separated according to their molecular size and/or charge. The molecules to be separated are placed in depressions (or sample "wells") in a thin porous gel slab (Fig. 10.6), which is then covered by a buffered solution and placed in a horizontal electrophoresis chamber (Fig. 10.1, 10.8).

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

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

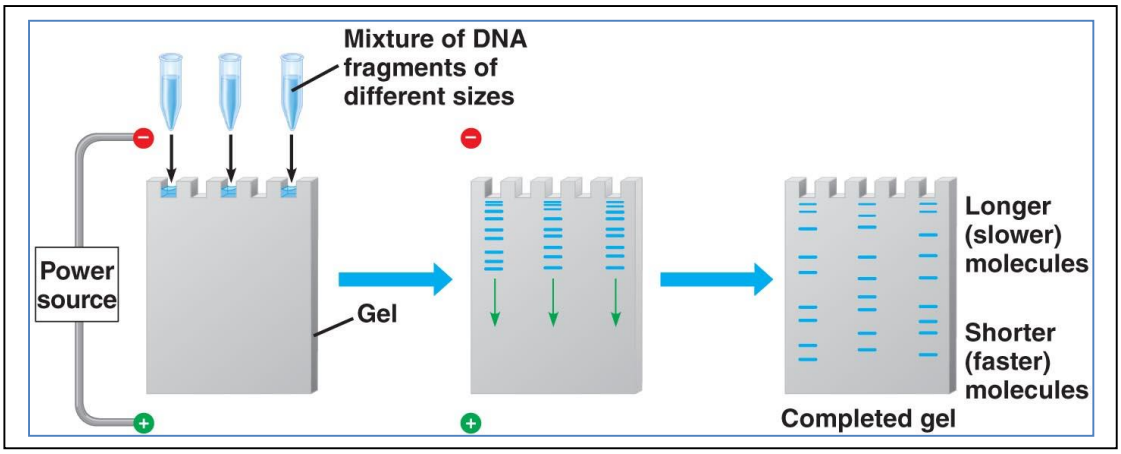


Fig. 10.6. The separation of DNA fragments in gel electrophoresis.

In the experiment we are conducting today, the DNA from three suspects has been digested with a few restriction enzymes in two separate reactions. Using agarose gel electrophoresis, these samples will form bands, which are then compared to DNA samples from a crime scene that have also been digested with the same few enzymes and run simultaneously in the same agarose gel.

The flow chart below (Fig. 10.7) outlines the procedure used for the restriction enzyme digestion of DNA obtained from Suspect 1.

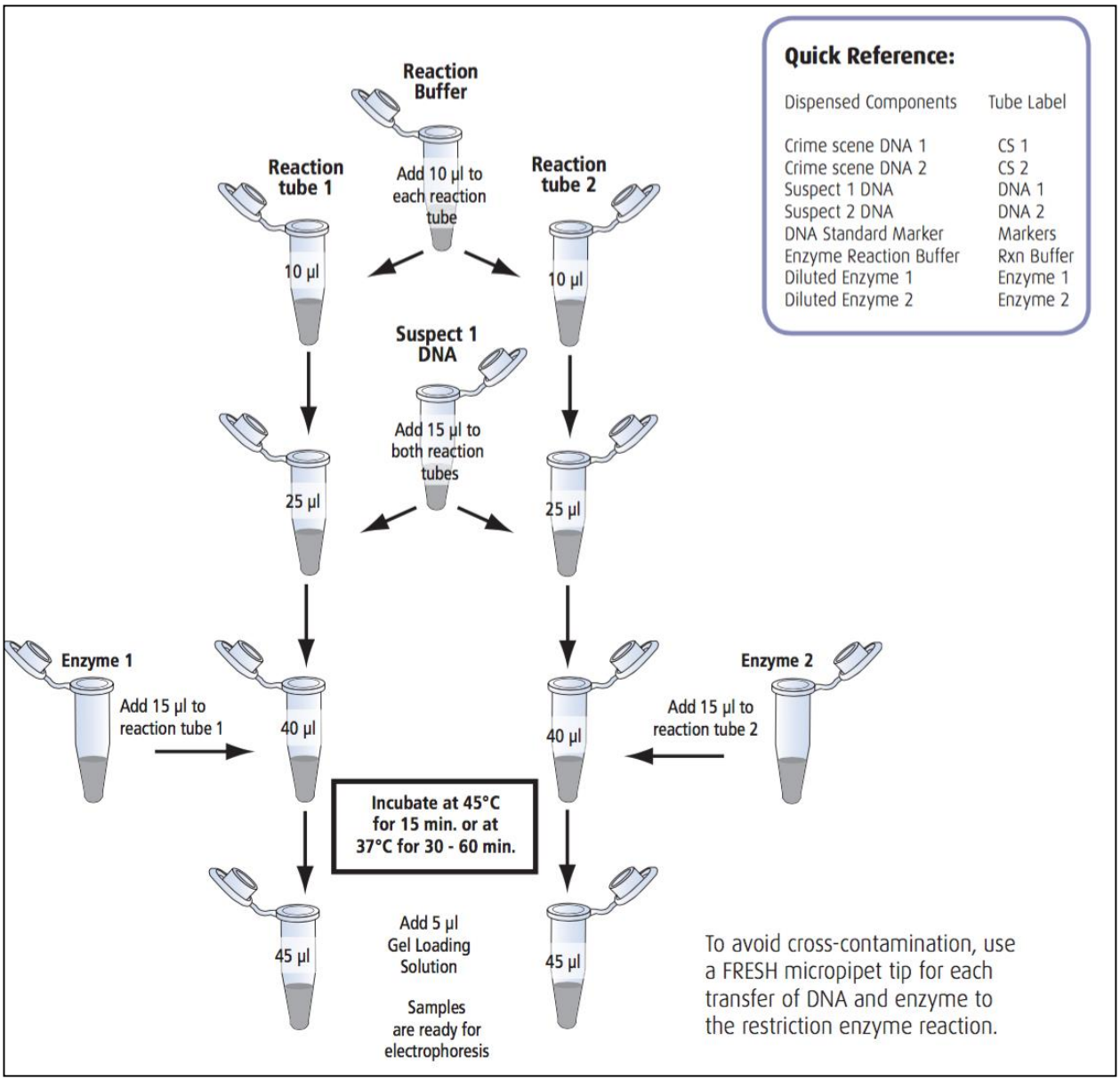


Fig. 10.7. Procedure to prepare DNA samples from Suspect 1 in this experiment for gel electrophoresis and DNA fingerprinting analysis. The DNA from Suspect 2 is digested in the same manner, using reaction tubes 3 and 4 (not shown).

The final step after a gel has run is simply a matter of lining up the sample profiles side-by-side and comparing them for the presence or absence of segments with particular bands. The more bands any given samples have in common, the more likely it is they came from the same person.

Principle Components of the Experimental Set-up

Your instructor will discuss and demonstrate how the **gel electrophoresis chamber** and its components (Fig. 10. 8, left; below) function.

Power Supply: This high voltage power source (Fig. 10.8, right) connects to the electrophoresis chamber and sets up an electric field between two electrodes – one positive and one negative. DNA-fragment samples loaded into the wells of an agarose gel are negatively charged and move through the gel toward the positive electrode (aka anode) as the agarose gel matrix separates the DNA molecules by size.

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

For the experiment described here, we will set the voltage on our power supplies to 150.

Fig. 10.8. Gel electrophoresis apparatus (left) and power supply (right).

Agarose, the main component of our **gels,** is a polysaccharide polymer extracted from seaweed. It is available as a powder, which is mixed with a buffered solution, heated until it dissolves, and then poured into molds where it solidifies (in about 20 minutes) into a gel slab (much like Jello®). A serrated "comb" placed in the mold before the agarose is poured causes **sample wells** to form in the finished gel.

TAE (Tris/Acetate/EDTA) Buffer covers the gel in the electrophoresis chamber and contains ions that carry the current through the apparatus. It also maintains a constant pH for the experiment.

Restriction Enzymes are used to cut the large pieces of DNA in our samples (the ones found at the crime scene and the ones from our two suspects) into small fragments that can migrate through the agarose gel.

Micropipettes are used to dispense all the fluids in this lab. These devices are designed to transfer small amounts of liquid (< 1 ml). The scale on micropipettes is in microliters (millionths of a milliliter; 1000 µl = 1 ml). The micropipettes we use in this lab are made by Edvotek and are delicate instruments that cost about $200 apiece. They are used with disposable sterile plastic tips.

The maximum value on our micropipettes is 50 µl.

The **Gel Loading Solution** serves two purposes. First, it provides a visible dye that helps with gel loading and makes it possible to gauge how far the gel has run as the current is applied. Second, it contains a high percentage of glycerol to increase the density of the DNA samples that are placed in the wells. This causes the samples to settle to the bottom of the wells rather than diffuse into the buffer.

InstaStain® Blue cards. These cards contain a small amount of blue DNA stain. When the card is placed in water, the DNA stain is released. This solution stains the gel and its DNA.

A **White Light Box** is used to visualize the banding pattern on the gels (Fig. 10.9, below).

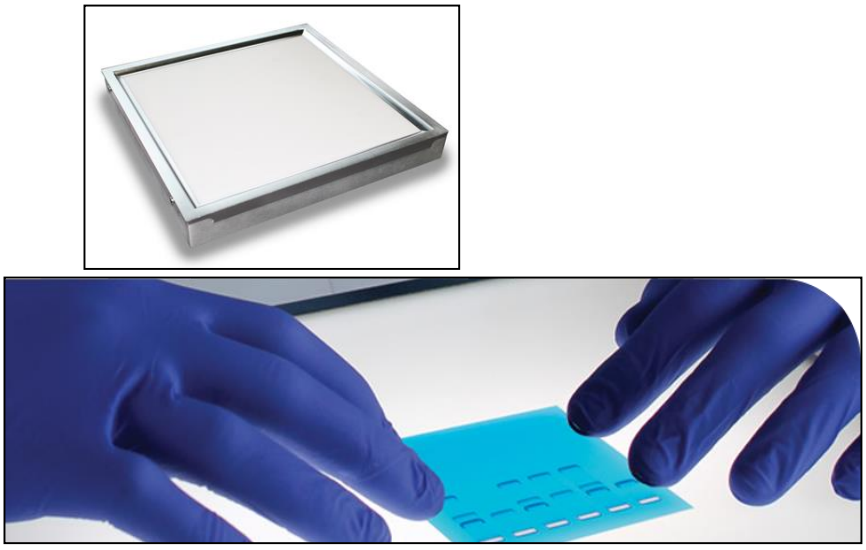


Fig.10.9. White light box (left) and visualization of DNA bands on gells.

Lab Safety

Gloves and goggles should be worn throughout the duration of this lab.

Exercise caution when using electrical equipment and any device (such as a water bath) that produces heat.

Wash hands thoroughly with soap and water at the end of lab.

Gel Preparation

Shortly after lab starts, pre-mixed liquid agarose gel will be poured into each group's casting mold by your instructor. Prior to pouring the gel, a comb (that will produce six wells) should be positioned in the groove near the negative electrode, which is indicated on the bottom of the mold. (Consequently, the DNA placed in the wells formed by the comb can migrate to the positive electrode once an electric current is applied.)

The gel will harden in about 20 minutes. The liquid agarose is clear and colorless. The hardened gel will be off-white and semi-opaque (cloudy). Prepare the DNA samples as you wait for your gel to solidify.

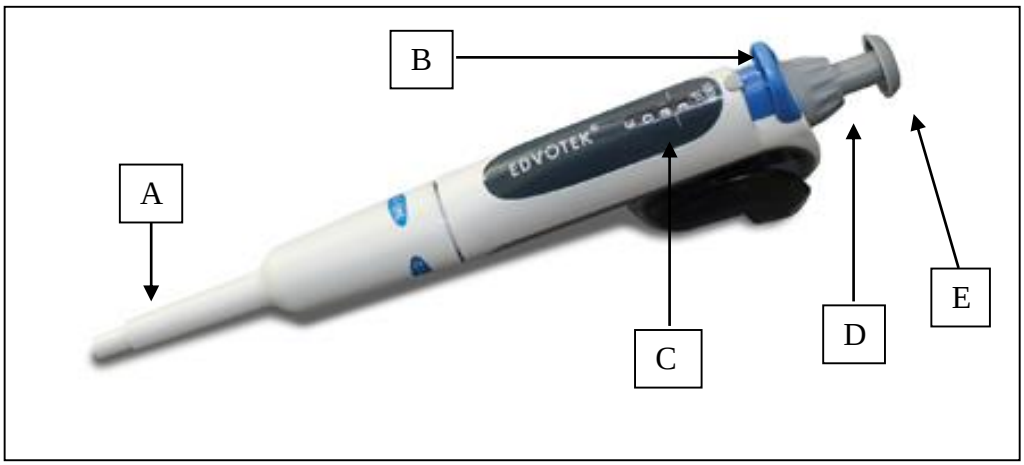
Preparing the DNA samples

1. Use a Sharpie to label four empty micro-test tubes 1 through 4 for the four restriction enzyme digestion reactions you will perform. Put your group number, or some other identifying mark, on each of the 4 tubes.

2. Gather together the 8 other tubes on your tray, each of which should contain a very small amount of liquid and be labeled, respectively, as follows:

- **CS** (Crime Scene DNA, tube B): the material in this tube has already been prepared for you
- **DNA 1** (Suspect 1 DNA, tube C)
- **DNA 2** (Suspect 2 DNA, tube D)
- **DNA 3** (Suspect 3 DNA, tube E)

How to use a Micropipette (your instructor will demonstrate this technique at the beginning of lab)



1. Adjust the dial (D) on the pipette so that the numbers (C) indicate the volume you wish to dispense. The volume that can be dispensed by our devices is measured in *micro*liters (µl).

Note: the numbers *above* the line on the volume indicator are whole numbers – such as "15" for 15 µl. The numbers *below* the line indicate *hundredths* of a microliter. Consequently, it would be possible to dispense 15.35 µl, or a similar volume, using our devices.

2. Place a tip on the dispensing end of the micropipette (A).

3. Gently press the plunger (E) until you feel resistance. This is the first level of resistance.

4. Continue to hold this level of resistance and insert the tip into the solution you wish to pipette.

5. Slowly release the plunger to draw up the solution.

6. Once the plunger is raised, remove the tip from the solution and place the tip and pipette into the vessel where the solution will be dispensed.

7. Slowly press the plunger down all the way -- past the first level of resistance until it cannot be released any further.

8. While still holding down the plunger, remove the tip from the solution THEN release the plunger. (The point here is just to eject all the solution in the tip. No other fluid should be sucked into the tip.)

9. Eject the tip by pressing the lever (B).

Loading the Gel

1. Retrieve your hardened gel. It should be off-white and semi-opaque.

2. Gently remove the black rubber bumpers from the mold. Then gently remove the comb by lifting it up slowly. Avoid tearing the gel. Leave the gel in the plastic mold (without the comb and black bumpers).

3. Place the mold in the gel chamber. It should only be able to fit in the chamber in one direction – with the wells facing the negative electrode (black wire).

4. Pour TAE Buffer into the chamber until the gel is completely covered.

4. Load 40 µl of each DNA sample you will run into its appropriate well, as follows (Table 10. 2):

Table 10.2. Material that will be run in each lane of your gel

Gel Lane (left to right)	Micro test tube	Contents
1	A	Sandard markers
2	B	DNA from crime scene
3	C	DNA from suspect 1
4	D	DNA from suspect 2
5	E	DNA from suspect 3
6		blank

Running the Gel

1. Close the lid of the electrophoresis chamber and connect the electrodes to the power supply – "black to black" and "red to red."

2. Set the power source to 150 amp. The red light will come on. Run the gel for 25-30 minutes, longer if possible.

3. Periodically check that the current is flowing correctly and the DNA fragments (indicated by the blue dye from the loading gel) are migrating toward the positive (red) electrode.

4. Turn off the power supply when the gel has finished its run.

Staining the Gel

1. Remove the mold from the chamber. Pour excess TAE back into the chamber. Slide the gel into a plastic "weigh boat" and add enough warm water to cover the gel.

2. Place an Insta®Stain Blue card, blue side down, on the gel. Weigh down the card with a beaker filled with water.

3. Wait 15 minutes.

4. Pour off liquid (which should be bluish) into the waste beaker provided. Gently rinse the gel several times by gently running water over it.

5. Place the gel on the light source and observe and compare the banding patterns.

Analysis

1. Lane 2 represent the same Crime Scene DNA digested by two different restriction enzymes. They should yield distinctly different DNA banding patterns.

2. Lanes 3, 4, and 5 represent DNA from Suspect 1, 2, and 3 respectivly. The suspect's DNA has been digested with the same restriction enzymes as was the DNA in Lane 2.

To determine which suspect was at the crime scene, the banding patterns of the suspect's DNA samples should match the banding patterns in lane 2.

Questions

1. Based on your DNA evidence, which suspect was at the crime scene?

2. Does a positive identification in this regard mean that that suspect committed the crime?

3. Could a potential suspect have been identified if only one restriction enzyme (ENZ 1) has been used to fragment the DNA?

EXERCISE 12: SURVEY OF PLANT AND ANIMAL DIVERSITY

I. Diversity of the Plant Kingdom

A. Introduction to Kingdom Plantae

All modern and terrestrial plants are descendant of algae that adapted to a terrestrial habitat roughly 500 million years ago. Compared to water, land is an erratic habitat where temperature and moisture availability may change abruptly and dramatically. What were the adaptations that these primitive plants needed to survive:

- Surrounded by air, the land plant is in constant threat of desiccation and must have waterproofing, usually in the form of a waxy **cuticle** layer.
- While algae could obtain nutrients from the surrounding water, land plants needed to extract minerals (and now even water) from the soil; **roots** adapted to take on this task.
- Evolution of a **rigid structural support** allowed plants to grow against gravity to new heights and better compete for sunlight.
- Yet, height must have coevolved with a **vascular (transport) tissue**; the internal plumbing system needed for the transport of water and nutrients from the roots skyward.
- Reproduction now became problematic as well, as sperm could no longer swim through open water to encounter the egg. Thus evolved sperm cells protected within **pollen grains** that could instead use wind, and later, unwitting animals to be carried to the egg.
- And what to do with the next generation? Conditions on land are much less predictable than in water, possibly favorably dry or hot after fertilization occurs. Packaging the immature (and dormant) plant in a **seed** allowed it to survive until favorable growth conditions (or the correct season) arrived.

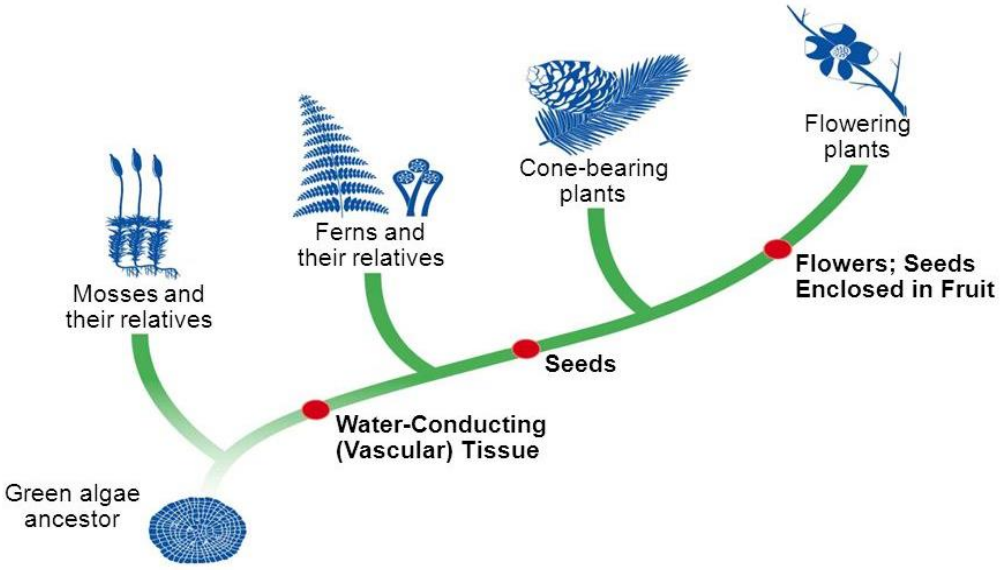
These were the traits that appeared during the evolution of plants and they can be traced through a study of the different groups of plants that exist on earth today.

B. Classification of Modern Plants

There are four major plant groups that evolved from algae:

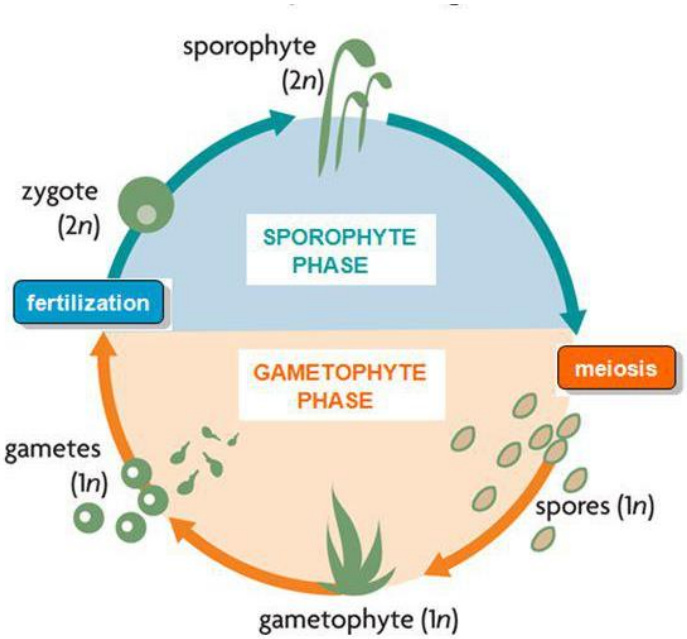
1. **Bryophytes** – include the mosses and their relatives
2. **Seedless vascular plants** – include the ferns and their relatives
3. **Gymnosperms** – include the conifers or cone bearing plants
4. **Angiosperms** – include the flowering plants which now predominate

The figure below demonstrates the evolution of plants and the major characteristic that separates each group.



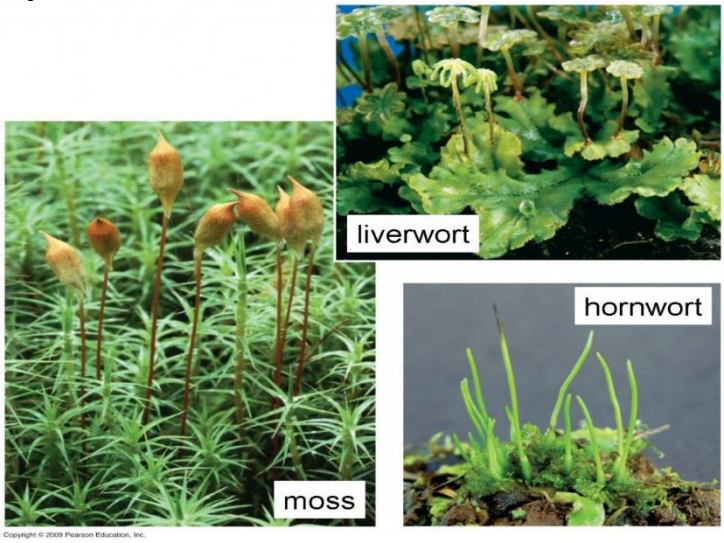
C. Plant Life Cycles

Sexual reproduction among plants is a complicated and intricate process. In animals, the adult forms (male and female) have specialized organs that produce reproductive cells called **gametes**. Upon fertilization, the egg and sperm combine to produce a zygote that can grow into another male or female adult. Unlike animals, plants have two alternative life stages. The figure below shows the **alternation of generations** as it occurs in a moss. Note: the **sporophyte phase** produces spores and the **gametophyte phase** produces sperm or egg gametes.

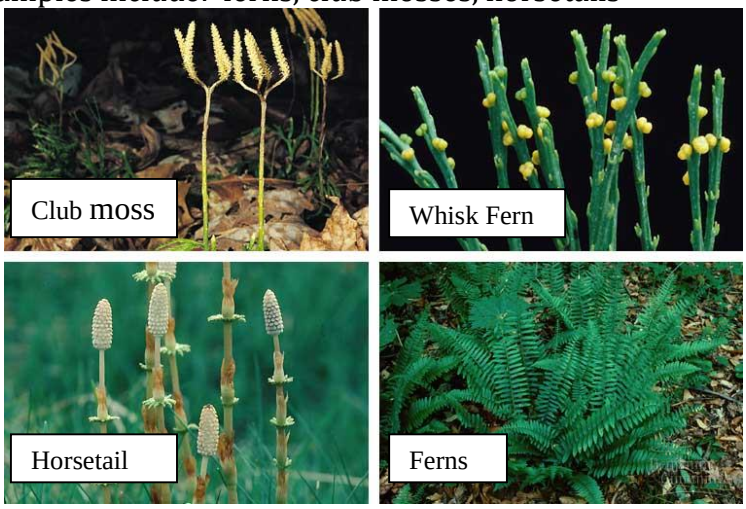


D. Characteristics of Plant Groups

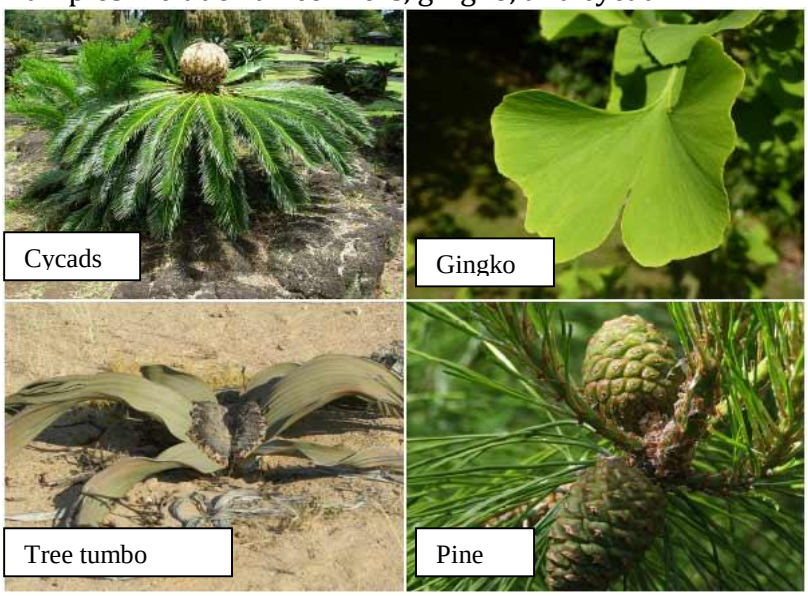
1. **Bryophytes** – also known as the "seedless, non-vascular plants".
    - Bryophytes disperse through spores, not seeds. Sperm must swim to the egg.
    - Bryophytes lack a vascular system
    - There are no tree-sized bryophytes because water could never travel adequately from the rootlets to the very top.
    - The gametophyte phase is dominant. Bryophytes are the only group of plants where the gametophyte phase is larger than the sporophyte phase.
    - Examples: mosses, liverworts, and hornworts



2. **Seedless Vascular Plants –** are believed to have evolved from moss-like plants 200-400 million years ago. This group is distinguished by:
    - Presence of a vascular system
    - A dominant sporophyte phase
    - Dispersal through spores, sperm must still swim to the egg
    - Examples include: ferns, club mosses, horsetails



3. **Gymnosperms** – also known as the "naked seed plants". Seed plants broke the dependence on external water in reproduction. The seed is a rugged package for dispersal within the tough seed coat contains the dormant plant embryo as well as stored food. This group is distinguished by:
    - Presence of a complex vascular system.
    - A dominant sporophyte phase.
    - The pollen grains and ovules are produced within cones. No need to swim to the egg.
    - They produce seeds although the seeds are not encased by an outer layer (or fruit). The name gymnosperm means "naked seed".
    - Examples include: all conifers, gingko, and cycad



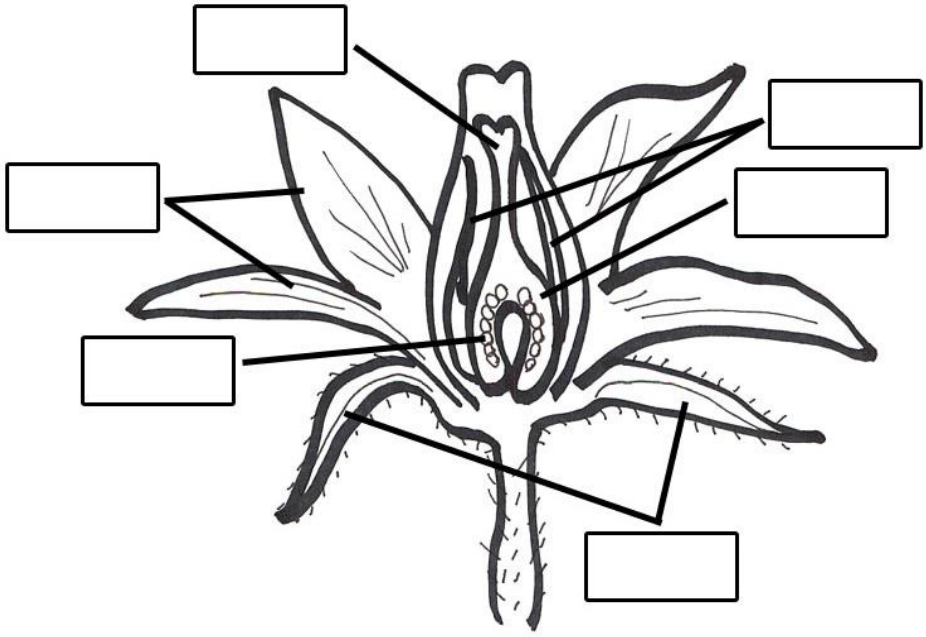
4. **Angiosperms** – also known as the "flowering plants". Through their color, smell and edible nectar, flowers attract insects and animals that unwittingly carry pollen between flowers to achieve pollination. This is much more efficient than merely releasing pollen into the wind like the cone-bearing plants. Likewise, fruits may also help recruit animals to disperse the seeds. Characteristics for this group include:
    - Presence of a complex vascular system.
    - A dominant sporophyte phase.
    - The pollen grains and ovules are produced within flowers.
    - They produce seeds that are encased by an outer layer called a fruit.
    - Examples include: apple tree, strawberry bush, orchid, lily

E. Plant Diversity Activities

1. Using the provided collection of plant specimens and the chart on the following page, identify the group to which each unknown plants belongs, give other examples of the group, and identify whether the group demonstrates the distinguishing characters by giving a "yes" or "no" in each column.

Specimen	Group	Examples	Produces Spores?	Produces Seeds?	Vascular System?	Produces Pollen?	Produces Flowers?	Produces Fruit?
#1								
#2								
#3								
#4								

2. Your instructor will go over the parts of a flower using the flower model. Label the parts of a typical flower using the terms listed below:



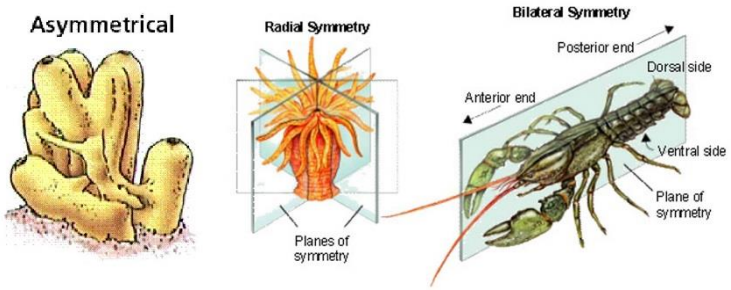
ovary	ovule	petals
pistil	sepals	stamens

II. Diversity of the Animal Kingdom

A. Introduction to the Animal Kingdom

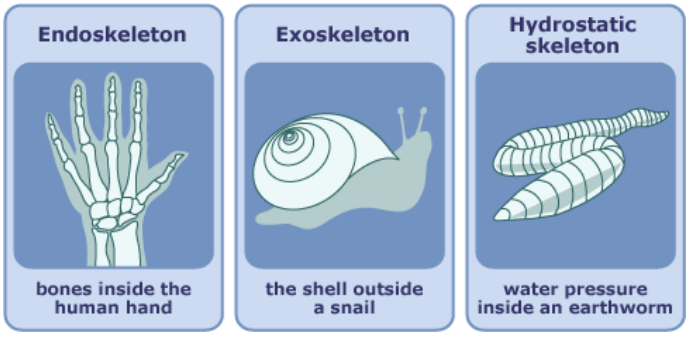
To provide some guidance, some of the anatomical traits that often, but not always, reflect homologous similarities are described below. As you observe the various specimens on display consider the following characteristics:

- **Body symmetry -** The shape of most animals is either **bilaterally symmetrical** or **radially symmetrical**, although a few primitive organisms are **asymmetrical**.

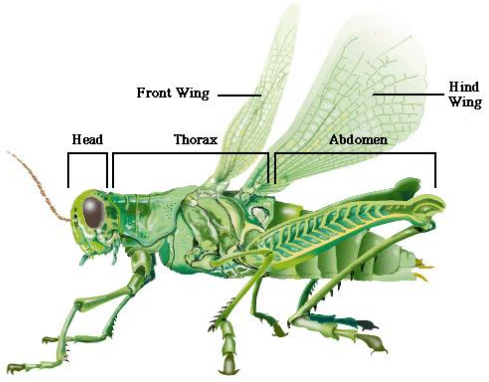


- **Type of body skeleton -** Animals such as a worm or sea anemone do not possess a rigid skeleton. The bodies of these organisms are supported by the pressure

exerted by internal fluids, referred to as a **hydrostatic skeleton**. However, other animals have evolved various types of rigid body skeletons. Insects and spiders evolved a hard outer casing, called an **exoskeleton**. Animals such as humans evolved an internal framework called an **endoskeleton**.



- **Segmentation -** The bodies of many organisms are composed of distinct units, and are said to be segmented. In more primitive organisms, such as earthworms, most of the segments are identical. However, in more advanced organisms certain segments evolved specialized functions and structures, such as the **head, thorax and abdomen**.



- **Presence of body appendages -**Legs, tentacles and antennae are examples of some of the various appendages that animals possess. These appendages serve many different functions; some facilitate movement or manipulation of objects in the environment, while others are sensory in nature, such as the antennae of insects.
- **Body covering -** The presence of hair, fur, feathers or scales are other useful characteristics. Some animals possess a shell along with other body coverings, which is different than an exoskeleton. Whereas an exoskeleton completely encases all of the appendages of an animal, an animal living in a shell can extend part of its body out from under the shell. Thus, a clam can extend its "foot" out from between its protective shell.

B. Classification of the Invertebrates

There are now about 36 recognized phyla in the animal kingdom, and all of them, including our own Phylum Chordata, contain invertebrate species. It is very difficult to determine relationships among invertebrate organisms. It is obvious from invertebrate diversity that many "evolutionary experiments" were tried and ended in extinction. Also

many organisms in the current phyla have diverged very far from the main line (or lines) of animal evolution. We will only consider 6 invertebrate groups:

1. **Characteristics of six invertebrate groups:**

	Porifera	Cnidaria	Worms	Mollusca	Echinoderm	Arthropoda
	Simple →					Complex
Describe	Pore-bearing	Stinging Cells	-Flatworms -Roundworms -Segmented Worms	Soft-bodied	Spiny Skin	Jointed Appendages
Examples	 Sponges	 Jellyfish, Sea Anemone, Corals, Portuguese Man-of-War	 Planaria, Tapeworm, Fluke, Pinworm, Hookworm, Heartworm, Earthworms, Leeches, Vinegar Eel	 Snails, Slugs, Clams, Oysters, Scallops, Mussels, Squid, Octopus	 Starfish, Sea Urchins, Sand dollars, Sea Cucumbers	 Crayfish, Crabs, Butterflies, Spiders, Scorpions, Beetles
Body Symmetry	Asymmetric or Radial	Radial	Bilateral Cephalization	Bilateral Cephalization	5-part Radial	Bilateral Cephalization
Digestion (Feeding)	Filter Feeding	Digestive Cavity (1 opening)	Digestive Cavity (1 opening) & Digestive Tract (2 openings)	Digestive Tract (2 openings)	Digestive Tract (2 openings)	Digestive Tract (2 openings)
Circulation (Circulatory System)	Diffusion	Diffusion	Simple—Diffusion & More complex—Closed Circulatory	Open Circulatory & Closed Circulatory—Squid, Octopus	Water Vascular System	Open Circulatory
Excretion (Excretory System)	Diffusion	Diffusion	Simple—Diffusion Complex—Present	Present	Water Vascular System	Present
Respiration (Respiratory System)	Diffusion	Diffusion	Diffusion	Gills, Vascular Lung	Water Vascular System	Tracheal tubes, Book Lungs, or Gills
Nervous (Response to Environment)	None	Nerve Net	Ganglia (Cephalization), Primitive Brain	Ganglia, Brain	Nerve Ring	Brain
Reproduction (Reproductive System)	Asexual, Sexual	Asexual, Sexual	Asexual, Sexual, Hermaphrodite	Sexual, hermaphrodite and Separate Sexes	External Fertilization	Internal and/or External Fertilization
Movement (Skeletal and Muscular)	Sessile	Muscles	Muscles	Muscles	Endoskeleton and Water Vascular System	Exoskeleton and Muscles
NOTES	-Simplest of all Animals -Medical research (antibiotics—Ex: strep throat)	-Cnidocytes-stinging cells -2 forms: polyps ↑ medusa ↓ -Coral	1st to have: bilateral symmetry, segments, organs, cephalization, and separate sexes -Some parasitic	-Medical research for anti-cancer drugs -Environmental monitors (filter feeders) -Known for muscular foot	-Regenerate -All marine (only found in salt water) -Defense Mechanisms: Spines, Shedding of Arms, Evisceration	-Molt to grow -Exoskeleton made of chitin -Land and water animals

2. Invertebrate Animal Activity:

Using the collection of invertebrate animals on display, identify the major group to which each specimen belongs, give other examples of the group, and identify the major characteristics of that group:

Specimen	Group	Symmetry	Skeleton	Segments	Appendages	Respiration	Digestion	Excretion
#1								
#2								
#3								
#4								
#5								
#6								

C. Classification of the Vertebrates

Vertebrate evolution began around 500 million years ago (mya) during the early Paleozoic era with the evolution of fish, followed by amphibians and then reptiles during middle Paleozoic. Some lizard-like reptiles of the Mesozoic evolved into small hair-covered mammals, birthing their young rather than laying eggs, while awaiting the demise of the dinosaurs before rising to prominence during the Cenozoic. However, before this eventual demise, the dinosaurs gave rise to another group, the birds which, like the mammals, survive to the present day. All vertebrates belong to the phylum Chordata. The characteristics of the six most common classes are outlined below:

1. **<u>Characteristics of 6 most common classes of phylum chordata</u>:**

Class	Examples	Characteristics
Chondrichthyes	Sharks, skates, and rays	1. Cartilaginous skeleton 2. Placoid scales 3. 2-chambered heart 4. Stiff immovable fins 5. Ectothermic 6. Lay eggs in water or live young 7. Lack a swim bladder 8. Respiration by gills
Osteichthyes	Bass, catfish, tuna, salmon, coelacanth	1. Bony skeleton 2. Cycloid, ctenoid, or ganoid scales 3. 2-chambered heart 4. Moveable fins supported by spines or rays 5. Ectothermic 6. Lay eggs in water 7. Frequently possess a swim bladder 8. Respiration by gills
Amphibia	Frogs, salamanders, newts, caecilian	1. Bony skeleton 2. Moist skin without scales, hair, or feathers 3. 3 chambered heart 4. Legs that lack claws on their toes (except caecilians) 5. Ectothermic 6. Lay eggs in water 7. Respiration by gills in immature stage, lungs by adults
Reptilia	Lizards, snakes, turtles, crocodilians	1. Bony skeleton 2. Skin with scales but no hair or feathers 3. 3 chambered heart (except alligators and crocodilians which have 4 chambered heart) 4. Legs that possess claws on their toes (except snakes) 5. Ectothermic 6. Lay amniotic eggs inside a leathery shell 7. Respiration by lungs
Aves	Finch, ostrich, penguin, eagle, duck	1. Bony skeleton is lightweight due to hollow bones 2. Skin with feathers 3. 4 chambered heart 4. Hind legs with claws on their toes 5. Forelimbs modified to form wings 6. Endothermic 7. Lay eggs inside of a hard shell 8. Respiration by lungs

Mammalia	Dolphin, humans, opossum, duck-billed platypus	1. Bony skeleton 2. Skin with hair 3. 4 chambered heart 4. Nails or claws on their toes 5. Endothermic 6. Possess sweat glands, oil glands, and mammary glands 7. Respiration by lungs 8. Retain young in womb and give live birth 9. Heterodont dentition (different types of teeth)

2. <u>Vertebrate Animal Activity</u>

Using the collection of vertebrate animals on display, identify the major group to which each specimen belongs, give other examples of the group, and identify the major characteristics of that group:

Specimen	Class	Examples	External Covering	Body Temp	Reproduce	Respiration	Heart	OTHER
#1								
#2								
#3								
#4								
#5								
#6								

Exercise 13: Human senses

Senses in the animal kingdom vary greatly from one animal to another. Some species have incredible abilities such as the barn owl who can spot a mouse in a field, at night, from hundreds of yards away! Or an alligator who can feel the ripples in the water as small as those made from one drop of water hitting the surface.

While the animal kingdom provides some incredible example of how sensory organs evolved differently for different organisms. Humans as a whole tend not to be overly remarkable in any of our main senses (sight, hearing, smell, taste, or touch). While we may not be able to detect the specific chemical cues of drugs hidden in a bag of coffee like a dog we do share the same basic physiology as all of these animals.

In the human body our peripheral nervous system passes information on to the spinal cord and ultimately the brain (which is referred to as the central nervous system). Today's investigation is a chance to explore our human senses and test hypotheses based on them.

SMELL

Inside your nose are many chemoreceptors. These specialized cells collect chemicals in the air and and stimulate the peripheral nervous system. In 2002 neuroscientist Pamela Dalton found that women became more smell-sensitive after a few exposures to a particular scent. Using different scents, we will test the hypothesis that women are better able to identify smells than men.

1. Open one vial at a time from the smell kit, sniff each scent and then write the name of smell you guess in table 1. (refer to odor selection list for possible smells)
2. After answering all questions, score your answer sheet and record your score (number correct out of 10).
3. Record all answers on board and calculate the average for male and female

Table 1: Individual results for smell	
Vial #	Answer Choice
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
# Correct	

Table 2: Class Results for gender differences in smell	
Male Score	Female Score

VISION

In your eyes you have special cells called photo receptors which contain pigments that absorb light. Thanks to the lens of the eye light is bent to allow for vision at many different distances. To do this the lens must change shape, its called accommodation and is made possible by muscle contractions. As people age the lens hardens making it harder to change the shape and thus they have trouble focusing on things up close. To test this we can look at a near point accommodation and compare it to known averages

1. Calculate the average near point data on your data sheets for each age group.
2. Based on the averages you calculated, predict your own near point in cm.
3. Locate a sharpened pencil and a metric ruler that measures at least 30cm.
4. Hold the tip out at arms length in front of you
5. Close your right eye on tip of pencil
6. Slowly move the pencil towards your face and stop when tip becomes blurry.
7. Your partner should then measure the distance from your eye to the pencil with the ruler
8. Compare your results with those known averages

Table 3: Near point of 10 year olds

Test	Near Point (cm)
1	11
2	6
3	8
4	8
5	10
6	7
7	10
8	9
9	12
10	8
Average	
Standard Deviation	

Table 4: Near point of 20 year olds

Test	Near Point (cm)
1	8
2	8
3	10
4	11
5	10
6	12
7	12
8	14
9	12
10	12
Average	
Standard Deviation	

Table 5: Near point of 30 year olds

Test	Near Point (cm)
1	13
2	15
3	10
4	12
5	13
6	14
7	12
8	14
Average	
Standard Deviation	

Table 6: Near point of 40 year olds	
Test	Near Point (cm)
1	21
2	16
3	17
4	19
5	20
6	21
7	17
8	15
9	18
10	20
Average	
Standard Deviation	

To calculate the standard deviation of those numbers:

1. Work out the average
2. Then for each number: subtract the average and square the result.
3. Then work out the mean of those squared differences.
4. Take the square root of that average and we are done!

Table 7: Near point of 50 year olds	
Test	Near Point (cm)
1	30
2	35
3	40
4	34
5	50
Average	
Standard Deviation	

Table 5: Near point of 70 year olds	
Test	Near Point (cm)
1	45
2	120
3	150
Average	
Standard Deviation	

Table 8: Near point of 60 year olds	
Test	Near Point (cm)
1	50
2	100
3	45
4	75
5	95
6	67
7	104
8	89
9	98
10	90
Average	
Standard Deviation	

Table 10: Your Near point	
Test	Near Point (cm)
Right Eye	
Left Eye	

TOUCH

One of the functions of the skin is to convey information about how things feel. It determines hot from cold, painful or not, rough or smooth. Cells that detect touch and pressure in your skin called mechanoreceptors. Our ability to detect

more details of objects is related to the number of mechanoreceptors in an area. If the number of receptors varies over the body then so should the the skins sensitivity.

To test this hypothesis you will have a person use a two pointed object and lightly poke your partner in various locations on their body to determine at what point they can detect 2 individual points.

1. Press the ends of a caliper together
2. One partner will turn their head so not to look, while the other then lightly pokes this partner with the caliper
3. The poked partner indicates if they can feel one point or two
4. If only one point is distinguishable, then the points are spread slightly apart and steps 2-4 are repeated until the poked person can distinguish 2 points
5. After the two points are distinguished measure the distance between the points on the caliper and record below
6. Record your results on the board and fill out the class data chart to find the average and range of results.

Table 11: Individual Results for Skin Sensitivity

Area of Body	Two-Point Threshold (mm)
Back of Upper Arm	
Forearm Palm Up	
Forearm Palm Down	
Back of Hand	
Palm of Hand	
Index Finger Tip	
Back of Neck	

Table 12: Class Results for Skin Sensitivity

Area of Body	Sample Size	Average Two-Point Thresh	Range Two-Point Threshold (mm)
Back of Upper Arm			
Forearm Palm Up			
Forearm Palm Down			
Back of Hand			

Palm of Hand			
Index Finger Tip			
Back of Neck			

Summary:
Did males or females on average have a higher score on the smell test?

What is a standard deviation? Why would a scientist use that and not just the average or range?

Did the sample size affect the standard deviation of scores?

Based on the near point averages what range are you in?

Do you think the near point you calculated for the 70-year old group is accurate? Explain.

Where was the part of the body with the smallest two-point threshold value? Where was the largest? Explain.

EXERCISE 14: Natural selection

Ancient Greek, Romans and Chinese all had philosophies which formed the basis for evolution. In the 1700's geologist began to notice that there was a pattern in fossils within rock strata, which led many to begin looking at the fossil record comparatively and seeing changes in organisms over time. By the 1800's evolution was becoming well studied and solidified in the scientific community; however the mechanism of how it worked was heavily debated.

In the early part of the 1800's a well-known French biologist named Jean-Baptiste Lamarck came up with the first full mechanism for how evolution worked. He stated that all organisms evolved based on acquired characteristics in a progression from simple to complex forms. However innovative Lamarck was, his theory had many flaws, but remained the most complete answer to the mechanism of evolution at the time. In 1859 a man named **Charles Darwin** published his now world famous book *On the Origin of Species*. In his book he proposes a new mechanism for evolution called natural selection, which is widely accepted amongst the scientific community as the main mechanism for evolution. **Natural selection** is defined as a difference, on average, between the survival or fecundity of individuals with certain phenotypes compared with individuals with other phenotypes. What this means is an organism that has an adaptation that allows it to survive to reproduce more will be more likely to pass on that adaptation than another of the same species without that adaptation.

In nature we see rapid evolution by natural selection in predator and prey populations. This is because there is heavy pressure for the prey to escape or avoid predation (by camouflage, change in behavior, change in size etc…) and heavy pressure for the predator to continue to predate on this ever changing prey. In this experiment we will be simulating a predator population that has multiple methods for gathering prey and also a varied prey population with different levels of camouflage and sizes.

Procedure

Each group will have 2 of each feeding types: Spoons, probes, Tweezers, Scoop, Scissors. Divide these up amongst your group members so that each feeding type is used at least once. There will also need to be one person designated as the time keeper.

Habitat set up
On each bench spread out your cup of gravel evenly in the habitat area. Then place your cup of 100 beans of each type in the habitat as well.

Predation
For one minute the predators (you) will collect the bean bunnies with your feeding mechanism **and only your feeding mechanism (no hands)!** After one minute count and record the total of each bean type you "ate" for each feeding mechanism and record this on the provided answer sheet.

Mating time!
For the remaining beans we assume everyone mates equally. Use the sheet provided to calculate how many of each bean to add back to your table for generation 2 (3, 4, and 5). This should always reach 400, our **carrying capacity** (the total number of individuals an ecosystem can contain based on available resources).

For the predators we assume consumption of less than 25% of the total beans consumed is insufficient to sustain life (those mechanisms go extinct). When we loose predators in nature we open up space for existing predators to take over (as seen in existing wolf and coyote populations). Use the attached sheet to redistribute feeding mechanisms.
Continue this for 4 generations.

Prey	A	B	C	D	E beginning population in next generation	F to be added back to habitat
Example	beginning bunnies	% of population	remaining after predation	% left	Multiply D x 400	Subtract C from E
black	100	0.25	50	0.27778	111	61
red	100	0.25	12	0.06667	27	15
large white	100	0.25	19	0.10556	42	23
small brown	100	0.25	99	0.55	220	121
Totals	400	1	180	1	400	220
Generation 1						
black						
red						
large white						
small brown						
Totals	400	1		1	400	
Generation 2						
black						
red						
large white						
small brown						
Totals	400	1		1	400	
Generation 3						
black						
red						
large white						
small brown						
Totals	400	1		1	400	
Generation 4						
black						
red						
large white						
small brown						
Totals	400	1		1	400	

EXERCISE 11: HUMAN GENETICS

Were the babies switched? – The Genetics of Blood Types

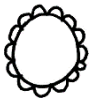
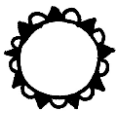
Courtesy of Dr. Jennifer Doherty and Dr. Ingrid Waldron, Department of Biology, University of Pennsylvania, 2016.

Two couples had babies on the same day in the same hospital. Denise and Earnest had a girl, Tonja. Danielle and Michael had twins, a boy, Michael, Jr., and a girl, Michelle. Danielle was convinced that there had been a mix-up and she had the wrong baby girl, since Michelle had light skin, while Michael Jr. and Tonja looked more like twins since they both had dark skin.

Danielle insisted on blood type tests for both families to check whether there had been a mix-up. To interpret the results of these tests, you will need to understand the genetics of blood types.

▪ Genetics of Blood Types

The ABO blood type system is the major blood type classification system that determines which type of blood can safely be used for a transfusion. The four blood types in the ABO system refer to different versions of carbohydrate molecules which are present on the surface of red blood cells.

People with:	**Have:**
Type A blood	Type A carbohydrate molecules on their red blood cells 
Type B blood	Type B carbohydrate molecules on their red blood cells 
Type AB blood	Both type A and type B carbohydrate molecules on their red blood cells 
Type O blood	Neither type A nor type B carbohydrate molecules on their red blood cells 

These different blood types result from
different alleles of a gene in the DNA that give the directions for making
different versions of a protein enzyme that puts
different types of carbohydrate molecules on the surface of red blood cells.

Allele	**Gives the directions for making a version of the enzyme that:**
$\mathbf{I^A}$	puts type A carbohydrate molecules on the surface of red blood cells
$\mathbf{I^B}$	puts type B carbohydrate molecules on the surface of red blood cells
i	is inactive; doesn't put either type of carbohydrate molecule on the surface of red blood cells

1. Each person has two copies of this gene, one inherited from his/her mother and the other inherited from his/her father. Complete the following table to relate genotypes to blood types.

Genotype	This person's cells make:	Blood Type
$\mathbf{I^A\,I^A}$	the version of the enzyme that puts type A carbohydrate molecules on the surface of red blood cells.	
i i	the inactive protein that doesn't put either type A or type B carbohydrate molecules on the surface of red blood cells.	
$\mathbf{I^A}\,\boldsymbol{i}$	both the version of the enzyme that puts type A carbohydrate molecules on the surface of red blood cells and the inactive protein	**A**

2. In a person with the $\mathbf{I^A}\,\boldsymbol{i}$ genotype, which allele is dominant, $\mathbf{I^A}$ or ***i***? Explain your reasoning.

3. For the genotypes listed below, which type(s) of enzyme would this person's cells make? What blood type would the person have?

Genotype	Will this person's cells make the version of the enzyme needed to put this carbohydrate on the surface of his/her red blood cells?	Blood Type
$\mathbf{I^B\,I^B}$	Type A __ yes __ no; Type B __ yes __ no	
$\mathbf{I^B}\,\boldsymbol{i}$	Type A __ yes __ no; Type B __ yes __ no	
$\mathbf{I^A\,I^B}$	Type A __ yes __ no; Type B __ yes __ no	**AB**

Codominance refers to inheritance in which two alleles of a gene each have a different observable effect on the phenotype of a heterozygous individual. Thus, in codominance, neither allele is recessive — both alleles are dominant.

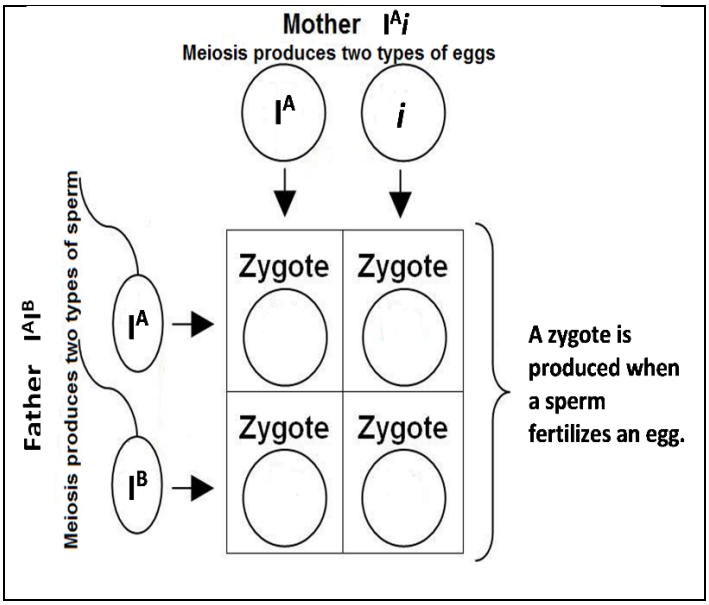
4. Which of the genotypes listed above results in a blood type that provides clear evidence of codominance? Explain your reasoning.

This expanded version of a Punnett square will show how meiosis and fertilization result in the inheritance of a gene.

5a. The mother has type _____ blood and the father has type _____ blood.

5b. Complete this chart to show the genetic makeup of each zygote produced by fertilization.

A baby develops from a zygote by repeated rounds of mitosis, so each cell in a baby's body has the same genetic makeup as the zygote. This is the genotype of the child.



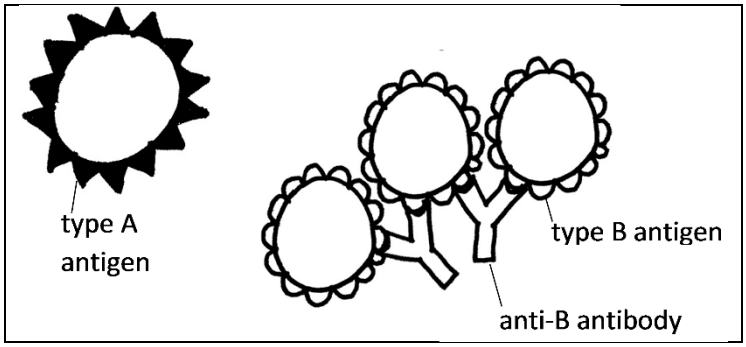
5c. Draw a Punnett square in the usual format for this same mother and father.

5d. Write in the blood type for each child.

Note that meiosis and fertilization can produce a child who has a different blood type than either parent.

▪ Understanding Blood Type Tests

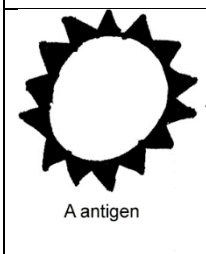
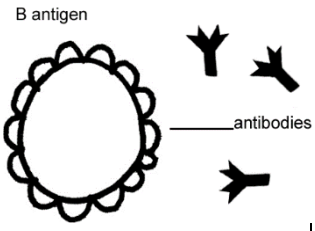
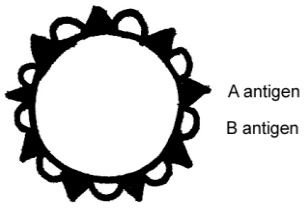
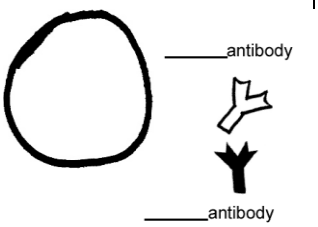
The type A and type B carbohydrate molecules are called **antigens** because they can stimulate the body to produce an immune response, including **antibodies**. Each specific type of antibody binds to a specific antigen. For example, anti-B antibodies bind to type B antigens, but not to type A antigens.



Normally, your body does *not* make antibodies against any antigens that are part of your body. For example, a person with type A blood

- does *not* make anti-A antibodies against the type A antigen on his/her red blood cells
- does make anti-B antibodies against the type B antigen which is *not* present on his/her red blood cells.

6. Fill in the blanks in this chart.

If you have type A blood, you have:	If you have type B blood, you have:	If you have type AB blood, you have:	If you have type O blood, you have:
• type A antigens on the surface of your red blood cells and • _____ antibodies in your blood.	• type B antigens on the surface of your red blood cells and • _____ antibodies in your blood.	• both type ___ and type ___ antigens on the surface of your red blood cells and • neither anti-A nor anti-B antibodies in your blood.	• neither type of antigen on the surface of your red blood cells and • both _____ and _____ antibodies in your blood.
			

If you are given a blood transfusion that does not match your blood type, antibodies in your blood can react with the antigens on the donated red blood cells. This reaction can cause the donated red blood cells to burst and/or clump together and block blood vessels. A transfusion reaction can be fatal. To prevent this from happening, doctors test whether donated blood is compatible with a person's blood before they give a blood transfusion.

7. Explain how a type B blood transfusion could be fatal for a person with type A blood. Begin with the antibody-antigen reaction in a person with type A blood who has been given a type B blood transfusion. (Hint: See the figure at the top of the page.)

To test blood types, you will first mix a blood sample with a solution that contains anti-A antibodies. If the blood sample has type A antigens, they will react with the anti-A antibodies, and this will result in clumping, also known as **agglutination**. Then, to test whether this blood has type B antigens, you will mix a second sample of this blood with a solution that contains anti-B antibodies.

8. To prepare to interpret the blood type tests, fill in the following chart.

Blood type	**Will this blood type clump if mixed with**	
	anti-A antibody?	**anti-B antibody?**
A		
B		
AB		
O		

Procedure

- Your group will need a blood-typing tray or other testing surface for each person listed below.
- For each person, you will use two separate blood samples to test for the A antigen and test for the B antigen. Place two drops of the person's blood in each of the testing locations on the testing surface.
- Place two drops of anti-A antibody solution on the appropriate blood sample and place two drops of anti-B antibody solution on the other blood sample.
- Mix each blood sample with the antibody solution with a clean toothpick and. Discard each toothpick after you have used it.
- If your testing surfaces are transparent, place them on a white background so you can more easily see whether there was a clumping reaction. For each person, record the results of both tests in the table below.
- Write in the blood type and possible genotypes of each person.

Results

	Reacts with anti-A antibody (Yes or No)	**Reacts with anti-B antibody (Yes or No)**	**Blood type (A, B, AB, O)**	**Possible genotype or genotypes**
Michael (father of twins)				
Danielle (mother of twins)				
Earnest (father of daughter)				
Denise (mother of daughter)				
Michael Jr. (boy twin)				
Baby girl 1 (girl twin, according to hospital)				
Baby girl 2 (daughter of Earnest and Denise, according to hospital)				

▪ Summing Up

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

9a. Draw Punnett squares in the space below that show the possible genotypes for Michael and Danielle and their children. Write in the blood type for each genotype to show the possible blood types for Michael and Danielle's children.

9b. Draw Punnett squares that show the possible genotypes for Earnest and Denise and their children. Write in the blood type for each genotype to show the possible blood types for Earnest and Denise's children.

9c. Who are the parents of each baby girl? How do you know?

9d. Were the babies switched?