Summer 2019

Foundations of Biology Lab Manual (Georgia Highlands College)

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GEORGIA HIGHLANDS COLLEGE

LABORATORY MANUAL

Foundations of Biology – BIOL1010K

(Revised Summer 2019)

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.

Lab 1: THE SCIENTIFIC METHOD

INTRODUCTION: What is science and how do we "do" science?

Science is how we gain knowledge about the natural world. Typically, it pertains only to what we can investigate or observe using our senses -- or instruments that extend the ability of our senses. As a science, **biology** concerns itself with understanding the **unity and diversity of living things** -- the **2,300,000, or so, described (and millions of undescribed) species** with which we share planet Earth.

Ideally, the **SCIENTIFIC METHOD** is a **process** that describes *how* scientists perform investigations to provide a systematic and rational approach to answer questions about the natural world. The goal here is to eliminate bias -- and **be as objective as possible** -- in what we study. **Preconceived ideas that can't be tested using the scientific method have no place in science.**

The goal of today's lab is to familiarize you with the steps of the **scientific method.**

You will use these steps to determine the effects of caffeine and alcohol on the heart rate of a small **aquatic** organism known by the **Latin name** *Daphnia magna* (**common name:** "water flea"; Fig. 1). This is an ideal **model organism** because its body is transparent, allowing its internal organs to be viewed with the help of a **dissecting microscope** (Fig. 2).

Figure 1. *Daphnia magna,* water flea
Credit: Dieter Ebert, own work
https://creativecommons.org/licenses/by-sa/4.0/deed.en

Figure 2. Dissecting microscope
Credit: Sarah Greenwood, own work
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Model organisms are non-human species used in research to investigate biological processes. Information learned in studies of model organisms can often be applied to other species, including humans. We use model organisms to learn about many different processes, including genetics, cellular mechanisms, and growth and development. There are certain characteristics that make a species an ideal model organism. For example, it must be easy to manipulate for study, inexpensive and easy to rear, and produce lots of offspring. Some commonly used model organisms include *Drosophila melanogaster* (fruit fly), *C. elegans* (roundworm), and *E. coli* (bacteria). The best model organism to use for a study depends upon the question being investigated. In this study, *Daphnia magna* is a good model organism because of its transparent body, which allows for ease of measuring heart rate and gathering data on the effects of caffeine and ethanol.

STEPS OF THE SCIENTIFIC METHOD

The scientific method consists of: 1) making an **observation**, 2) **asking a question** based on that observation, 3) predicting a logical answer to that question (stated in terms of an **hypothesis)**, 4) the **design of a controlled experiment** to see if the hypothesis is supported or not, and 5) the **collection, analysis and interpretation of data** generated during the experiment.

If the **conclusion of an experiment** is such that a hypothesis is *not* supported, then another hypothesis must be developed along with another experiment designed to test it. Ultimately, the results of successful experimentation are **published in peer-reviewed journals** (along with detailed methods used to obtain them) so that other researchers can verify or replicate the experiment. See Fig. 3, below.

An **idealized version of the scientific method** appears below (Fig. 3). It is considered "idealized" because it is important to note that **chance** plays an important role in science. Often, the initial observations that result in important discoveries are stumbled on by accident rather than sought out.

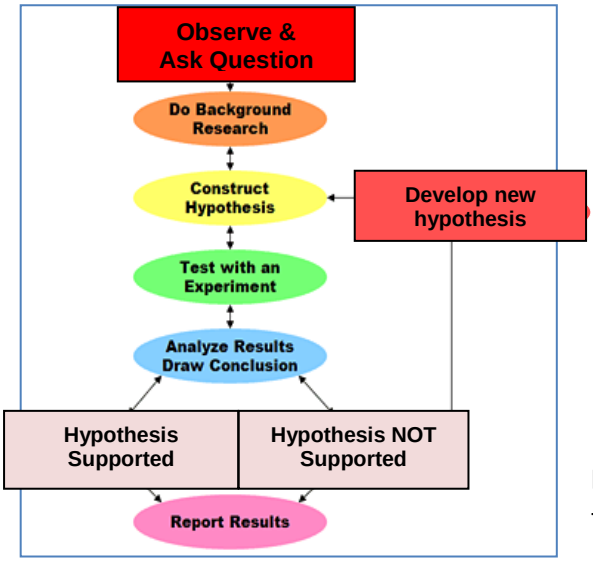


Figure 3. Idealized steps of the Scientific Method

MATERIALS & METHODS

Materials and Supplies

Daphnia magna specimens
Dissecting microscope
Watch glass
Cotton balls
Petroleum jelly
Wooden applicator sticks
Dissecting pins
Plastic pipettes
Test solutions (water, and alcohol and caffeine of varying concentrations)
Paper towels
Watch/clock with a second hand, or alternative electronic device
Event counter
Calculator
Diagram of a *Daphnia*

Step 1: Observation of *Daphnia* in watch glass

Making and recording observations (often referred to as **DESCRIPTIVE SCIENCE**) is the first step in the scientific method. Start by making general observations on water fleas.

1. Remove a **dissecting scope** from the storage cabinets, as instructed, plug it in, and look through the eye pieces (**objective lenses**).
2. Obtain a **watch glass**, which will be used to put the *Daphnia* on which you conduct your experiments. Place a small amount of cotton, finely teased apart, on it. Use a pipette to remove a *Daphnia*, and some of the water it's in, from its jar (at the front of the lab) and place it on the cotton. Make sure the *Daphnia* is totally covered by water.
   *__ALTERNATELY__, you may use a dab of petroleum jelly to immobilize your *Daphnia*. Use a wooden applicator stick to place a **very small** amount of petroleum jelly onto the watch glass.
3. Take the watch glass and water flea back to your lab station to view under the microscope. Use the **top light only** and the **focusing knobs** and to bring your specimen into focus.
4. Use the dissecting pin to gently maneuver the *Daphnia* onto its side so that you can clearly view its heart. *When using petroleum jelly, be careful not to suffocate your specimen in the jelly.
5. Look carefully at your water flea. Refer to the chart that depicts *Daphnia* morphology to identify the animals' various parts.

6. **Make a rough sketch of the *Daphnia's* body.** Label the following parts: head region, eye, heart, digestive tract/midgut, thoracic appendages (actually leg-like structures that also function as gills), etc. On your drawing, indicate which body parts are moving. Do you see any eggs or young? Provide a rough size scale for your drawing.

7. Once you have found and observed your *Daphnia*'s heart, count the number of heartbeats in one minute. The heartbeat of a healthy specimen is about 2 to 5 beats per second. Because it is so fast, count the heart beat for 15 seconds and then multiply that figure by 4. Use an **event counter** if necessary. Record the following information:

    a. Heart beat per 15 seconds: _____

    b. Heart rate per minute: _____

Step 2: Formulation of Questions and Hypotheses

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

A **hypothesis** is a testable explanation for a set of observations based on available data. It is a **tentative answer to the question you are asking** based on **knowledge** about what you are observing and asking. (This knowledge can be pre-existing or information from a published resource.)

In this lab, you need to formulate several hypotheses about how you believe various test solutions will affect the heart rate of a *Daphnia* based on your **prior knowledge** of how these solutions affect humans. After you formulate the hypotheses, you will test predictions based on these hypotheses.

Note: Hypotheses are usually written as **"If….., then…."** statements.

Example: **If** I put *Daphnia* in warm milk, **then** their heart rates will decrease. We'll put them to sleep.

Formulate hypotheses to describe what you think will happen to *Daphnia* in the test solutions.

a. **Water** (from the *Daphnia* culture jars)

b. **Ethyl Alcohol** (in four concentrations: 2%, 4%, 6%, 8%)

c. **Caffeine** (in three concentrations: 1%, 2%, 3%)

Step 3: Experimental Design

The next step in the scientific method is to **test predictions based on your hypotheses** by designing one or more **experiments** that allow you to collect the best **data**[1] to answer your question.

Before doing this, it is necessary to **define the variables** with which you will be dealing.

- An **independent variable** is the condition or event under study. It's the **pre-determined condition *the investigator* sets (and can vary)**. Only one independent variable is tested at a time, so that an observed response is attributable to *just* that variable. In today's lab, **the independent variables are the test solutions to which your animals are subjected**.

- A **dependent variable** is the condition or event that occurs (**the data collected**) in response to the specified, pre-determined, independent variables that are set. In our *Daphnia*, a **dependent variable is the number of heart beats that occur in response to a specific test solution**.

- Controlled variables are any conditions or events that *could* potentially affect the outcome of an experiment. Consequently, they *must* be **held constant (controlled)** and **never varied**. In the case of our *Daphnia*, an example of a controlled variable would be the temperature of the water in which the *Daphnia* are tested. This variable needs to be controlled because *Daphnia* hearts beat faster in warm water than they do in cold water.

[1] The word **data** is plural; the singular form of the word is **datum**

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

a. What will be your **independent variables**? List all of them:

b. What will be your **dependent variables**? Be specific with your answers.

c. What other **variables** *should* be **controlled**?

Importance of a Control Group

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

Step 4: Procedure

1. Your group will test the effects of **plain water** and **JUST ONE of the test solution categories** – either the alcohol (in all concentrations) ***OR*** the caffeine (in all concentrations).
2. The test solutions are as follows:
    a. Water from the *Daphnia* container
    b. Alcohol (2%, 4%, 6%, 8%)
    c. Caffeine (1%, 2%, 3%)
3. To test a solution, you need to remove most of the existing water covering the *Daphnia* in your watch glass. "Wick it away" with a small piece of paper towel at the same time you add your test solution with a pipette. Do not add more than 5 ml of each test solution. Also, always keep the water flea submerged in fluid! **If your Daphnia dies at any point, you need to re-start the experiment with a new specimen.**
4. Add the first test solution (water from the *Daphnia* container), wait 1 minute, and count the heartbeats for 15 seconds. Record your data in the chart below and calculate the number of beats per minute.
5. Add the second test solution (more water from the *Daphnia* container) by wicking the previous water sample away as described above. Wait 1 minute, then count the heartbeats for 15 seconds. Record your data and calculate the number of beats per minute.

6. Repeat these steps 6 more times. Use your data to calculate an **average value** for the effects of water on the heart rate of your water flea. This part of the experiment is the **control** for your **experiment**. It serves as a **baseline** against which you can compare the results from the *Daphnia* you subject to the alcohol or caffeine.
7. Next, test all the other solutions your group has been assigned – either alcohol or caffeine. Start with the lowest concentration and progress to the highest concentration.
8. Note: Be sure to keep all the steps of your experimental protocols exactly the same (add only 5 ml of solution, always wait one minute before counting, record the heart beats for 15 seconds, etc). Due to time constraints, **do only one run for each test solution.**
9. When your tests have been completed, use a pipette to transfer your *Daphnia* to the recovery beaker in the front of the lab.
10. **Wash and dry all the glassware you used and put it back where you found it. Dispose of used pipettes in a garbage can. Make sure lids are on all your solution bottles. Clean up any mess made may made and wipe down your lab bench with the cloths provided.**
11. Compile class data as directed by instructor.

Record your individual data in Table 1, below:

Table 1. Baseline (control) heartbeats for *Daphnia*

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

Average of 8 treatments		

Substances tested by your group: _____

Test substance 1 first, followed by 2, 3, and 4 (lowest to highest concentrations).

Record your results below, in Table 2.

Table 2. *Daphnia* heartbeats when subjected to test substances

Substance Concentration	Beats per 15 seconds	Beats per 1 minute

Compile class data in the chart below.

Drug Concentration	Group #1	Group #2	Group #3	Group #4	Group #5	Group #6	Group #7	Group #8	Class Avg.
Average Water Treatment									
2% alcohol									
4% alcohol									
6% alcohol									
8% alcohol									
1% caffeine									

2% caffeine									
3% caffeine									

Step 5: Data Analysis, Display and Interpretation

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

A **graph** is a **diagram showing the relationship between independent and dependent variables.**
When making graphs, the following rules should be observed:

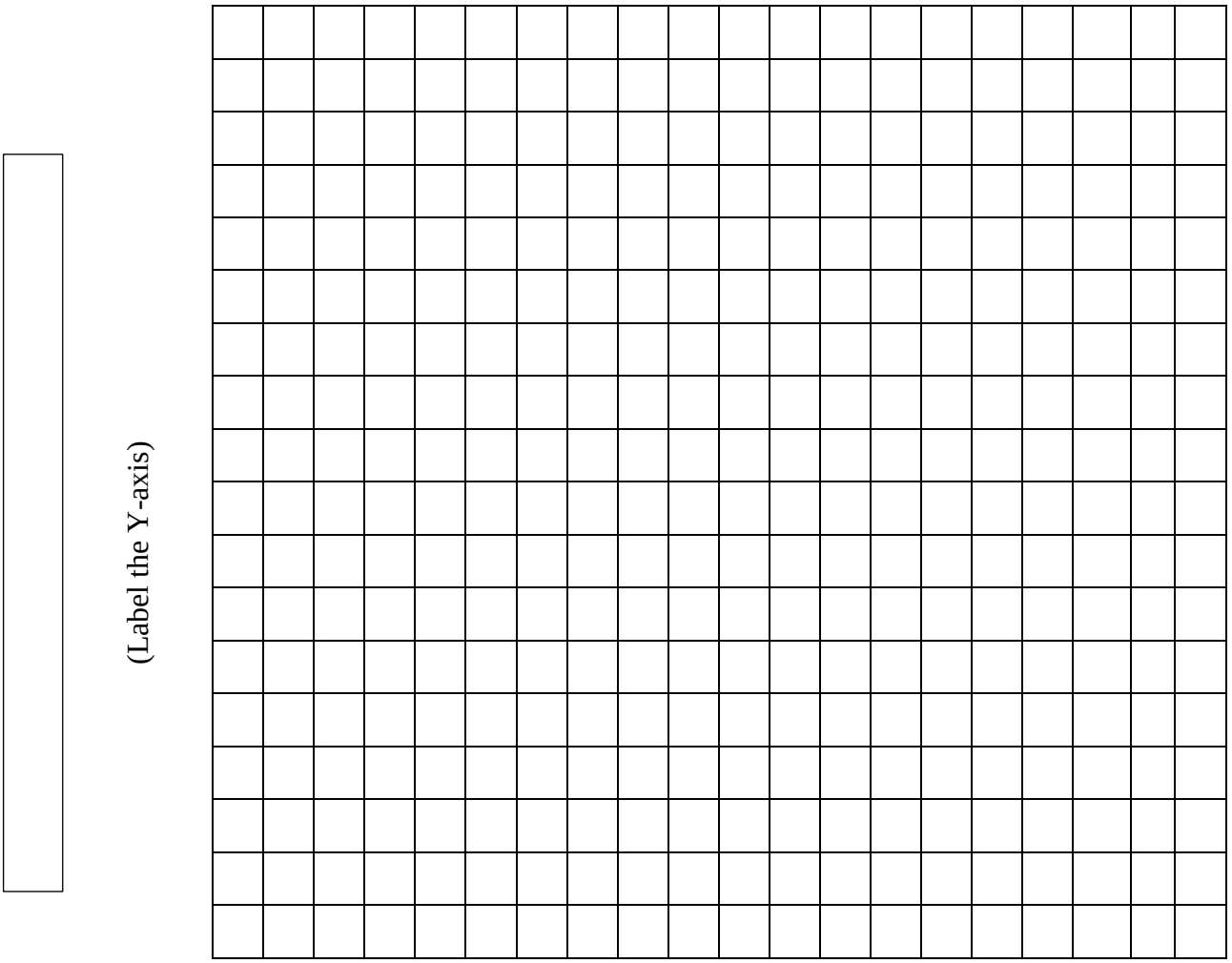
1. The **independent variable** is usually plotted on the **X-axis** (horizontal axis) and the **dependent variable** is plotted on the **Y-axis** (vertical axis).
2. Each axis should be **labeled properly** with the name of the variable and the units of measurement.
3. Data intervals must be evenly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

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

Use the grid provided below to graph your individual results.

_____

(Create a title)



(Label the X-axis)

Interpret Your Results

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

Did the results of your experiments **support** or **refute** your hypothesis?_____

Lab 2: ORGANIC MOLECULES

Introduction

Organic compounds contain carbon and, strictly defined, include very simple molecules such as carbon monoxide (CO) and carbon dioxide (CO_2) that behave more like inorganic molecules than organic ones. However, for our studies, we define **organic molecules** as **containing hydrogen along with carbon**.

Biologically important organic compounds also contain the four other "building " block" elements: **oxygen (O), nitrogen (N), phosphorus (P) and sulfur (S)**.

The four main groups of biologically important compounds are the **carbo-hydrates**, **lipids**, **proteins** and **nucleic acids**. These compounds are also known as biological "macromolecules" and all but the nucleic acids are the common food categories listed on Nutrition Facts panels ("food labels," right). The organic molecules, their functions, and the elements that comprise them appear in Table 1.

Table. 1. Comparison of carbohydrates, lipids, proteins and nucleic acids

Type of Molecule	Chemical Components	Function	Examples
Carbohydrates *Mono*saccharides - "*One* sugar"	C, H, O	Nutrient/energy source	Glucose, fructose (fruit sugar), ribose, deoxyribose, galactose
*Di*saccharides - "*Two* sugars"		Nutrient/energy source	Sucrose (table sugar), maltose, lactose (milk sugar)
*Poly*saccharides - "*Many* sugars"	Chitin also has N	Energy storage (short term) Structural support	Starch (plants), glycogen (animals) Cellulose (plants), chitin (animals, fungi)
Lipids - Fats - Phospholipids - Steroids	C, H, O, P, N (contain P, N)	Energy storage (long term) Cell membrane structure Cell membrane component	Triglycerides Cholesterol, sex hormones
Proteins	C, H, O, N, S	Structural components Movement Regulate metabolism Transport oxygen Regulate blood glucose Protect against disease	Keratin Actin, myosin Enzymes Hemoglobin Insulin Antibodies
Nucleic Acids	C, H, O, N, P	Store & transmit genetic info	DNA, RNA ATP

		Cell's "energy currency"	

In this lab, we will use **chemical indicators** (aka ***test reagents***) and chemical tests to detect the presence of two kinds of carbohydrates (reducing sugars and starch), lipids and proteins. Chemical indicators are substances that react in a characteristic fashion (often a color change) if a particular molecule is present.

Each test will include a **positive control**, a **negative control**, and one known substance. Each group of students will also test one unknown substance for its contents.

A ***positive* control** is a test substance that produces a positive result. It shows what a "positive" looks like. It contains the compound for which we are testing and all the appropriate chemical indicator(s).

A ***negative* control** is a test substance that produces a negative result. It shows what a "negative" looks like. It usually contains *just distilled water* (**dH_20**) and the appropriate indicator(s). To be valid, a negative control is placed through ***all*** *the physical steps* of a positive control – such as heating, changing of pH, etc., if required.

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

Record the number of your unknown here: _____

Testing for Carbohydrates

Monosaccharides (single-ringed sugars) and ***most* disaccharides** (double-ringed sugars formed by joining two monosaccharides) are **reducing sugars**. This means they have a **carbonyl group** (a C double-bonded to an O) as shown below in the monosaccharides **glucose** and **fructose** (Fig. 2, below). Note: These sugars can also occur as linear structures. **Sucrose** is not a reducing sugar.

Fructo

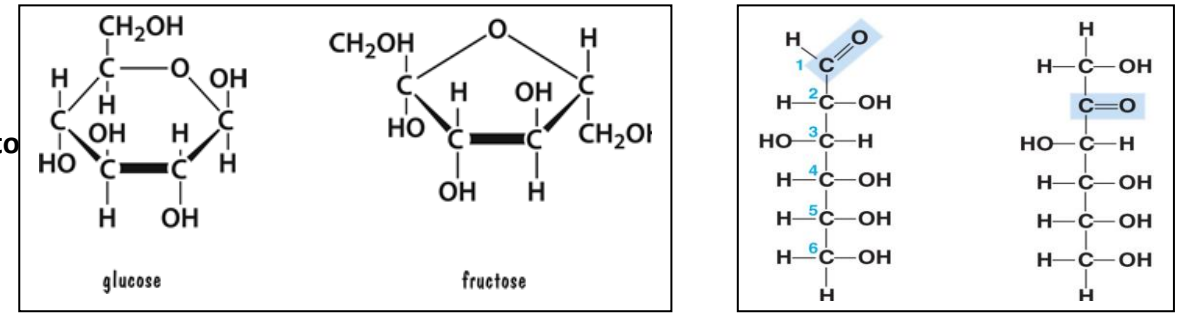


Figure 2. Glucose and fructose in ringed or linear forms

Benedict's reagent (a solution of copper sulfate, sodium carbonate and sodium citrate) **is the indicator used to test for the presence of reducing sugars**. In the absence of such sugars, Benedict's is a bright

royal blue color, and clear (not cloudy). However, when heated in the presence of a reducing sugar, it accepts electrons from the sugar and changes color. It also becomes cloudy as it forms a **precipitate** (an insoluble solid that emerges from a liquid solution) of cuprous oxide.

The degree of color change depends on the amount of reducing sugar present (Fig. 3). A change from blue to yellow, or green, indicates a small amount of reducing sugar. A change from blue to red, or orange, indicates a large amount of reducing sugar.

No sugar Trace amount Moderate amount Large amount

Figure 3. Results of Benedict's test for reducing sugars

From left to right, above:

- **Negative control**: Benedict's reagent + distilled water (after heating)
- **Negative reaction (minimal reducing sugars)**: Benedict's reagent + unknown (after heating)
- **Positive reaction (some reducing sugars):** Benedict's reagent + unknown (after heating)
- **Positive control (large amount of reducing sugar):** Benedict's reagent + glucose solution (after heating)

Polysaccharides, like starch and glycogen, are very long chains of monosaccharides and **do not react with Benedict's reagent**. Consequently, a different indicator is required to test for their presence.

Lugol's reagent (aka Lugol's Iodine) (I_2KI), an amber-colored clear liquid, indicates the presence of **starch,** the *highly digestible* storage polysaccharide of plants. The starch molecules interact with iodine to produce a blue-black color (Fig. 4, below). **Glycogen**, the storage polysaccharide in animals reacts to a lesser extent with Lugol's to produce a red-brown or reddish-purple color.

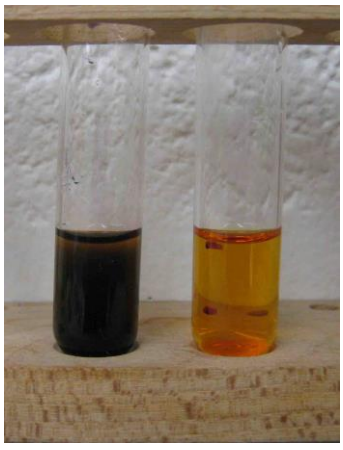


Figure 4. Positive (left) and negative (right) results of Lugol's iodine tests for starch

Carbohydrate Test 1 - Benedict's Test for Reducing Sugars

Materials Required

Test Tube Rack	Grease pencil	Distilled water (dH_2O)
4 test tubes	Pasteur Pipettes	Starch solution
Test tube holder	**Benedict's reagent**	Unknown
Boiling water bath	Glucose solution	

Procedure

1) Use the grease pencil to number **four clean test tubes 1 through 4**.
2) Use a pipette to **transfer 1 mL of the following solutions to the numbered tubes**.

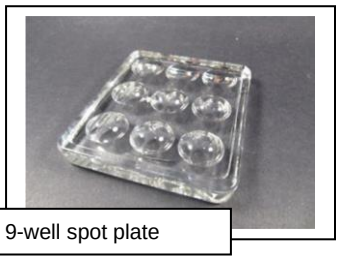
Tube 1	Tube 2	Tube 3	Tube 4
Glucose (positive control)	dH_2O (negative control)	Starch	Unknown # ___

3) Add an **equal amount (1 mL) of Benedict's reagent** to each tube. Swirl to mix. Record color of material each tube in Table 3, below (*before* doing anything else to it).
4) Place the tubes in the test tube rack, in a boiling water bath (on the instructor's lab bench) for 5 minutes. Record any color changes, or changes in appearance in Table 3. Use color change information to determine whether or not each solution contained reducing sugars.
5) **Discard contents of test tubes in the location indicated by your instructor**. Use test tube brushes to **wash tubes with soapy water. Rinse thoroughly and shake out excess water. Return test tubes to tray.**

Table 3. Results of Benedict's Test for Reducing Sugars

Tube #	Material Tested	Color (solution plus indicator) *before* boiling	Color *after* boiling	Reducing sugar present? (Yes or No)
1				(pos control)
2				(neg control)
3				
4	Unknown # _____			

Carbohydrate Test 2 - Lugols's Iodine Test for Starch


9-well spot plate

Materials Required

9-well spot plate	Toothpicks	Glucose solution
Laminated grid	**Lugol's Iodine**	dH_2O
Pasteur pipettes	Starch solution	Unknown

Procedure

1) Place your spot plate on the laminated sheet with the 9-square grid.
2) Use a Pasteur pipette to transfer 5 drops of the following solutions to the appropriately numbered well.

Well 1	Well 2	Well 3	Well 4
Starch solution (positive control)	dH_2O (negative control)	Glucose	Unknown # _____

3) Add **5 drops of Lugol's Iodine** to each well. Mix using a toothpick. Record the color of the liquid in each well in Table 4.
4**) Discard contents of spot plate as instructed. Wash your spot plate, with soapy water. Rinse and dry.**

Table 4. Results of Lugol's Test for Starch

Well #	Material Tested	Color ***before*** adding Lugol's	Color ***after*** adding Lugol's	Starch present? Yes or No
1				(pos control)
2				(neg control)
3				
4	**Unknown # _____**			

Testing for Proteins

Biuret reagent (a light aquamarine liquid) is **used to test for <u>proteins</u>**. Proteins are made by linking together **amino acids** by **peptide bonds**. Note that **a positive Biuret reaction only occurs at an elevated pH**; therefore, **a strong *base*** (**<u>NaOH</u>**) needs to be added to the Biuret reagent, turning it a turquoise color.

Copper ions in the Biuret reagent react with peptide bonds causing a color change from its original color to purple or pink (**Fig. 5**). Proteins with short peptide chains turn pink; those with longer chains turn purple. Other types of molecules can cause color changes, but <u>*only the purple or pink colors indicate the presence of peptide bonds.*</u>

Pepsin (listed below as a known material to test) is a digestive enzyme. Most enzymes are proteins.

Figure 5. Positive result of Biuret test for protein
(Creative Commons: Ozone aurora/Philip Evans - Own work)

Test 3 - Biuret Test for Proteins

<u>Materials Required</u>

9-well spot plate	Toothpicks	Albumin	Unknown
Laminated grid	**Biuret Reagent**	dH_2O	
Pasteur pipettes	**NaOH**	Pepsin	

<u>Procedure</u>

1) Place your spot plate on the laminated sheet with the 9-square grid.
2) Use a Pasteur pipette to transfer 5 drops of the following solutions to the appropriately numbered well.

Well 1	Well 2	Well 3	Well 4
Albumin (positive control)	dH_2O (negative control)	Pepsin	Unknown # _____

3) Add **5 drops of Biuret and 3 drops of NaOH** to each well. Mix using a toothpick. Record the color of the liquid in each well in Table 5.
4) **Discard contents of the spot plate in location indicated by your instructor. Wash your spot plate, in the sink at the back of the lab, with soapy water. Rinse thoroughly and dry. Return to tray.**

Table 5. Results of Biuret Test for Protein

Well #	Material Tested	Color ***before*** adding Biuret & NaOH	Color ***after*** adding Biuret & NaOH	Protein present? Yes or No
1				(pos control)
2				(neg control)
3				
4	**Unknown # ____**			

Testing for Lipids

Lipids (fats, oils, waxes and steroids) are a varied and diverse group of organic molecules that are **hydrophobic.** They *cannot* dissolve in polar solvents such as water. They *can* dissolve in nonpolar solvents (such as ethanol).

Most of the lipids in living things are **triglycerides**, which consist of three fatty acids bonded to one glycerol molecule. Fats are *solid* triglycerides at room temperature; oils are *liquid* triglycerides at room temperature. This designation depends on the **degree of saturation in the fatty acids** in the triglycerides. Solid fats are generally described as **saturated fats**; liquid oils are described as **unsaturated fats**.

Test 4 – Test for Lipids

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

Ethanol is an amphipathic molecule; it has both polar and non-polar ends. Because of the non-polar component of the molecule, ethanol can dissolve lipids; however, because of polar component, ethanol can also mix with water. The **ethanol emulsion test** works because of the

amphipathic nature of ethanol. When lipids are present in a sample, they dissolve in ethanol, and the mixture remains clear. However, when added to water, the lipids are forced out of solution and appear as tiny fat droplets, which reflect light and appear whitish (Fig. 6).

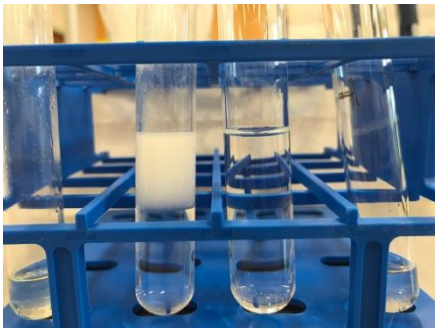


Figure 6. Positive ethanol emulsion test (left) and negative ethanol emulsion test (right).

Materials Required

Test tube rack	Pasteur pipettes	dH_2O
8 test tubes	Ethanol	Heavy cream
Grease pencil	Vegetable oil	Unknown

Procedure

1) Use the grease pencil to number **four clean test tubes 1 through 4**.
2) Use a pipette to transfer 1mL of each substance into a clean test tube.
3) Use a clean pipette to transfer 2 mL of ethanol into each tube. Use a gloved finger to cap the tubes and shake well to mix.
4) Allow the contents to settle for about 30 seconds.
5) Use a clean pipette to remove the top half of the solution and transfer it to a clean test tube.
6) Add 2mL of dH_2O to each tube, and observe the results.
7) Use the appearance of a milky whitish layer as a positive indication of the presence of lipids in the sample.
8) **Discard contents of test tubes in the location indicated by your instructor**. Use test tube brushes to **wash tubes with soapy water. Rinse thoroughly and shake out excess water. Return test tubes to tray.**

Table 6. Results of ethanol emulsion test for lipids

Spot #	Material Tested	Lipid present? Yes or No
1	Oil	(pos control)
2	dH_2O	(neg control)
3	Heavy cream	
4	**Unknown # ____**	

Testing Unknown Substances

1) Record the data from your unknown in Table 7, below. Note that the natural color of the unknown can influence the color of your results. Therefore, think in terms of how the color of your test reagent might change when added to a colored substance.
2) Determine what compounds (reducing sugar, starch, lipid, protein) are present in your unknown.
3) Use data from other lab groups to complete the table.

Table 7. Analysis of Unknown Substances; Class Results

Unknown	Benedict's Test (+ or -)	Lugol's Test (+ or -)	Biuret Test (+ or -)	Ethanol Emulsion Test	Compounds Present
1					
2					
3					
4					

Can you guess what each of the unknowns are?

_____
_____
_____

After completing the experimental portion of this lab, please:

- Cap and return all test reagent bottles to the trays provided for your lab bench.
- Discard used pipettes in trash cans.
- Dispose of all test materials in the locations indicated by your instructor.
- Wash and dry (as best you can) all glassware used and return to the tray on your lab bench.
- Wipe down your lab bench

LAB 3: Microscopy

Many biological specimens are too small to be seen with the naked eye. They must be magnified to be studied. Consequently, the basic operation and care of **microscopes** is an important skill in biology.

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

Compound light microscopes (Fig. 1) pass light through two lenses to magnify specimens (usually very thin *slices* of a specimen) mounted on a **glass slide** and placed on the flat surface (**stage**) of the microscope.

The first set of lenses (**oculars**) is located at the top of the microscope -- where we place our eyes – and magnifies a specimen ten times (10x). The second set of four lenses is located on a **revolving nosepiece**. Collectively known as **objective lenses**, these can magnify a specimen four times (4x), ten times (10x), forty times (40x), or 100 times (100x), respectively. Magnification values are etched on the sides of the metal casings that protect the lenses. The maximum magnification attainable with this type of microscope is 1000x – determined by multiplying magnification value of the ocular lens by that of the objective lens.

The light source in these microscopes can be controlled at a variety of points on the microscope, which you will see as part of this lab.

Stereomicroscopes (aka **dissecting microscopes;** Fig. 2) allow objects to be viewed in three dimensions at lower magnifications than possible using a compound light microscope. They are used to study entire small organisms or other opaque objects that can be viewed only by reflected light cast on a specimen. It is also possible to view multiple samples in a petri dish placed on the microscope's stage (such as we did with the *Daphnia* we observed several weeks ago) with light that is projected from below.

Ocular lenses on a stereoscope magnify by a factor of 10x. There is a great deal of variation in stereo-microscopes and the manner in which they achieve higher magnification. This will be discussed in lab.

Figure 1. Compound light microscope

Figure 2. Stereomicroscope

Electron Microscopes

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

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

Scanning electron microscopes, analogous to stereomicroscopes, allow a specimen's surfaces to be observed in detail. The object coated with a thin film of metal. An electron beam excites surface electrons on the specimen and produces a three-dimensional image.

Electron microscopes are expensive and require special training. They cannot be used to observe live cells.

Image Inversion

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

Dissecting microscopes, work in a similar manner, but include an additional magnification system that makes the final image appear to be upright.

The Compound Light Microscope

Proper practice for handling and use of compound light microscopes is as follows:

1. **Carry the microscope upright with one hand supporting the base.** Care should be taken not to bump the microscope on the microscope cabinet, chairs, tables, or other obstacles. **Gently** place your microscope on your laboratory bench and remove its protective plastic cover.
2. **Do not push or slide the microscope across the table.** This causes vibrations that can loosen screws or misalign microscope parts.
3. **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 your station drawers).

4. **Always begin viewing a slide using the scanning (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 will probably need to focus only slightly, as most light microscopes are **parfocal**, meaning that the image remains nearly in focus as you change lenses (from lowest to highest power).
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 material from the stage. Lower the stage and move the lowest power objective into position. Bundle the electrical cord securely (not around the arm of the microscope), replace the plastic cover, and put the microscope back in the storage cabinet.

Materials:

- Compound light microscope, stereomicroscope
- Prepared slides: letter "e", colored threads, human epithelial (cheek) cells, onion epithelial cells
- Clear plastic ruler

In this exercise, you will identify and learn the function of various microscope parts, practice focusing the microscope, and learn to use the microscope as a measuring tool. Use Fig. 3, below, to identify and learn parts of the microscope listed in Table 1, below.

Table 1: Microscope parts and functions

Microscope Part	Function
Arm	Supports the body tube and lenses. Use the arm and base to carry the scope.
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. Ocular magnification is **10x**.
Body Tube or **Turret**	Holds the ocular at one end and 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 objective lenses (aka *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 (4x),** followed by **low power (10x)** and **high**

	power (40x) . 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 **manipulator knobs** adjacent to or below the stage. Allows 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 (4x) objective.**
Fine Adjustment Knob	Located within the coarse adjustment knob. Allows fine focus of specimen.

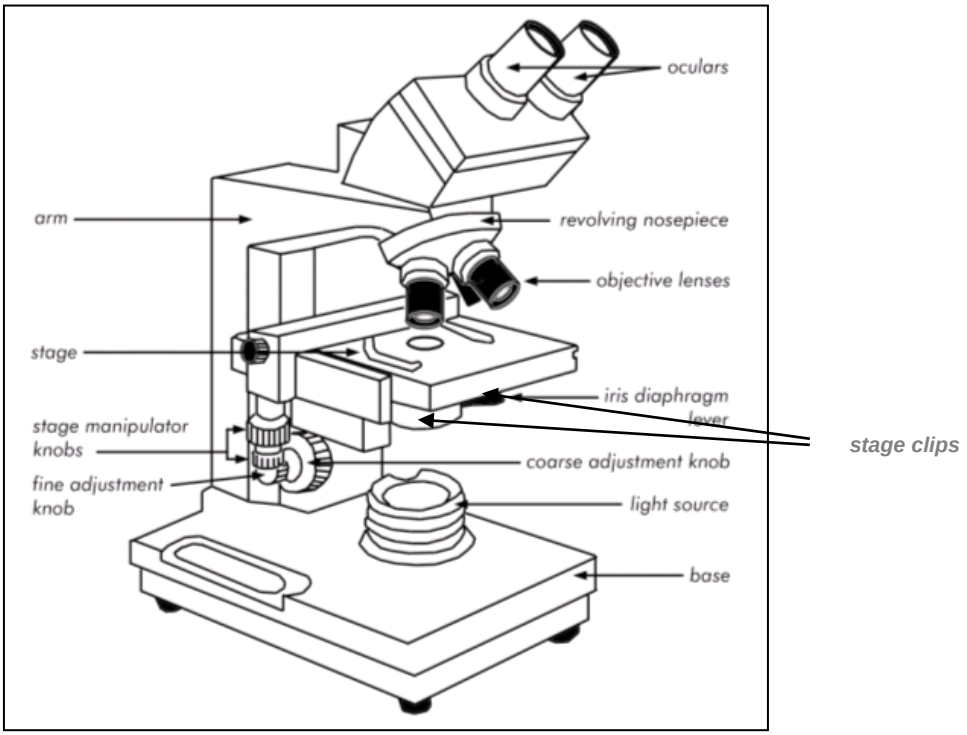


Figure 3: The binocular compound light microscope**.**

Calculating Magnification

When using a compound light microscope, both the **ocular** lens and **objective** lens help magnify the image. Therefore, to calculate the **total magnification** of an image, the contribution of each lens must be taken into consideration. Total magnification can be calculated using a simple formula:

Total magnification = Ocular lens power x Objective lens power

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

Table 2: Total magnification calculations using a compound light microscope

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

Closely related to the topic of magnification, is **resolution**, or **resolving power**, which is the ability of a microscope to distinguish two adjacent structures as distinct, or separate. The higher the resolution, the better the clarity and detail of the image.

1. Focusing & Image Inversion

a) Obtain a slide of the letter "e." Look at the letter "e" and draw what you see in the space below. Place the slide on the **stage**. Secure with the **stage clips**. Make sure that the **scanning objective (4x)** is clicked into place. Use the **stage manipulator knobs** to center the letter "e" over the **light source**.

Letter "e" as seen on hand-held slide

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 through the microscope**.

Letter "e" as seen through microscope

d) Compare what you drew above to the previous drawing you made. How do the two drawings differ?

e) When you view the letter "e" using the **scanning objective**, what is the **total magnification**?

f) 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.

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

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

i) Compared to **scanning magnification**, does the letter appear larger?

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

2. ***Diameter* of field**

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 (4x)** 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 Fig. 4).

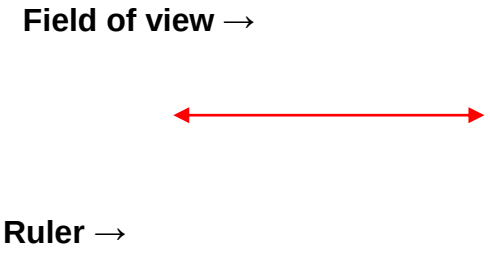


Figure 4: Measuring field diameter using scanning (4x)

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 (10x)** 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.

3. ***Depth* of field**

Obtain a **slide with colored threads** mounted together. Place it on the stage and focus using **scanning (4x) objective**. The center of your field should be 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 power (10x) 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.

4. Microscopic Observations

Obtain a slide of **human epithelial cells** and observe under the microscope. Locate the **nucleus, cytoplasm** and **cell membrane**. Draw and label what you see, below.

What is the approximate size of one cell?

How do you know this?

Obtain a **slide of onion skin (epithelial) cells** and observe under the microscope. Locate the nucleus, cytoplasm and cell wall. Draw and label what you see, below.

Estimate the size of one cell:

The Stereomicroscope (aka Dissecting Microscope)

Your instructor will describe how to use the stereomicroscope. Refer to the Fig. 5, below:

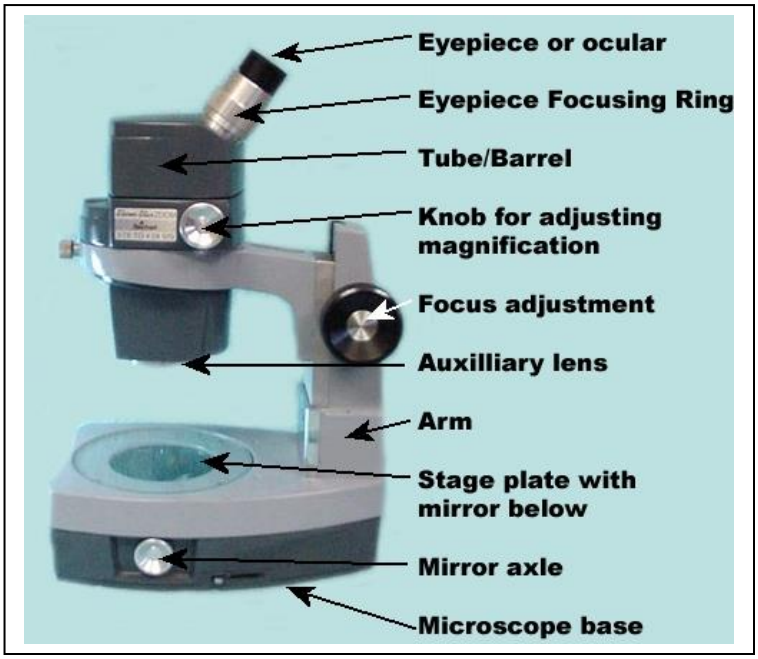


Figure 5. Parts of a Stereomicroscope (aka dissecting microscopes)

Use the **stereomicroscope** to view the variety of materials provided for you (feather, insects in alcohol, butterfly wings, crystals, etc.).

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

Lab 4: CELL STRUCTURE & FUNCTION

Background

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 semipermeable nature of the cell membrane (aka, plasma membrane). The cell membrane controls what enters and exits the cell, and therefore serves a very important cellular function. You will also explore the concept of **tonicity**, which refers to the solute concentration of a solution, and its inherent ability to influence the rate and direction of osmosis.

Diffusion and Osmosis

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.

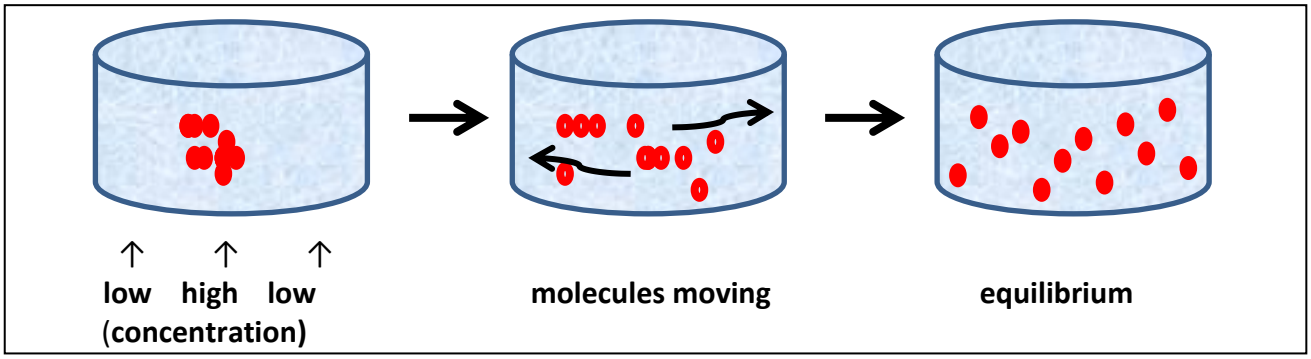


Figure 4.1 Diffusion of molecules from an area of high concentration to low concentration. Equilibrium is reached when the molecules are equally distributed.

Osmosis a specific type of diffusion: the diffusion of water molecules across a semipermeable membrane. Like other molecules, water molecules diffuse down a concentration gradient, from an area of higher water concentration to an area of lower water concentration. This means that water will move across a semipermeable membrane, like the cell membrane, in the direction of

the higher solute concentration. (In solution, high solute concentration = low water concentration; conversely, low solute concentration = high water concentration.) You will practice with this concept in **Part 2: Tonicity**.

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

In 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. The solute is this experiment is **sucrose**, ($C_{12}H_{22}O_{11}$); sucrose molecules are too large to pass through the pores of the dialysis tubing, but the solvent molecules (H_2O), are small enough to pass easily.

Part 1: Diffusion Across a Membrane

Materials:

- Dialysis tubing
- Plastic clips
- 250 mL beakers
- Electronic balance
- Sucrose solutions (15% and 30%)
- Graduated cylinder
- Wax pencil

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). *This step may be done for you; check with your instructor.
2. 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

3. Set beakers aside.
4. Remove one piece of dialysis membrane from the soaking water and open it, forming a tube. Close one end of the tube with a plastic clip or piece of string (Fig. 2)

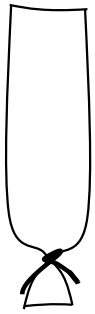


Figure 2. Dialysis tube

5. Fill the tube with 10 mL of H2O. Remove excess air, and close the other end of the tube with a plastic clip or piece of string (Fig. 3). Set aside on a paper towel.

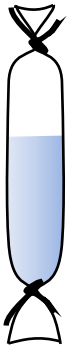


Figure 3.

6. 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
7. Rinse off the outside of the bags with water and carefully blot dry.
8. Weigh each bag to the nearest 0.5g. Record the weights in Table 4.2, in the column labeled "0 min."
9. Place each bag in the corresponding beaker (Bag #1 in Beaker #1, etc.).
10. Set a timer for 5 minutes.
11. At the end of 5 minutes, remove each bag from its beaker, blot excess fluid, and record the mass in **Table 1**.
12. Return the bags to the appropriate beaker, and wait another 5 minutes.
13. Repeat steps 12-13 every 5 minutes and record the weights in Table 1.

Table 1. Osmosis – Mass over time for dialysis bags					
			Mass (g)		
Time (min)	0	5	10	15	20
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 2**. Calculate the **rate** (g/min) of osmosis for each bag by dividing the weight change by the time change. Since all 4 bags were recorded for a total of 20 minutes, the time change for all 4 bags is 20 minutes. Record the rate of osmosis for all 4 bags in Table 2.

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

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** (water) concentration, water moved from the area of _____ concentration to the area of _____ concentration across a selectively permeable membrane, which is defined as _____.

Part 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 three test tubes. In one test tube, a potato strip is soaking in water. In another tube, a potato strip is soaking in 10% sodium chloride (NaCl). In a third test tube, a potato strip is soaking in 0.9% 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? _____

Which solution is isotonic to the inside of the potato cell? _____

What happened to the potato strip in the isotonic solution? _____

_____

Lab 5: ENZYME FUNCTION

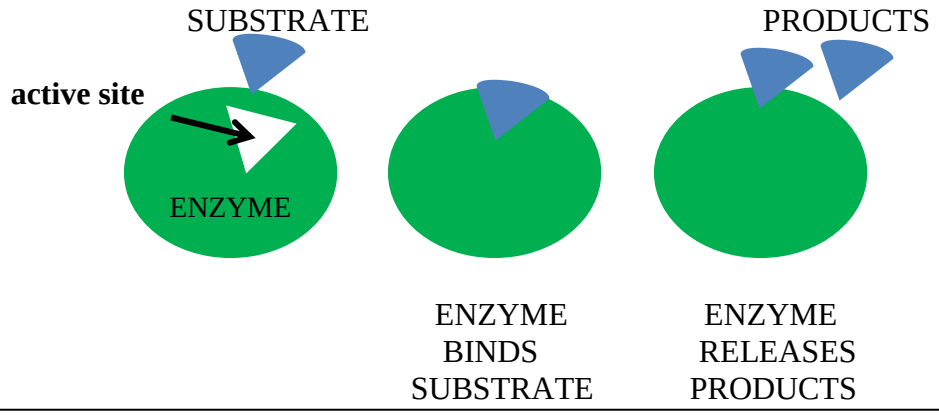
In every living cell, many chemical reactions are performed. In chemical reactions, the **reactants** are molecules that undergo a change, which results in the **products** (Figure 5.1). The arrow stands for the change that produced the product(s). As you can see in the figure below, the number of reactants 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 catalyze (speed up) chemical reactions. They work by binding to the reactants and converting them to a different molecule (the product). Enzymes are specific to a type of reactant, and therefore can catalyze only one type of reaction. This **enzyme specificity** is the result of the particular shape of the enzyme that only permits binding to one type of reactant, much like a key fits a lock (Figure 5.2). The reactants in an enzymatic chemical reaction are called **substrate(s)**. Notice how the shape of the enzyme fits its substrate. The location where the enzyme binds a substrate is called the **active site** because the reaction occurs here. At the end of the reaction, the product is released, and the enzyme can then bind to more substrate. Thus, an enzyme can perform the reaction over and over again, as long as there is substrate present.

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.



Most enzymes are complex proteins that function most efficiently within a specific range of temperature and pH. Extremes in temperature or pH will **denature** the enzyme by permanently altering its chemical structure. Even a small change in the protein's structure will change the enzyme's shape enough to prevent the substrate from binding, and thus keep the reaction from occurring. In this laboratory, you will 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; 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; wait at least 20 seconds.

5. Measure the height of the bubble column (in millimeters); 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; 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 ^{o}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 could 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; wait 10 seconds.
5. Measure the height of the bubble column (in mm); 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); 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; wait 10 seconds.
13. Measure the height of the bubble column (in mm); 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, or a level of acidity or alkalinity at which it functions best. A higher or lower pH affects hydrogen bonding and can alter the structure of the enzyme, leading to reduced activity.

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?
_____

Lab 6: CELLULAR RESPIRATION AND EXERCISE

Background:

Cellular respiration is the process by which the chemical energy in organic molecules like glucose is converted into **ATP**, the form of energy that is usable by organisms.
The chemical equation for the cellular respiration is shown below:

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

(Reactants) (Products)

Note that **oxygen (O_2)** is a reactant; molecules of oxygen are required for the energy conversion reaction to proceed. Glucose may only be **oxidized** completely if sufficient oxygen is available.

Terrestrial animals (including humans) are constantly breathing in oxygen in order to convert glucose into ATP. This process is quite efficient at producing the required ATP for energy in a mammalian body. However, the complex chemical reactions involved in the conversion of glucose to ATP has a number of byproducts, including water and **carbon dioxide (CO_2)**. CO_2 is considered a cellular waste product.

Regardless of our individual activity levels, we are always performing energy conversion reactions. Even we are resting, we are very busy at the cellular level. With increased physical activity, the demand for energy also increases, and there is a corresponding increase in the need for oxygen in order to breakdown more glucose for energy. This is why we breathe more heavily and at an increased rate when we are exercising. Increasing our intake of oxygen is only half of the equation; the other half involves exhaling cellular waste products, specifically carbon dioxide. As activity levels increase and more glucose is converted into ATP, more CO_2 is produced as a byproduct.

Bromothymol blue (BTB) is an pH indicator; when BTB is exposed to an acidic solution, it changes color from blue to yellow. When CO_2 (gas) mixes with liquid water (H_2O), CO_2 reacts with water molecules to form carbonic acid (H_2CO_3). Because carbonic acid gives off H^+ ions in solution, it is considered an acid; it **lowers the pH** of the solution. We can use a pH indicator like BTB to measure the drop in pH (Fig. 1).

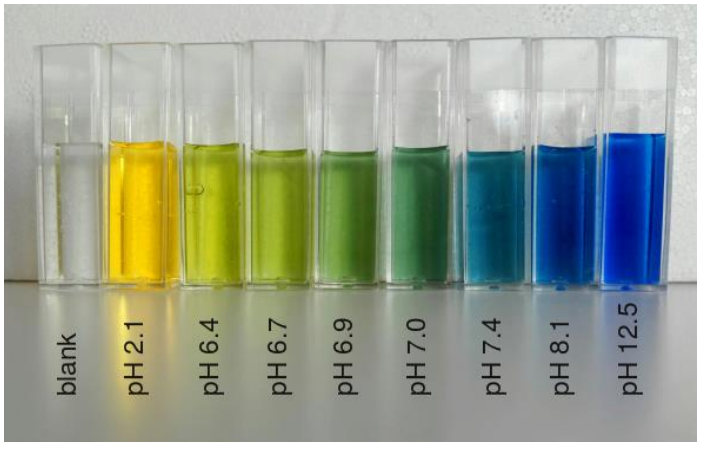


Fig. 1. Bromothymol blue at different pH levels

Purpose

To investigate how exercise influences the rate of cellular respiration, we will measure carbon dioxide production by your body at rest compared with your body after exercising. The more active you are, the more glucose your muscles will need to convert to ATP, which translates to higher O_2 use and subsequently, higher CO_2 production. The more CO_2 you exhale into the BTB solution, the faster you will see a color change from blue to yellow.

Procedure

A. Assembling your Respiration Chamber

Insert straw through the stopper so that it extends about 2 inches out the bottom side. Using a graduated cylinder, measure 10 ml of Bromothymol blue indicator and add it to the side arm flask. Insert the stopper on top. Double check that the straw does not actually touch the liquid.

B. Baseline CO_2 Measurement

Sit or stand calmly for 1 minute, trying to limit movement. After a minute, have your lab partner time you while you exhale normally into your straw. Stop recording when the BTB solution turns completely yellow. ***DO NOT INHALE THROUGH THE STRAW!!***

Repeat the experiment at rest **2 more times**. Between each experiment, remove the stopper and swirl the BTB solution to return it to its original blue color. **Record your results in Table 1.**

In addition to measuring your CO_2 output, you will also collect data on your breathing rate and heart rate, as they both play a role in transport and removal of CO_2 from the body.

To measure your breathing rate, count your number of breaths for 30 seconds and multiply by 2 (1 breath = one full inhale and the following exhale). **Record your breathing rate in Table 2.**

To measure heart rate, you will use the provided portable heart rate monitor. Simply press the device against the inner wrist and it will provide a heart rate reading in beats per minute (BPM). **Record your heart rate in Table 3.**

C. Increased Muscle Activity Measurements

The step test is a simple low impact/ low stress way to increase muscular activity. To perform the step test, you simply step up and down on a standard size box for an extended time. For this part of the experiment you will perform measurements at increasing intervals to determine if there is an increase in CO_2 production.

To start, one lab partner will perform the step test for **1 minute**. The other lab partner will assemble the respiration chamber for use at the end of the first minute. When the first minute is complete, repeat the procedure used in part B (Baseline CO_2 Measurements) to calculate the amount of time it takes to turn BTB from blue to yellow. **Record your values in Table 1.**

As in part B, you will also record your breathing rate and heart rate in **tables 2 and 3**, respectively, after the step test.

Repeat the procedures for a **2-minute step test** and record all your data. Make sure to reset the BTB solution in between each test.

All procedures and data collection should be completed on both lab partners (as possible). Additionally, collect data from 2 other lab groups and calculate average values for each data point.

Table 1: CO_2 Production (time to change colors in seconds)

	Student 1	Student 2	Student 3	Student 4	Student 5	Student 6	**Average**
Resting 1							
Resting 2							
Resting 3							
Average							
1 Minute							
2 Minutes							

Table 2: Breathing rate (breaths per minute)

	Student 1	Student 2	Student 3	Student 4	Student 5	Student 6	**Average**
Resting 1							
Resting 2							
Resting 3							
Average							
1 Minute							
2 Minutes							

Table 3: Heart rate (beats per minute)

	Student 1	Student 2	Student 3	Student 4	Student 5	Student 6	**Average**
Resting 1							
Resting 2							
Resting 3							
Average							
1 Minute							
2 Minutes							

What conclusions can you make based on your results?

Lab 7: Isolation of Photosynthetic Pigments

Background:

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

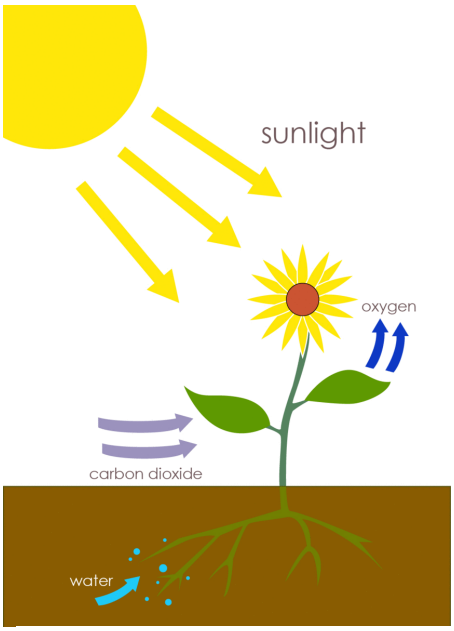


Figure 1. Photosynthesis
Credit: [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)]

Sunlight Energy

$$H_2O + 6\ CO_2 \Rightarrow 6\ C_6H_{12}O_6 + 6\ O_2$$

water + carbon dioxide — glucose + oxygen

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

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

Different pigments absorb different wavelengths of light, and therefore appear different colors to us. During the spring and summer months, when plants are most productive, they

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

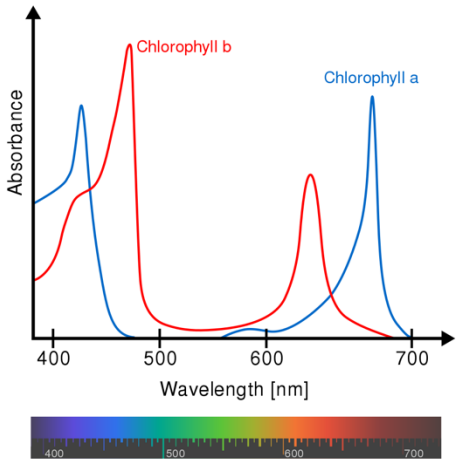


Figure 3. Absorption spectrum of chlorophyll *a* and chlorophyll *b*
Credit: Chlorophyll_ab_spectra2.PNG: Daniele Pugliesiderivative work: M0tty
[CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)]

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

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

In this lab you will first extract pigments from spinach leaves and then separate pigments from one another using a technique called **chromatography.** Chromatography is used to separate chemicals based chemical properties, such as **polarity**. Recall from OpenStax Concepts of Biology, Chapter 2: Chemistry of Life, that molecules and compounds are classified as **polar** or **nonpolar**, and that "like dissolves like." In other words, polar compounds dissolve in polar solvents, but not in non-polar solvents, and vice versa. An example of this concept is seen when oil, a non-polar substance, comes in contact with

water, a polar substance. The two substances do not mix; they are repelled by one another. Chromatography works by separating chemicals according to their varying degrees of polarity. In chromatography experiments, there are two "phases," the stationary phase, which does not move, and the mobile phase, which travels across the stationary phase.

In this experiment, you will use **paper chromatography**. Paper, which is made of cellulose, is very polar, and acts as the stationary phase. The solvent you will use is a mixture of petroleum ether and absolute acetone, both of which are nonpolar; the solvent acts as the mobile phase. Extracted plant pigment is applied to the paper, and the paper is placed in a tank of solvent. As the solvent moves up the paper through capillary action, it carries the pigment along with it. Pigments that are more polar are attracted to the polar water molecules in the stationary phase (the paper), so they move more slowly as the solvent travels up the paper. Nonpolar pigments are more strongly attracted to the nonpolar solvent and tend to stay in solution longer, thus moving farther up the paper. Since different pigment molecules have different molecular structures and varying degrees of polarity, this technique works well to separate pigments from one another, giving us a clear look at each pigment individually.

Procedures:

Part 1. Pigment Extraction

1. Obtain spinach leaves. Tear leaves into small pieces, discarding the large midvein. Weigh out approximately 4.0 grams of leaf tissue.
2. Place leaf tissue plus a pinch of clean quartz sand into a clean mortar and pestle; grind to a fine pulp.
3. Add 6mL absolute acetone to the pulp and continue to grind.
4. When leaf tissue is thoroughly ground into a paste-like consistency, allow the solution to rest for 1 minute. Carefully pour off the liquid portion of the mixture into a clean test tube, leaving the pulpy remains in the mortar.

Part 2. Pigment Separation

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

Paper chromatography requires that the atmosphere within the tank be completely saturated with solvent. Be sure that the aluminum foil covering the beakers stay in place before and during chromatogram development.

1. Prepare chromatography sheet: On one side of the sheet, write your initials in an upper corner. Place the chromatography paper onto the template sheet with the dark paper line at the bottom. A faint line should be visible through the chromatography paper. With a **pencil**, lightly trace the template line onto the chromatography paper. This line will serve as a guide when applying extract to help

ensure that the extract is applied evenly, in a straight line and at a level above the solvent in the chromatography tank.

2. Apply pigment: Using a capillary pipette, apply the pigment extract to the paper in a linear series of small dots (follow the line you created as a guide for application). **NOTE: Leave a 1 cm margin at each edge that is free of pigment extract. DO NOT TOUCH THE PAPER; oils from your skin can interfere with the process.**
3. After you have completed the pigment line across the chromatography paper, go back and re-apply pigment over the first line. Repeat the procedure until ***all*** the pigment extract is used. Allow sample to dry.
4. Once your sample has dried, take your 1000 mL beaker and chromatography paper to the fume hood. Add 100mL of tank solution to the beaker, then roll the paper so that the line faces inward and toward the bottom of the beaker. Place the rolled paper into the beaker so that it is in the tank solution. **The tank solution should not be higher than the line on your chromatography paper.**
5. Cover the beaker with aluminum foil and observe as the tank solvent (the mobile phase) travels up the paper (the stationary phase).
6. When all of the pigments are clearly separated and **before** the solvent front has reached the top edge, remove the chromatogram.
7. You should be able to see at least four distinct bands. (There may be as many as six bands.)
8. Make a sketch of the chromatogram in the space below, noting the color of the bands. Use Table 1 below to help you identify each pigment. Label each band with the name of the pigment.

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

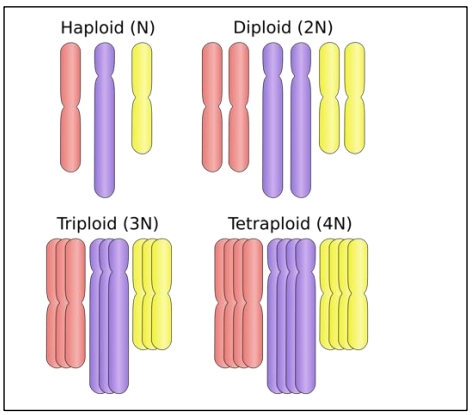
Pigment	**Band Color**
Beta-Carotene	Orange
Xanthophylls	Yellow
Chlorophyll A	Blue/Green
Chlorophyll B	Yellow/Green

LAB ACTIVITY: DNA EXTRACTION FROM STRAWBERRIES

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

Strawberry fruit tissue is an excellent type of tissue to use for demonstration of DNA extraction. First, ripe strawberries are soft and juicy; as the fruit matures, the cells fill up with water and sugar, which make the fruit so delicious! Second, as the strawberry ripens, a series of chemical reactions take place within the cells that lead to the breakdown of long-chain polysaccharides, like cellulose and pectin, that make the cell wall tough. Lastly, cultivated strawberries (*Fragaria* x *ananassa*) are the product of a hybridization between two other strawberry species, and they have an **octoploid** genome, meaning they have eight sets of chromosomes inside their cells. That translates to lots of molecules of DNA, which increases our yield and makes the DNA easier to visualize.

Strawberry flowers; photo from Wikimedia Commons



Illustrations of **ploidy levels**; N=number of chromosomes in one set.
Strawberries (*Fragaria* x *ananassa*) are **octoploid** (8N)

Illustration: Wikimedia Commons

Objective: Extract DNA from the cells of strawberry fruit tissue.

Materials:

- Plastic zipper bag
- 1 fresh strawberry
- 10 mL DNA extraction buffer
- Gauze squares
- Funnel

- Ice cold ethanol
- Plastic transfer pipettes
- Clear glass test tube
- Wooden skewer
- Microcentrifuge tubes

To make DNA extraction buffer (this part has been done for you), combine the following:

- 45 mL DI water
- 5mL liquid dish soap
- 0.75 g NaCl (table salt)

Procedure:

1. Place strawberry into plastic zipper bag and add 10mL of DNA extraction buffer. Seal the bag tightly.

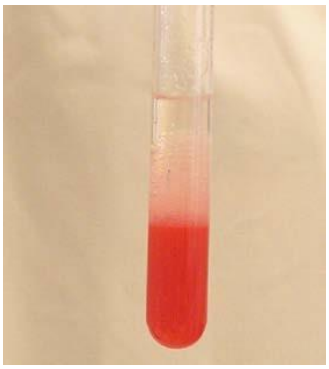
2. Gently, but thoroughly crush the strawberry inside the bag for about one minute.

3. Line the funnel with a gauze square. Place the funnel into the test tube.

4. Pour the strawberry juice/DNA extraction buffer mixture into the funnel so the juice passes through the gauze and into the test tube. Use the gauze to strain the strawberry mixture so that only the juice flows in to the tube and the pulp is retained in the gauze.

5. Discard the gauze and the strawberry pulp.

6. Layer an equal volume of ice cold ethanol on top of the strawberry juice mixture in the test tube using the plastic transfer pipette.



7. Hold the tube still at eye level (DO NOT shake) and observe what happens at the interface of the alcohol and the strawberry juice when you twirl your wooden skewer through the interface of the solutions.

8. Use your wooden skewer to transfer your strawberry DNA into a microcentrifuge tube. Add a small amount of ethanol to the tube to prevent your DNA from drying out.

You now have a clump of aggregated DNA molecules that you can see! This DNA contains all the genes within the strawberry genome.

Discussion:

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

Remember, there are two key ingredients in the DNA extraction buffer aside from the water: **dish soap** and **salt**. The dish soap acts to break up the lipid molecules that form the cell membrane and the nuclear membrane, which lyses the cell and releases the cellular contents, including DNA. The salt has two functions in the extraction process. It helps to neutralize the charge on the sugar-phosphate backbone, making DNA *less soluble* in water, and it also makes the DNA molecules stick to each other, so they are easier to visualize and remove from the solution.

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

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LAB 9: GEL ELECTROPHORESIS, RESTRICTION ENZYMES & DNA FINGERPRINTING

Purpose:

To 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. 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 three people (Suspect 1, Suspect 2, and Suspect 3) to the DNA found at the crime scene.

If the DNA sample from a suspect matches the DNA at a crime scene, then that signifies that the suspect in question *was present at* the crime scene (although he or she may not have actually committed the crime). If the DNA profiles from the crime scene do not match any of the suspects, then it can be concluded that none of those individuals were present at the crime scene.

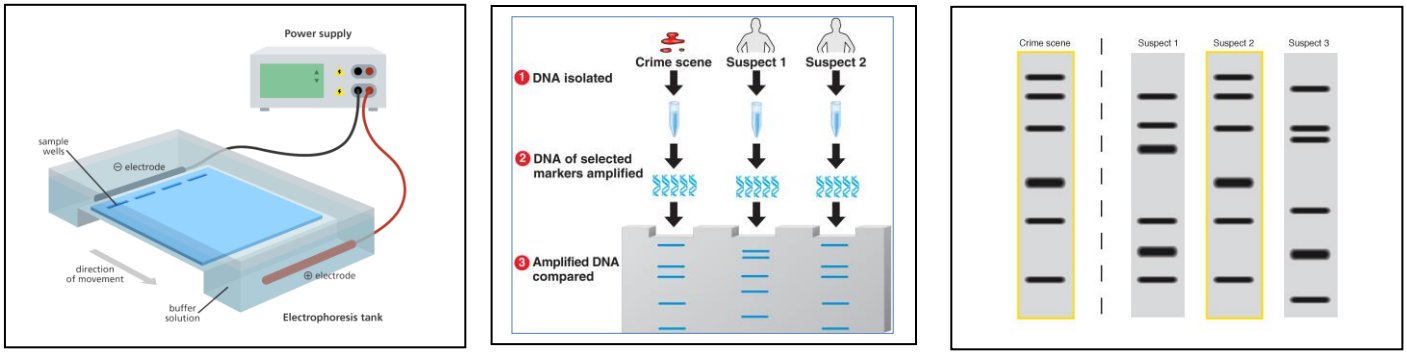


Fig. 1. Gel electrophoresis apparatus (left), (stylized) example of methodology used in this technique (center), and (stylized) example of gel from crime scene and three potential suspects (right). The point is to match characteristics of the DNA found at a crime scene with the DNA from potential suspects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

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 one such repeated unit (or simply **repeat**) that is 4 bp long. It might be repeated 3 to 100+ times as follows:
CTTGCTTGCTTGCTTGCTTGCTTGCTTG…..

Repeats are referred to by a variety of terms – sometimes confusing -- depending on their size. For example, sequence repeats of 10 to 80 bp are called **minisatellites** or **variable number tandem repeats** (VNTR). **Microsatellites**, also known as **short tandem repeats (STR)**, are smaller repeated units of 1 to 6 bp.
Regardless of their size (number of base pairs) or names, DNA repeats show greater variation from one person to another than other parts of our 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. 2) could exhibit the following variation in the length of a particular repeat sequence on the chromosomes they received from their parents:

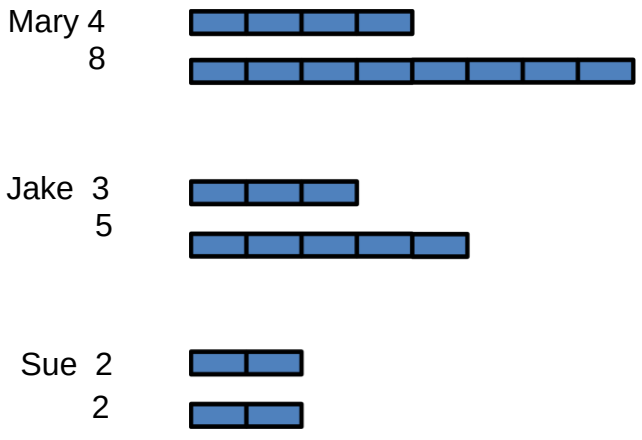


Fig. 2. Hypothetical variation in number of microsatellites in the DNA of 3 individuals. Blue rectangles represent microsatellite repeats on homologous chromosomes.

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

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

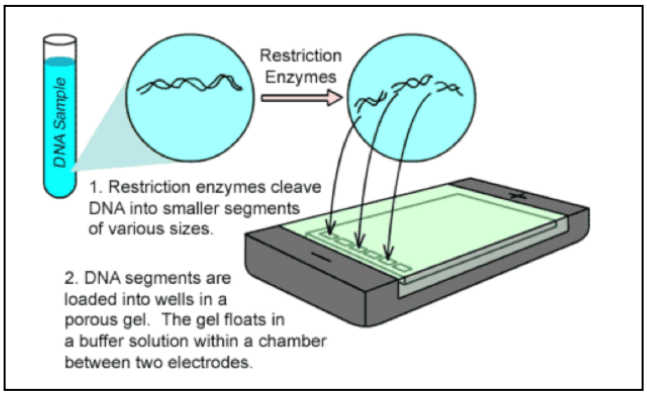


Fig. 3. How restriction enzymes are used in DNA fingerprinting.

Restriction Enzymes

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. 4).

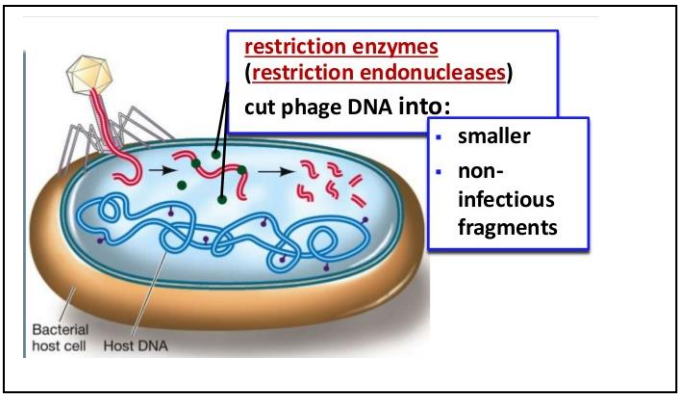


Fig. 4. How restriction enzymes function in bacteria.

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

Restriction enzymes are described by unique **acronyms** (abbreviations) that document the organism from which they were isolated. The first letter of the acronym is the first letter of the genus of the bacterium. The next two letters are the first two letters of the bacterium's species name. Additional letters and numerals indicate specific bacterial strains and their order of discovery. For example, *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. 5, top).

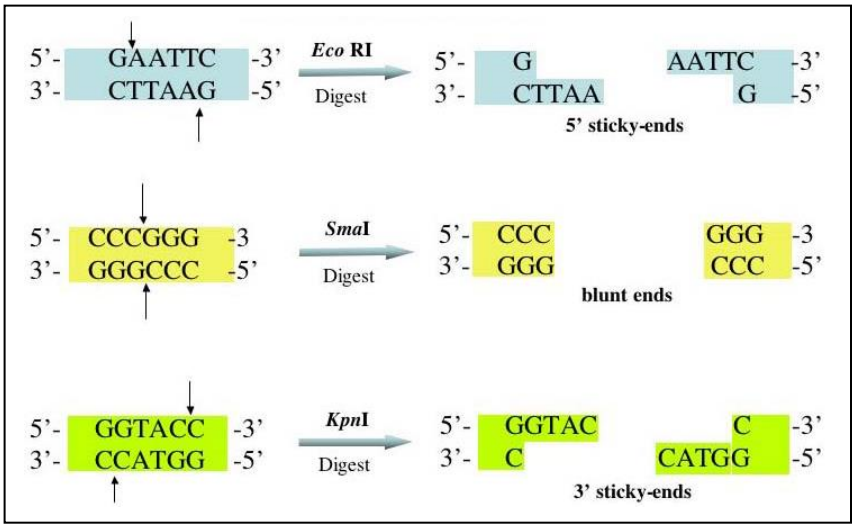


Fig. 5. Examples of how restriction enzymes can cut DNA.

It is important to note that the ends of the cleavage (cut) produced by *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.

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

Gel electrophoresis

Gel electrophoresis is a laboratory technique that allows macromolecules, such as DNA or RNA fragments or proteins, in a mixture to be separated according to their molecular size and/or

charge. The molecules to be separated are placed in depressions (or sample "wells") in a thin porous gel slab (Fig. 6), which is then covered by a buffered solution and placed in a horizontal electrophoresis chamber (Fig. 1; Fig. 7).

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 – away from the **negative electrode** (where the wells are located) toward the **positive electrode**. Shorter DNA fragments move more quickly -- and farther on the gel -- than do larger fragments.

The different sized 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 dye.

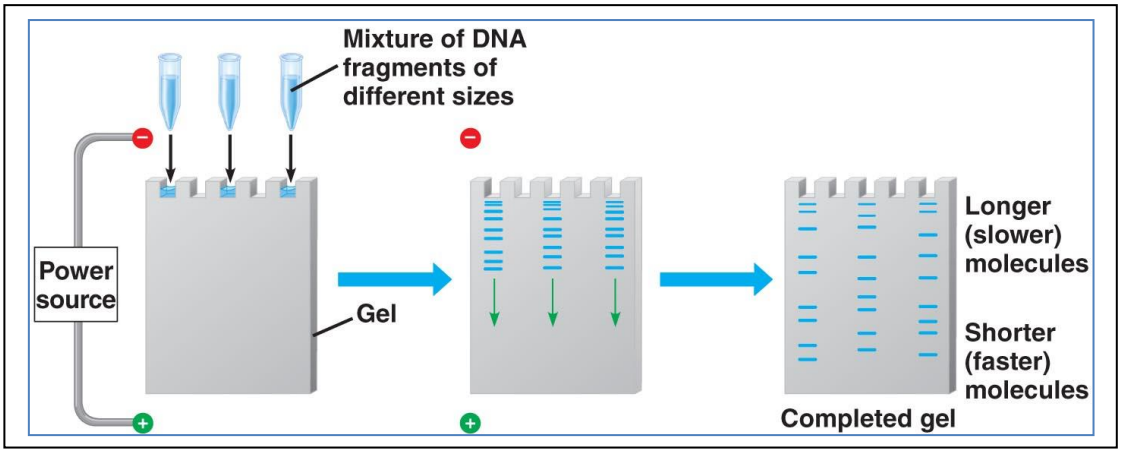


Fig. 10.6. The separation of DNA fragments in gel electrophoresis.

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 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. 7, left; below) function.

Power Supply: This high voltage power source (Fig. 7, 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 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 150V**.

Fig. 7. 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

(thousandths 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 blue dye is positively charged, and sticks to the negatively charged sugar-phosphate backbone of the DNA molecules. Instastain blue is non-toxic, and the stained DNA molecules are easily visualized when the gel is placed on a **white light box** (Fig. 8, below).

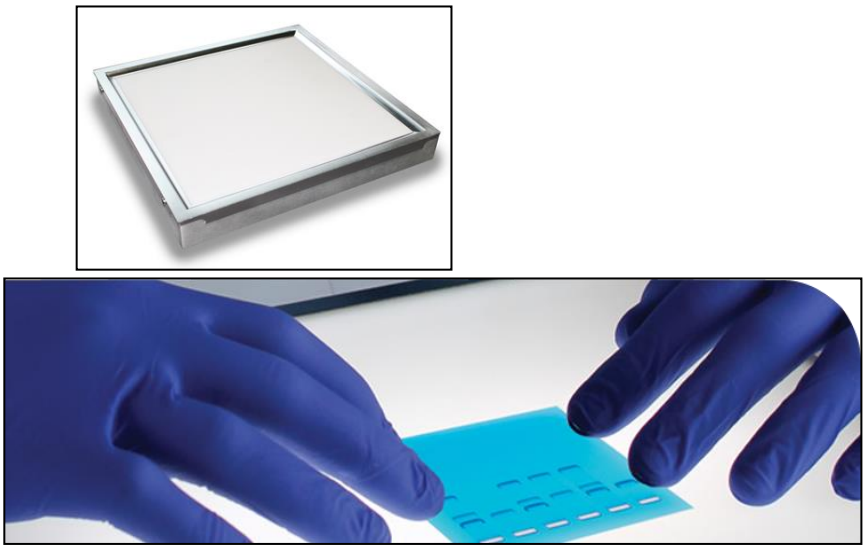


Fig. 8. White light box (left) and visualization of DNA bands on gels.

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.

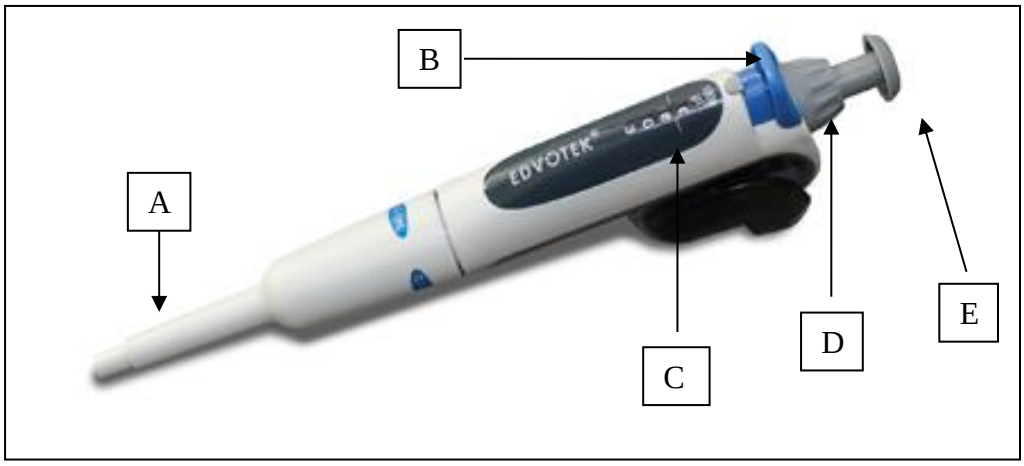
Wash hands thoroughly with soap and water at the end of lab.

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).

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, or the "**first stop**".

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.

5. Load 40 µl of each DNA sample you will run into its appropriate well, as follows (**Table 1**):

Table 1. Material that will be run in each lane of your gel

Gel Lane (left to right)	Micro test tube	Contents
1	A	Standard 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 150V. 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 InstaStain® 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.

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?

LAB 10: MITOSIS & MEIOSIS

Eukaryotic cells exist in a **cell cycle**, which consists of the separate phases you learned about in lecture. The first phase, **interphase**, is the time during which the cell performs its normal functions and prepares for cell division. Cells spend most of their time in this phase.

During interphase, **chromosomes** are not visible because they are **decondensed** (present only as a tangled mass of thin threads). The nuclear membrane is present, and visible, as is the **nucleolus**.

As interphase proceeds and cells make proteins, respire, etc.; they will also grow and replicate their DNA. The latter occurs specifically in **S phase**. At the beginning of S phase, chromosomes are single. By the end of S phase, each chromosome has doubled and consists of **sister chromatids**. In animal cells, interphase is also when the **centrosome** (consisting of **two centrioles**) is replicated.

Fig .1, below, shows a pair of **homologous chromosomes** at the beginning and end of S phase. Note that the chromosomes are pictured in a **condensed** form. In reality, both the unreplicated and replicated chromosomes would be decondensed, thread-like, and invisible when viewed under the microscope.

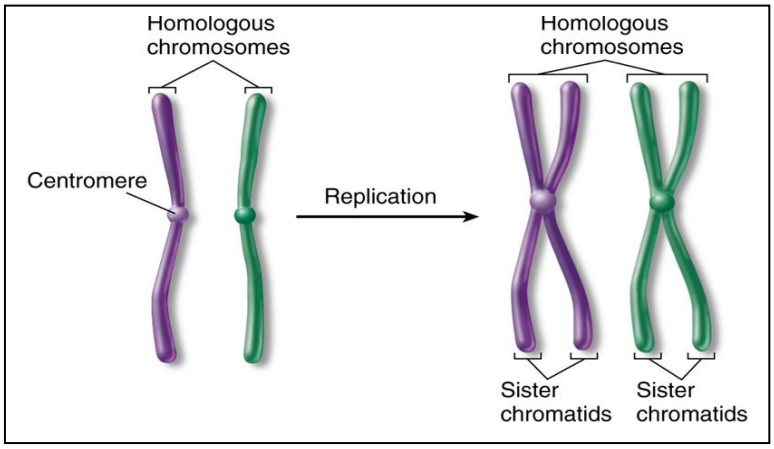


Figure 1. Homologous chromosomes at the beginning of S phase

Interphase is followed by **mitosis** (in a somatic cell) **or meiosis** (in reproductive cells), which is when the nucleus and cytoplasm divide. **This lab exercise examines both mitotic and meiotic cell division**.

MATERIALS:

- Physical models of dividing animal cells

- Posters (on whiteboard) depicting dividing animal and plant cells and human chromosomes
- Whitefish blastula slide
- Onion root tip slides
- Compound light microscope
- Pop beads

I. Mitosis

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

Mitosis is a complex process and highly regulated. It occurs in the following 4 separate phases:

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

Metaphase: Spindle fibers (called **kinetochore microtubules** or **kinetochore spindle fibers**) that emanate from the centromeres attach to the **kinetochore** (a proteinaceous area) on the sister chromatids (**Fig. 2**, below). The fibers pull, and otherwise manipulate, the chromosomes to align them on the plane that passes through the center of the cell (**metaphase plate**). This "plate" is not an actual structure; it merely signifies the location of replicated chromosomes prior to their impending separation.

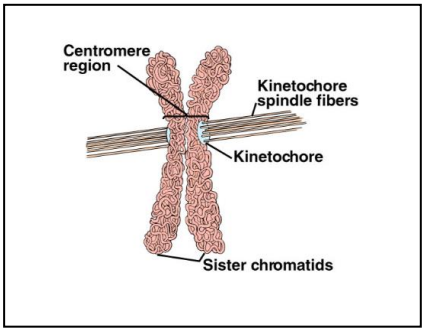


Figure 2.

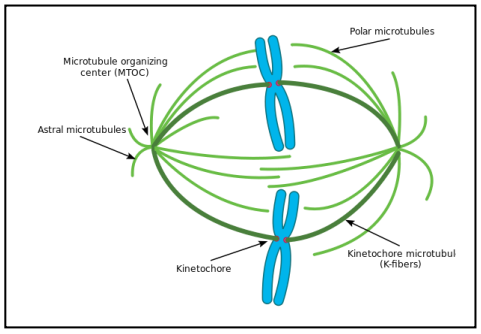


Figure 3.

Other **non-kinetochore spindle fibers or tubules (**aka **polar microtubules)**, emanating from the two centrosomes, elongate and eventually overlap with each other near the metaphase plate (**Fig. 3**, above).

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

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

Cytokinesis (splitting of the cytoplasm):

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

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

1. Examine a slide of dividing whitefish blastula cells; draw and label each stage, and the most visible components, of the cell cycle, which will look similar to what appears below (Fig. 4):

Mitosis in whitefish blastula cells:

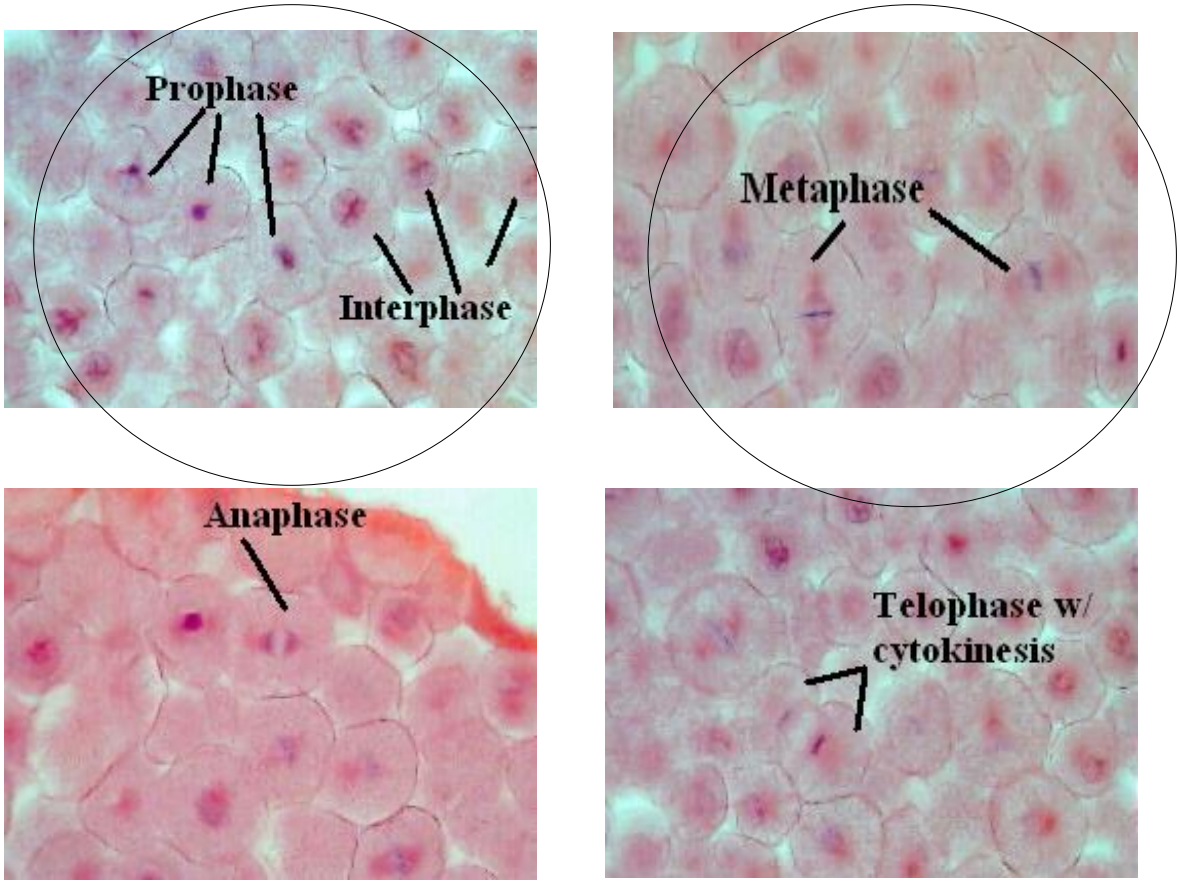


Figure 4. Dividing whitefish blastula cells.

Interphase

Prophase

Metaphase

Anaphase

Telophase

Cytokinesis

2. Examine a slide of <u>dividing onion root tip cells</u> and draw and label each stage, and the most visible components, of the cell cycle. It will look similar to Fig. 5, below.

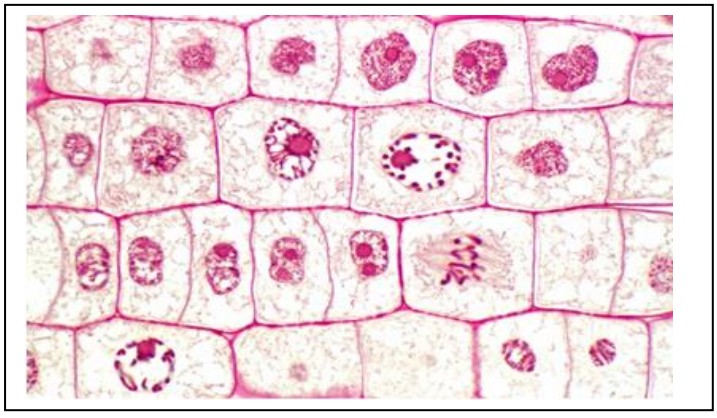
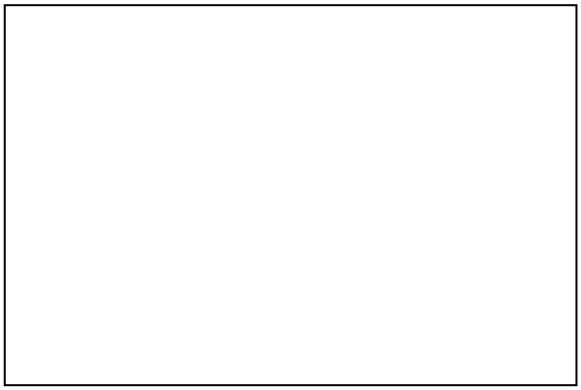
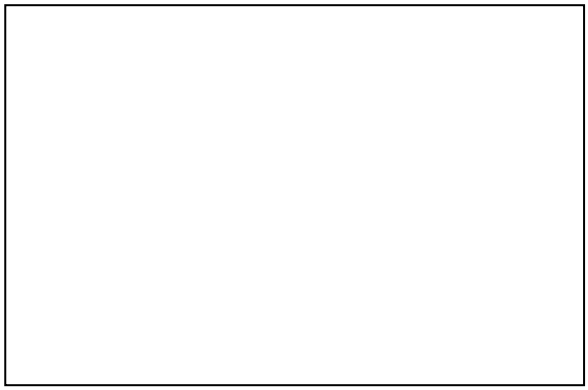


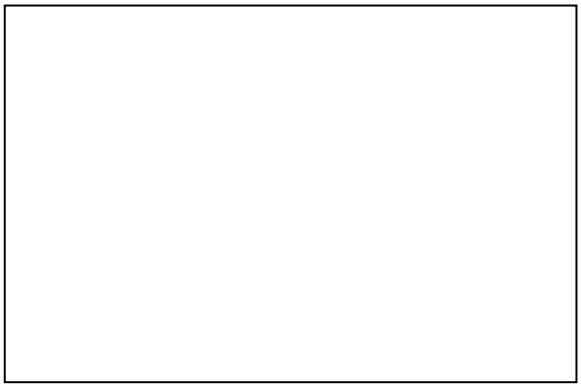
Figure 5. Diving cells in onion root tip



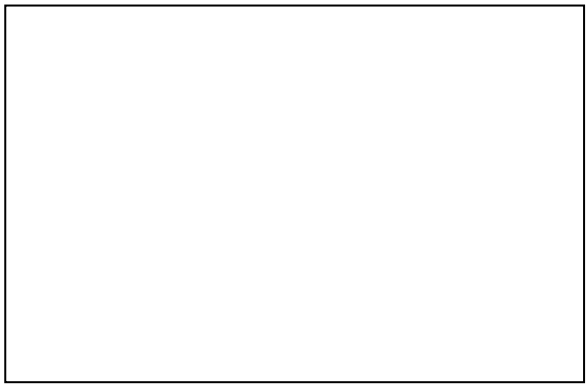
Interphase



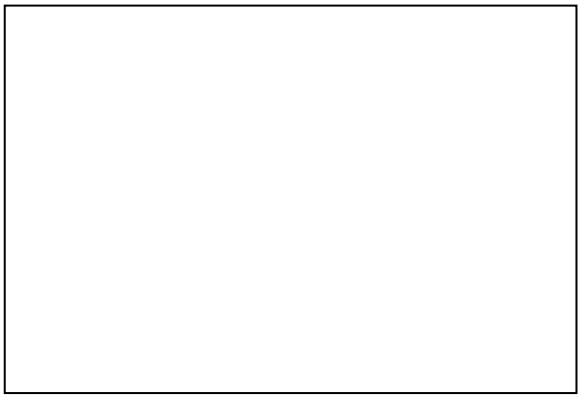
Prophase



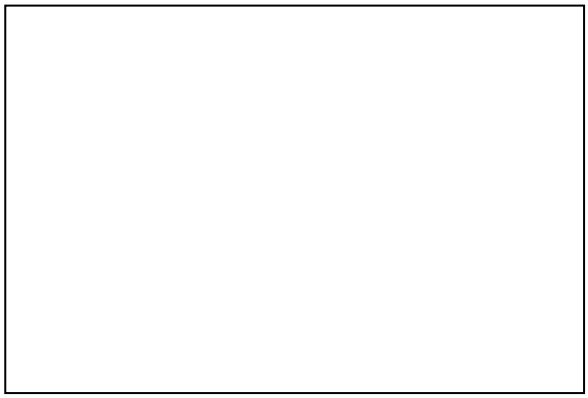
Metaphase



Anaphase



Telophase



Cytokinesis

For the next part of this activity, you will use pop beads to represent DNA (and chromosomes).

1. Obtain a bag that contains yellow and red beads connected to look **like the ones pictured in Fig. 6 below.**

   These beaded strands are intended to represent two pairs of replicated homologous chromosomes. One pair of chromosomes is longer than the others. Half of each pair is yellow and represents **maternal DNA** (genetic material contributed by a female's egg). The other half of each pair is red and represents **paternal DNA** (genetic material contributed by a father's sperm). Thus, for each pair of **homologous chromosomes**, one should be yellow and one should be red. Alert your instructor if the chromosomes in your bag differ from those pictured below.

2. The chromosomes pictured below are replicated. Recall that when the chromosomes are fully condensed, and consist of 2 chromatids joined at the centromere, they are in prophase. Place the chromosomes on your lab bench to represent their position in the cell nucleus. Use string to represent the nuclear membrane and the cell membrane.

3. 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 the 2 new nuclear membranes forming in telophase.

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

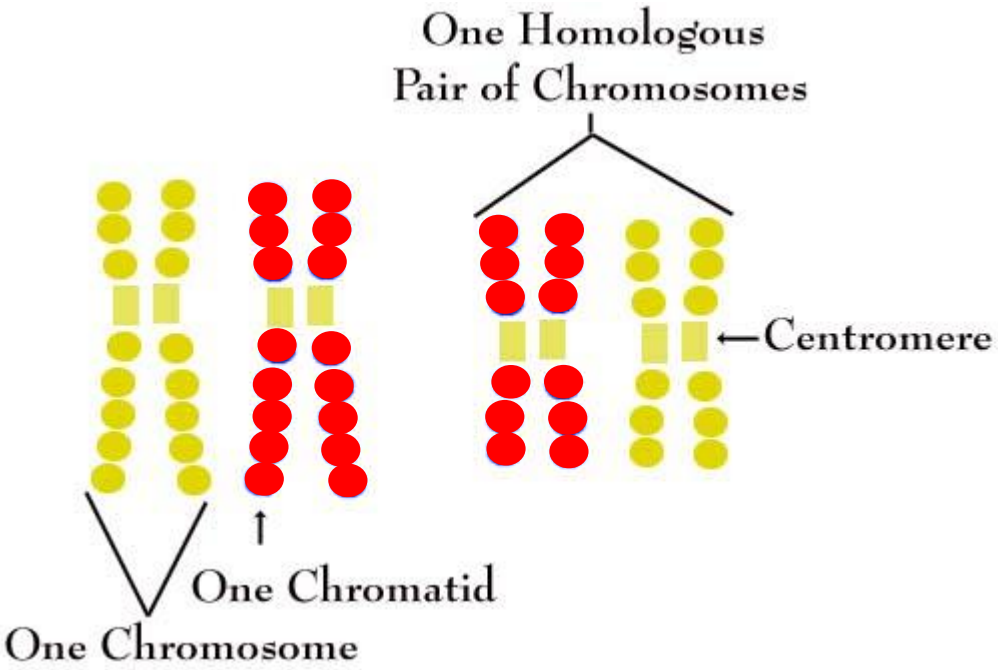


Figure. 6 Two homologous chromosome pairs – one long and one short. The yellow chromosomes represent genetic material contributed by a mother's egg. The red chromosomes represent material contributed by a father's sperm

II. Meiosis (aka "Reduction Division")

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

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

The stages of meiosis

Meiosis I	Meiosis II
Prophase I	Prophase II
Metaphase I	Metaphase II
Anaphase I	Anaphase II
Telophase I	Telophase II
Cytokinesis I	Cytokinesis II

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

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

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

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

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

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

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

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

During **Prophase II**, chromosomes (present as sister chromatids) are lined up on the equator of each daughter cell by the spindle fibers. This is completed by the end of **Metaphase II**. The centromeres separate and sister chromatids are pulled to each pole of the cell during **Anaphase II**. **Cytokinesis II** occurs after **Telophase II** to complete cell division and ultimately the production of four (4) daughter cells.

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

To fully understand the mechanism involved in meiosis -- the production of gametes -- you 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 7, or ask your instructor**.

2. Place the 4 chromosomes on your lab bench, 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 (I) for insulin production. At a different locus is a gene (D) for hair color. The different versions of the same gene ("I" and "i", or "D" and "d") are called **alleles**.

4. Some alleles are indicated with a capital (uppercase) letter and others with a lowercase letter. **Dominant** alleles (those that are always expressed) are in uppercase, while **recessive** (only expressed when no dominant allele is present) are in lowercase.

5. Recall that the red beads are paternal (from the father) and yellow beads are maternal (from the mother) DNA. The "cell" you are dealing with has 2 sets of genetic information (red and yellow chromosomes) and is diploid. In **Table 1**, list the **genotype** (genetic make-up) and **phenotype** (physical appearance) of your organism.

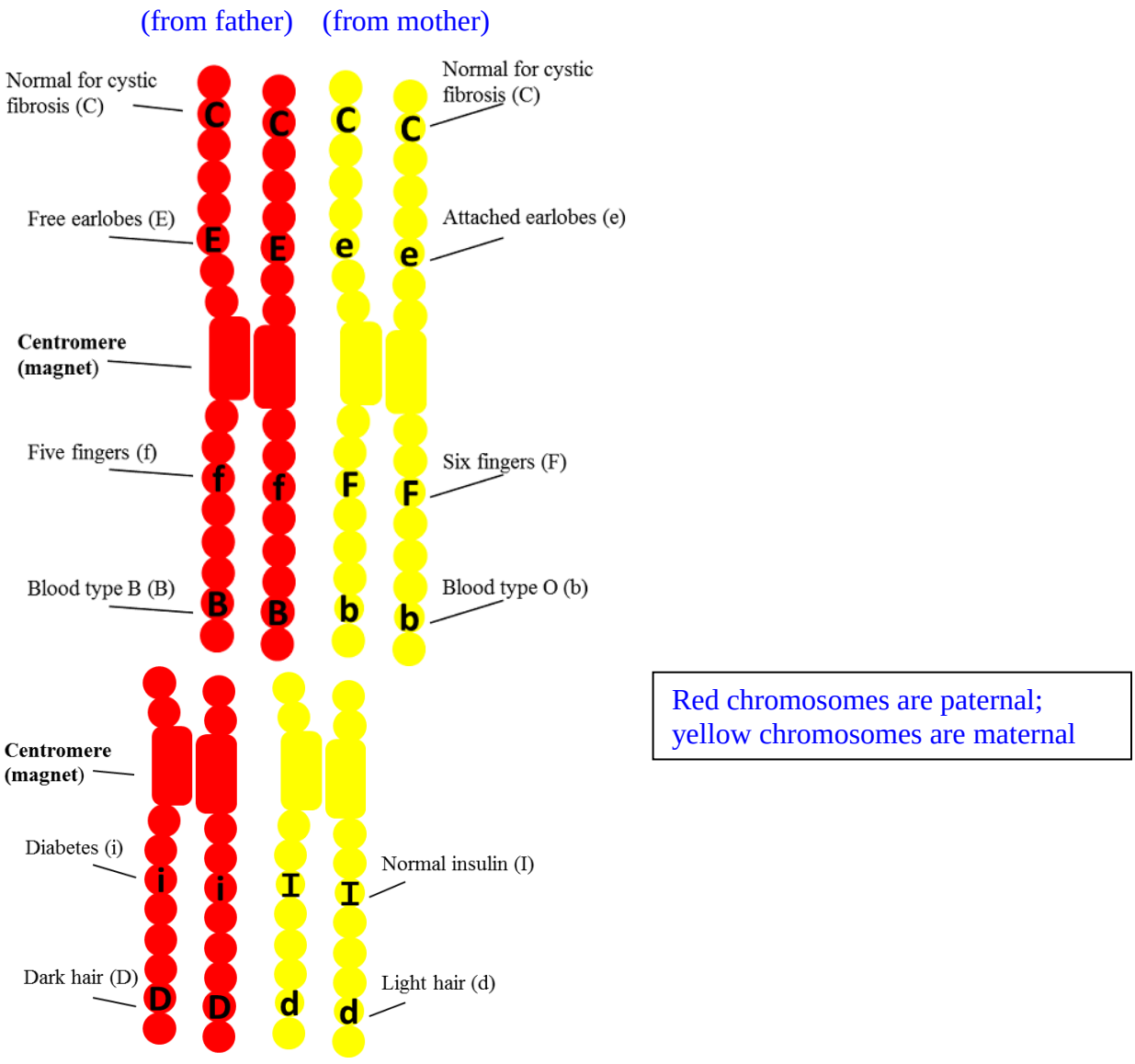


Figure 7. Homologous chromosome set up for Meiosis demonstration

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Table 1. List of characteristics	**Genotype of Organism**		**Phenotype of Organism**
TRAIT	Allele from mother (yellow)	Allele from father (red)	
Cystic fibrosis (C = normal, or c = cf)			
Ear Shape (E = Free or e = attached)			

Finger number (f = 5 or F = 6)			
Blood type (B = type B or b = type O)			
Insulin production ability (I or i)			
Hair color (D = dark or d = light)			

PROPHASE I – synapsis and crossing over

1. Put homologous chromosomes next to each other on your lab bench to simulate synapsis, the state whereby homologs 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 beads). If you do not, reassemble the chromosomes to match Figure 8.7, and try again (ask your instructor for help if needed). 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 the same? 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 metaphase I, homologous chromosomes are placed in the center of the cell by spindles with the homologs for each pair of chromosomes situated on opposite sides of the equatorial plane. Every pair of chromosomes is arranged independent of another; thus, there are several possible arrangements you can have (see Fig. 8, below). (**Note:** This is significantly different from what happens during mitosis).

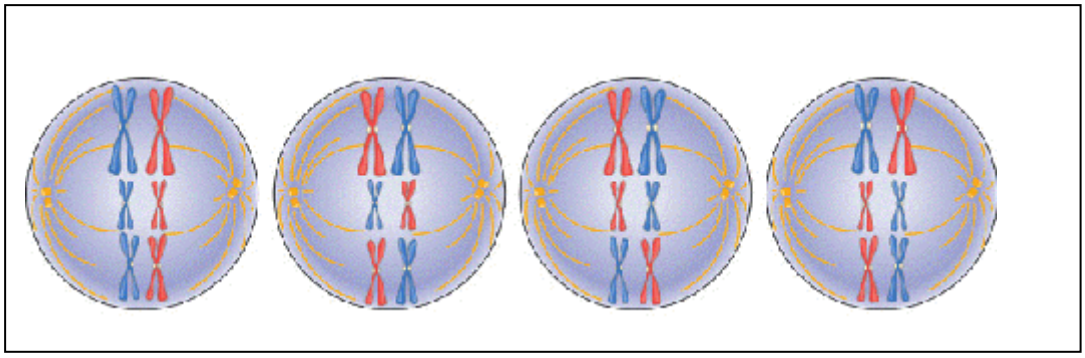
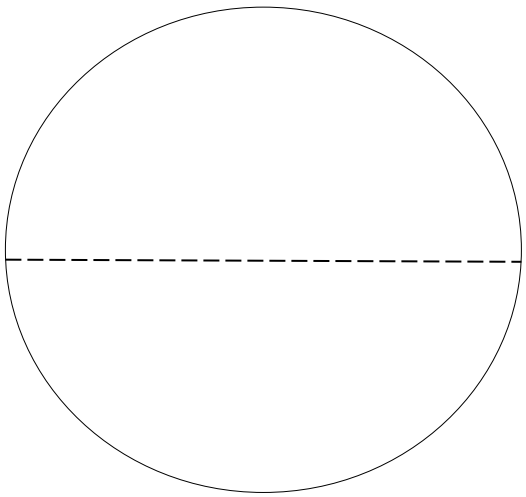


Fig. 8. Possible random orientation of chromosomes at Metaphase Plate during Independent Assortment. This figure represents 4 of the 8 possible chromosomal arrangements possible.

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



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

_____

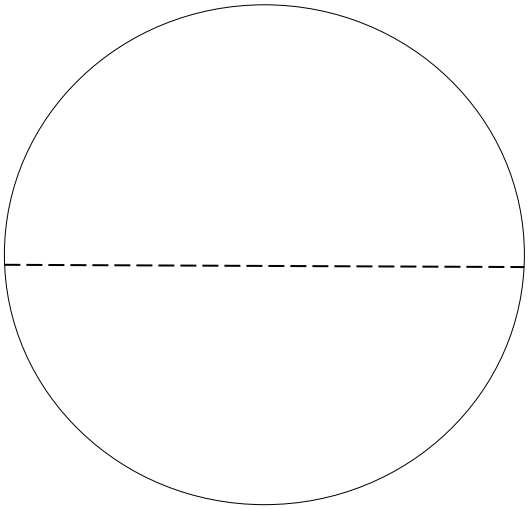
_____

_____

<u>ANAPHASE I</u> – Segregation

During this phase, homologous chromosomes separate. This is called **segregation.**

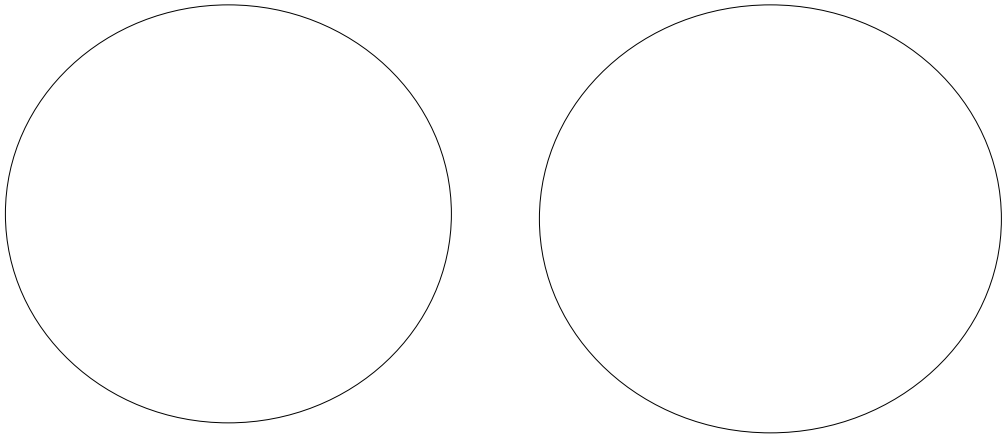
1. Segregate the 2 homologs of each pair of chromosomes by moving them to opposite ends of your "cell".
2. Draw the arrangement of the chromosomes below:



TELOPHASE I and Cytokinesis

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

1. Once the chromosomes reach opposite poles of the cell, a **cleavage furrow** (or **cell plate** in plants) forms, dividing the cytoplasm roughly in half. 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 your daughter cells.



Compare the genetic information in each of your daughter cells. Are they identical? In **Table 2**, list the genes present in of each of your daughter cells.

Table 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.

_____

_____

_____

<u>PROPHASE II</u> – Cells prepare for the second division

DNA replication does not occur after telophase I, when each daughter cell produced in meiosis I enters prophase II. This phase is much like prophase of mitosis. Chromosomes fully condense, nuclear membranes degrade, 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 align at the cell's equator

Chromosomes, still composed of 2 chromatids, are lined up on the metaphase plate.

1. Move the 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 (now 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 3**, list the alleles found in each of your four gametes.
3. Are your gametes different from one another? _____
4. Review meiosis I & meiosis II and identify the processes that contribute to the different allele combinations in your gametes. **List them:**

_____

Table 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		

<u>FERTILIZATION</u> – 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 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 4. Genotype & phenotype of offspring			
TRAIT	**allele from mother**	**allele from father**	**PHENOTYPE**
Cystic fibrosis			
Ear Shape			
Finger #			
Blood type			
Insulin			
Hair color			