Introductory Biology: Unity of Life

Laboratory Manual

11th Edition

BIOL 1408

by

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BIOL 1408 Lab Manual
Biology 1408 Lab Safety Rules and Information

General Rules and Information

Your lab class professor will provide the most current information to you during lab class. You are required to know and abide by this information.

Safety Video

Watch the safety video during class or else on your own at http://www.austincc.edu/biology/safetyvid.html.

Warning Labels Used in This Lab Manual

- Dispose of these materials in a Biohazard container in the lab.

- Dispose of these in the proper waste disposal area in the lab.

- Dispose of these materials as directed.

- Caution!
- Pay special attention to something.

Safety Training for Biology Hematology Labs

This information pertains to Lab 14. It will be provided by your lab professor.
Units of Measurement

In all scientific work, the International System of Units or SI units is used. You have probably already learned how to use these units in previous science classes when you learned the metric system.

Contrary to popular belief, scientists do not use the metric system in order to make life difficult for students like you—who have more experience using the English system of measurement that Americans usually use in everyday life.

Scientists use SI units because it standardizes their work with scientists working anywhere in the world. In other countries only the metric system is used, even for everyday measurements. Even the English no longer use the English system!

As a bonus, the metric system is much easier to use than the English system once you get used to it. (Would you rather divide by 10 to convert units or by 16 or 32?)

International System of Units (SI)

<table>
<thead>
<tr>
<th>Type of Measurement</th>
<th>Unit</th>
<th>Abbr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>Liter</td>
<td>L</td>
</tr>
<tr>
<td>Mass</td>
<td>Gram</td>
<td>g</td>
</tr>
<tr>
<td>Length</td>
<td>Meter</td>
<td>m</td>
</tr>
<tr>
<td>Time</td>
<td>Second</td>
<td>s</td>
</tr>
<tr>
<td>Amount of a substance</td>
<td>Mole</td>
<td>mol</td>
</tr>
</tbody>
</table>
### Metric Prefixes for Units of Measurement

<table>
<thead>
<tr>
<th>Prefix</th>
<th>Abbr</th>
<th>Relationship to basic SI unit</th>
<th>Means multiply by: (to convert to basic SI unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>micro-</td>
<td>μ</td>
<td>one millionth</td>
<td>$10^{-6}$ or 0.000001</td>
</tr>
<tr>
<td>milli-</td>
<td>m</td>
<td>one thousandth</td>
<td>$10^{-3}$ or 0.001</td>
</tr>
<tr>
<td>centi-</td>
<td>c</td>
<td>one hundredth</td>
<td>$10^{-2}$ or 0.01</td>
</tr>
<tr>
<td>deci-</td>
<td>d</td>
<td>one tenth</td>
<td>$10^{-1}$ or 0.1</td>
</tr>
<tr>
<td>kilo-</td>
<td>k</td>
<td>one thousand times</td>
<td>1,000</td>
</tr>
</tbody>
</table>

### Units of Measurement Most Often Used in BIOL 1408 Labs

<table>
<thead>
<tr>
<th>Name of unit</th>
<th>Abbr.</th>
<th>Relationship to standard SI unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liter</td>
<td>L</td>
<td>Standard unit</td>
</tr>
<tr>
<td>Milliliter</td>
<td>mL</td>
<td>1/1,000 of a liter</td>
</tr>
<tr>
<td>Microliter</td>
<td>μL</td>
<td>1/1,000,000 of a liter</td>
</tr>
<tr>
<td><strong>Mass measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram</td>
<td>g</td>
<td>Standard unit</td>
</tr>
<tr>
<td>Milligram</td>
<td>mg</td>
<td>1/1,000 of a gram</td>
</tr>
<tr>
<td>Microgram</td>
<td>μg</td>
<td>1/1,000,000 of a gram</td>
</tr>
<tr>
<td><strong>Length measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meter</td>
<td>m</td>
<td>Standard unit</td>
</tr>
<tr>
<td>Centimeter</td>
<td>cm</td>
<td>1/100 of a meter</td>
</tr>
<tr>
<td>Millimeter</td>
<td>mm</td>
<td>1/1,000 of a meter</td>
</tr>
</tbody>
</table>
Part 1: Use Your Senses

1. For this section of the handout, work as a group with all the people at your table, but write your own set of answers. Write the FULL names of your group partners below.

2. **DO NOT TOUCH THE OBJECTS ON THE TABLE YET.** Using only your other senses, predict the relative weights of the shiny round object, the flat white object, and the long object with numbers on it. Write your answer below.

   Heaviest =  
   Somewhere in between =  
   Lightest =  

3. Does everyone in your group agree? __________________________

4. Why did you rank the objects in that order?

5. **Now feel free to touch and handle the objects. DO NOT HARM THEM OR TAKE THEM APART.** Using any of your senses (and only your senses), write your predictions below concerning which is heavier etc.

   Heaviest =  
   Somewhere in between =  
   Lightest =  

6. Does everyone in your group agree? ____________

7. Did you change your mind about the original ranking? _______________  
   Explain why or why not.
8. In the space below, write a description of how you could test your predictions to see if they are accurate. **DO NOT HARM THESE OBJECTS OR TAKE THEM APART.**

9. Carry out your plan. Use the space below to write down exactly what you did.

10. What results did you get?

    Heaviest =  
    Somewhere in between =  
    Lightest =

11. **Trade** your group’s handouts and your three objects with those of another lab group. Have them check your results and have them fill in the following:

12. Do you agree with the above results from the other group? _____________

    Explain why or why not . . . be complete.

13. Sign your name here and return this handout and their three objects to the proper group.

14. Now that you have your handout and three objects back, write your **overall conclusions** about what you have learned so far in this lab exercise. Be sure to explain yourself. Discuss this with your partners before you write your conclusions, then show your answer to your professor.
15. **Types of Data You Have Now - And Should Always Use** (Wait for your Professor)

   Qualitative

   Quantitative

**Part 2: Oranges . . . or Something Similar**

For this section, continue to work as one lab group with the students at your lab table. Feel free to collaborate with your lab partners, but write your own answers.

16. Pick an orange - you may touch/handle it, but **DO NOT MARK IT OR DAMAGE IT IN ANY WAY**. Write as **complete** a description of your orange as you can in the space below.

17. *Wait for your professor’s directions before continuing.*

    Total time taken by the class ________________

18. Explain what the key items were that helped you.
19. *Wait for your professor’s directions again (different instructions this time!).*
   Total time taken by the class ________________

20. **Retrieve** your own orange and handout. Explain on your own lab handout how the descriptions from the other lab table could have been more helpful to you.

21. Use the space below to add to **your** original description of **your** orange to make it more complete. Be sure to examine your orange while doing this.

**Part 3: Collecting More Data**

22. Predict how many segments are inside your orange. ________________

23. What criteria did you use in order to make your prediction?

24. Write **your** prediction on the chalkboard, and fill in the chart below with all the class data.

<table>
<thead>
<tr>
<th>GROUP NO.</th>
<th>PREDICTED # OF SEGMENTS</th>
<th>ACTUAL # OF SEGMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CLASS AVERAGE</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
25. **Test your prediction**
Wash your hands and your orange and put the orange on a paper towel on your table. Neither you nor the tables are sterile. Peel your orange and count the segments - feel free to eat the segments if they do not touch the lab table. *Bon appétit!!!*

26. **Write your results on the chalkboard.**

   **Dispose of your waste in the regular trash.**

   **Clean your lab table.**

27. Compare your prediction with those of other groups. Are they the same, different, similar?

28. **Problem:** Assume that you own a catering business. Your client wants 350 salads, and on each salad wants seven of these orange slices.

29. How many oranges of this kind will you need to order? ____________
   Show your work here:

30. Assume that you have bought a container with 15,000 oranges, but these are not the same kind of oranges that you had in the lab. Using what you have learned from this lab exercise, describe how you could estimate the total number of orange slices there are in the entire container of oranges.
31. What are your overall conclusions about the specific number of segments in this kind of orange?

32. What evidence do you have to back up the conclusion you just wrote in the previous question?

The End!
LAB 1:
Introduction to Science

PPE Required - None

The impression of science that some students get from classes in school is that science is just facts to be memorized. Many of the facts are interesting but some seem obscure and irrelevant. The technical jargon is like a foreign language—but useless for ordering lunch in Paris.

For a scientist, on the other hand, science is a great job. You are pretty much your own boss. Your colleagues are smart and competitive—always challenging you to look at things in a new way. The work changes constantly, so it does not get boring. There is always a new question to answer, a new technique to learn, or another piece of equipment to build or repair. Scientists get to travel all over the world, for field research, to give talks, or attend conferences.

To a scientist, science is not a collection of facts but is something to do, and that process begins with a question. Suppose you ask, “Why does excess salt in the diet raise blood pressure in some people, but not in others?” The current answer is, “Nobody knows.” A nonscientist would say, “Well, I wish someone would figure it out and tell me.” A scientist would say, “How could a person find out the answer?”

Over the past few centuries, scientists have developed certain ways of approaching questions about the natural world (the weather, the movement of stars, the web of life in the oceans, our own bodies) that consistently give reliable answers. To understand better how this process works, you will begin thinking like a researcher in this exercise.

NOTE: The main task you face in this lab is to be creative while staying inside the allowed parameters. Your main obstacle is to move beyond assuming that there is just one possible solution to a problem.

Use a pencil for this lab!
Part 1: Everyday Problem Solving

Sometimes people get into an argument because each one has an explanation for something that happened which fits what was observed; but the different explanations don’t agree. Apply what you have learned during your life to the following situation.

You and your friends are in your car. The car key is in and turned to the point where electronics should work. The CD player (which worked the last time you tried it) is turned on, but the CD player is not producing any music. Think of four possible explanations for this and tell how you would test for each one.

<table>
<thead>
<tr>
<th>EXPLANATION</th>
<th>TEST FOR THAT EXPLANATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td></td>
</tr>
<tr>
<td>#2</td>
<td></td>
</tr>
<tr>
<td>#3</td>
<td></td>
</tr>
<tr>
<td>#4</td>
<td></td>
</tr>
</tbody>
</table>
Part 2: Writing and Testing Hypotheses

Part 2 Step 1: Observations

All science begins with observing the natural world, like you did with the CD player problem above. We have to know what happens before we can wonder how it happens and set about finding the answer to questions. For example, what happens if you place salt water in the bottom of a beaker of distilled water? Follow the instructions below to try it.

1. **Obtain two 100 mL beakers** and label one “S” and the other “E”. Put each one on a Kimwipe.

2. Add 60 mL of distilled or deionized water (dH$_2$O) to each beaker. Mix 2 teaspoons of salt into beaker “S” and stir until the solution is saturated, which means that no more salt will dissolve. Add 5 drops of food color to the salt water and stir it again.

3. Pour enough of the salt water into a test tube to fill it to 2 cm from the top. Place a straw in the test tube.

4. Holding your finger over the end of the straw, lift the straw out of the test tube and lower it into beaker “E” of distilled water. When the straw is resting on the bottom of the beaker, gently remove your finger from the straw and s-l-o-w-l-y lift the straw out of the beaker.

5. Draw your results for beaker E. Label the materials and colors.

You have just drawn your results. Your drawing represents the data you must use throughout the rest of this lab exercise. This is very important to remember!
Part 2 Step 2: Explaining Your Observations

So far we have a neat trick and interesting observations, but we do not have science. The goal of science is to **explain how the world works**.

6. Describe what you observed when you lifted the straw out of beaker E.

7. What do you think made that happen?

**This is the beginning of your first hypothesis** to explain your observations. An hypothesis must be consistent with your observations (data). Does this mean that you have **proved** that this explanation is the correct one? Not so fast!

**Part 2 Step 3: Alternative Explanations**

There is no reason to accept an explanation, or hypothesis as the correct one if other hypotheses explain the observations just as well. In order to convince others of the correctness of your explanation (hypothesis), you must not only show that observations are consistent with your hypothesis, but also **disprove all of the other reasonable explanations** that someone else might think of. That means you must rule out other factors that can explain your hypothesis.

For example: Imagine that your car will not start. Not only that, the gas gauge is on “Empty.” You might hypothesize that your car will not start because you are completely out of gas. Someone else might point out that perhaps your battery is so completely dead that the gas gauge will not work. You could then **disprove** their alternate hypothesis by turning on the radio, and if it plays, their alternate explanation is **disproved** and your original hypothesis is still the best one.

When a scientist publishes an explanation of an observation, other scientists will always read the article critically. If they can think of other reasonable explanations that fit the data, they will not accept the published explanation as a scientific fact yet.

**One explanation** for your salt water data could be, “Colored salt water stays on the bottom of a beaker of dH₂O because the **salt water** is more dense than dH₂O, and therefore is heavier than dH₂O.”

8. Consider your drawing (data) from Step 1. Write an **alternative explanation** that would explain your results.
9. Write one more alternative explanation that could explain your data.

You now have three possible explanations for what you observed. All of the explanations could account for what you observed when you placed colored salt water in the bottom of a beaker of salt water. But is one of them a correct explanation? How do you find out?

A correct explanation of something that you have observed in the natural world must:

- Be a reasonable, logical explanation of what you have observed
- Allow you to predict accurately what will happen in other situations that you have not yet observed.

You’ve been doing this all of your life although you may not have been calling it science. For example, babies seem to enjoy exasperating their parents by dropping things and throwing things over and over again. A baby will hold a toy and let go of it. Down! Cup of milk? Down! Cereal bowl? Down! Spoon? Down!

Baby is learning about gravity.

- Things go down!
- This radio I’m pulling toward the edge of the shelf will go down! too if I can just get it a little farther . . .

Think about what you have done so far:

- Started with salt water and distilled water
- Put food color in the salt water
- Placed a salt water/food color mixture at the bottom of distilled water.

Part 2 Step 4: Writing Your Hypotheses

Note: In this part of the exercise (Step 4) you will not actually do anything with the salt, food coloring, and water. You are planning what you will do later.

You need to consider which of your possible explanations would correctly predict what would happen in other situations, using the same materials.

In order to do this, you need to convert your explanations into specific statements called hypotheses.
A sample hypothesis has already been written for you, as an example, in Table 1-1. Note that it is written in such a way that it has three specific parts.

“A salt....distilled water” is a brief description of what will be done

“stays at the bottom” is a prediction of what will happen

“because....distilled water” is an explanation of why it happens that way.

Use exactly the same format described above, and write two more hypotheses in boxes #2 and #3 in the first column of Table 1-1. Your two new hypotheses must be based on your drawing data from Step 1 of this lab.

Your hypotheses must include only the same materials and techniques that you used in Step 1 of this lab.

Your hypotheses must be testable with only the materials used in Step 1 of the lab. These limitations allow you to control the variables described in your hypotheses.

Don’t introduce anything new, like stirring, mixing, etc.

10. Ask your professor to check your hypotheses before you continue.

11. Read the suggested tests you could perform, which are listed across the top row of Table 1-1. You’ve already done the first test.

12. For the first hypotheses on Table 1-1, assume that you would preform each test listed in the top row of the table. You are NOT going to perform the tests now, just predict what result you would expect IF that hypothesis is correct. Now, write your predicted results in the boxes under each suggested test.

13. Repeat the process above with the other two hypotheses. Remember, for each hypothesis, make your predictions based on the assumption of THAT hypothesis being correct.

14. Study the patterns of your predictions. They should be different for each hypothesis.
## Table 1-1

<table>
<thead>
<tr>
<th>Hypotheses</th>
<th>Possible Tests Which Could Be Done</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Place salt water/food color mixture at the bottom of distilled water.</td>
</tr>
</tbody>
</table>

For each of your three hypotheses, write predictions in the boxes below, of what would happen if you did each of the possible tests listed above.

### #1
A salt water/food color mixture placed at the bottom of a beaker of distilled water stays at the bottom because salt water is more dense than distilled water.

### #2

### #3

Part 2 Step 5: Testing your hypotheses

Now you need to find out if your predictions match what you will observe when you do the tests.

15. **Do the tests** listed in Table 1-2. As you do each test, write “Yes” or “No” in the box under each test, according to your actual results. Answer the questions under Table 1-2.

Dispose of these materials by washing them down the drain with plenty of water.

Rinse the beakers and test tubes and return them to your tray.

**TABLE 1-2**

<table>
<thead>
<tr>
<th>Tests To Do</th>
<th>Place salt water/food color mixture at the bottom of distilled water; it stays on the bottom of the beaker.</th>
<th>Place salt water/food color mixture at the top of distilled water; it stays on top.</th>
<th>Place distilled water/food color mixture at the bottom of salt water; it stays on the bottom of the beaker.</th>
<th>Place distilled water/food color mixture at the top of salt water; it stays on top.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did you observe this result?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

16. Which hypothesis (#1, #2, or #3) from Table 1-1 **accurately predicted** what happened when you did the tests in Table 1-2?

17. Therefore, which hypothesis from Table 1-1 is most likely a **correct** explanation for what you observed in Step 1 of this lab exercise?

As long as a hypothesis continues to be a reasonable explanation of our observations, and if we can disprove any alternate hypotheses, the more **confident** we become that the first explanation is correct. After our methods, data, and proposed explanation are **published**, other scientists may think of observations and tests that disprove our original hypothesis, or alternate explanations that have not been disproved.

However, if no one is able to collect data that contradict our original hypothesis, and more and more alternate explanations are disproved, our explanation becomes the **best explanation**. A **hypothesis** that has been repeatedly tested by many different researchers over a long period of time and not disproved will generally be accepted as the **correct explanation** by most scientists.

If, in addition, a thoroughly tested hypothesis unifies and **explains many different observations** of the natural world and continues to predict accurately what will happen in new circumstances, the hypothesis will be called a **theory** or a **model**. To a scientist, the words theory and model mean something almost opposite from our everyday use of the word. It means a far-reaching explanation that is **highly likely to be true**.

And that is about as close as human beings can get to scientific truth.

18. In everyday speech, when we say "I have a theory that . . .," the word "theory" means

19. On the other hand, a **scientific** theory or model is
**Part 4: Standard Lab Equipment**

When doing the lab exercises in this class, it is important that you are able to identify and locate the items you will need to do the lab exercises. The following items have been set out and labeled for you:

- Electronic balance
- Beaker
- Erlenmeyer flask
- Graduated cylinder
- 1-mL and 5-mL pipettes
- Pipette bulb
- Automatic pipetter/micropipetter with tips
- Pasteur pipette and bulb
- Disposable transfer pipette
- Cover slips
- Weigh boat
- Test tube
- Eppendorf tube
- Eppendorf tube "floatie" or carrier
- Petri dish (glass and plastic)
- Spot plate
- Squeeze bottle liquid dispenser
- Parafilm®
- Magnetic stir bars
- Microscope slides
- Kimwipes®

**Draw a sketch of each item which you do not already recognize.**
**Use the space below.**

The End!
LAB 2: Designing an Experiment

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

The foods we eat contain a variety of different organic compounds, such as carbohydrates, fats, oils, and proteins, which are complex organic molecules made by other living things. Our bodies use these organic compounds from our food both as an energy source and as a supply of raw materials to build our own organic compounds.

For example, glucose (a sugar) circulates through our bloodstream. It is the most basic energy source for all of our cells. Bread contains a large amount of starch. What’s the connection? Starch is a long chain of glucose molecules joined together. Plants make starch as a way to store energy for themselves for a rainy day. Unfortunately for them, their starch may be eaten by a human first.

As a bite of bread is chewed, swallowed and dissolved in digestive juices, the dissolved starch passes through our small intestine. Starch molecules are huge, however, and are much too big to pass into the cells lining the digestive tract. Enzymes break the starch into individual glucose molecules, which are small enough to enter the cells which line the small intestine. Then the glucose passes through the cells and moves on into the bloodstream. Once in the bloodstream, the glucose molecules can be carried to all the cells of the body to be used as an energy supply.

Inside potato cells are protein molecules called enzymes busily catalyzing (speeding up) chemical reactions needed by the potato for its own survival. When you eat a potato, the potato proteins/enzymes are too large to enter your intestinal tract cells. Your own enzymes break apart those proteins into individual amino acids, which are small enough to enter your cells. The amino acids are passed on to the blood stream which carries them to other cells. Those cells take in the amino acids and assemble them in different arrangements to make a human protein (maybe yet another enzyme).

Although your cells contain thousands of different enzymes which speed up all kinds of chemical reactions, digestive enzymes are used to take apart large food molecules into subunits that are small enough to pass into the cells lining your digestive tract and then move them into your blood stream. These enzymes are found in saliva, pancreatic fluid, or on the surfaces of the cells of the small intestine.
Examples of Organic Compounds

<table>
<thead>
<tr>
<th>Type of Organic Compound</th>
<th>Specific Examples</th>
<th>What You Get if You Take One Molecule Apart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Starch</td>
<td>100’s of glucose molecules</td>
</tr>
<tr>
<td></td>
<td>Sucrose (table sugar)</td>
<td>1 fructose + 1 glucose</td>
</tr>
<tr>
<td></td>
<td>Lactose (milk sugar)</td>
<td>1 galactose + 1 glucose</td>
</tr>
<tr>
<td>Proteins</td>
<td>Casein (milk protein)</td>
<td>100’s of amino acids</td>
</tr>
<tr>
<td></td>
<td>Collagen (gelatin)</td>
<td>100’s of amino acids</td>
</tr>
<tr>
<td>Lipids</td>
<td>Olive oil</td>
<td>1 glycerol + 3 fatty acids</td>
</tr>
<tr>
<td></td>
<td>Butter</td>
<td>1 glycerol + 3 fatty acids</td>
</tr>
</tbody>
</table>

Part 1: Digestive Enzymes Found in Pancreatin

Pancreatin is found in the pancreas of animals and is used to digest fats and oils (which are lipids). Pancreatin aids the digestion of lipids because it contains lipase, which is the digestive enzyme that performs the first steps to break apart lipid molecules.

The pancreas produces other digestive enzymes besides lipase. But which enzymes? Does it, for example contain amylase, which breaks down starch? Or sucrase, which breaks sucrose into simpler sugars? Or protease, which breaks down proteins into amino acids?

In this exercise, you will design experiments to determine which other digestive enzymes are found in pancreatin in addition to lipase. You should design experiments to look for evidence of the activity of at least two enzymes (other than lipase). You may do additional experiments to check for the presence of other enzymes if you wish and if there is sufficient time during the lab period.

Check the “Tests to use for organic compounds” in Part 2 of this lab when deciding how to do your experiment. As a source of organic compounds to try to digest, you may use any of the supplies that have been put out for this lab.

Review Lab 1. Design your experiment in such a way that, when you are finished, the reader is unlikely to think of a better explanation for your observations than your own.
Hints for Planning Your First Experiment

Starch is made of many glucose molecules. However, if you test certain starches for the presence of glucose, your test will indicate no glucose is present (a negative test result), because as long as their glucose molecules are attached to one another and make starch, they behave (and test) like starch, not glucose. This same general phenomenon applies to all organic molecules and their components.

- If you mix starch with amylase, the original organic compound (starch) disappears. We know this because the indicator for starch (Lugol’s iodine) would turn our solution dark blue before we add the amylase. As digestion occurs, the solution will become clear, which indicates that starch is no longer present.

- A new organic compound will appear. A Benedict’s test for the presence of glucose would be negative before we added the enzyme; but it would be positive after the enzyme had time to work and cut up the starch into separate molecules of glucose.

For some organic compounds, you may be able to test for both the original organic compounds and the products of digestion. For others, you may have to rely on one type of test OR the other.

Part 2: Tests to Use for Organic Compounds

Testing for the presence of the sugar glucose (Benedict’s test)

1. Fill a test tube to a height of 1 cm with the solution to be tested.

2. Add 5 drops of Benedict’s reagent.

3. Using a test tube holder, place the test tube in a hot water bath and heat it until the color changes, but no longer than 3 minutes.

4. Note the color of the precipitate formed. A positive result is indicated by a color change which may change from green to yellow to orange to red or brown depending on how much glucose is present.

Dispose of these materials by washing them down the drain with plenty of water.
Testing for the presence of **starch** using Lugol’s iodine

5. Fill a spot plate well ⅓ full with the solution to be tested.

6. Add 2 drops of Lugol’s iodine.

7. Note the color of the solution. A color change ranging from blue to black indicates the presence of starch.

   Place the liquids in the waste disposal area in the lab.

Testing for the presence of **proteins and amino acids**: The biuret test

8. Put 3 mL of the solution to be tested into a test tube.

9. Add 10 drops of 20% NaOH

10. Add 5 drops 10% CuSO₄.

11. **Violet** color indicates the presence of protein (one or more long chains of amino acids). **Pink** color indicates the presence of polypeptides (short chains of amino acids). **Blue** color indicates just amino acids (complete protein digestion).

   Place the liquids in the waste disposal area in the lab.

Testing for changes in the amount of **acid** present using Phenol Red

Phenol red is **red** in an alkaline solution (non-acidic), and is **yellow** in an acidic solution.

   Place the liquids in the waste disposal area in the lab.
Testing for changes in the amount of acid present using Methyl Red

Methyl red is red in an acidic solution and yellow in an alkaline (non-acidic) solution.

Place the liquids in the waste disposal area in the lab.

Part 3: Experiments and Recording Data

First Experiment

12. Choose a type of organic compound that you would attempt to digest using pancreatin. What have you chosen?

13. What new substances (products) would be formed if this organic compound is in fact digested by pancreatin?

14. a. How could you determine if these predicted products are present after you have attempted to digest this organic compound using pancreatin?

And/Or

b. How would you determine if the original organic compound is no longer present after you have attempted to digest it using pancreatin?
Second Experiment (Use a different organic compound now.)

15. Choose a second type of organic compound that you would attempt to digest using pancreatin. What have you chosen?

16. What new substances (products) would be formed if this organic compound is in fact digested by pancreatin?
17. a. How could you determine if these predicted products are present after you have attempted to digest this organic compound using pancreatin?

And/Or

b. How will you determine if the original organic compound is no longer present after you have attempted to digest it?

Lab Notebook Entries

Procedure

Results
Part 4: Reporting Your Results

On a separate sheet of paper, write up one of your two experiments using the standard format for scientific papers. There will be four parts, as listed below.

I. Introduction

Tell the reader what the experiment is about. First, clearly state your question—that is, what were you trying to figure out? Then explain, very briefly, how you went about answering the question and what you discovered. Yes, this really is a very short version of the rest of the report, but without all the details.

II. Methods and Materials

Explain exactly what you did. You should be as specific as possible. Times, amounts, temperatures, equipment, etc. should be included. This allows readers to judge for themselves whether or not your procedure would yield an accurate and reliable result. A few readers may want to repeat your experiments (often as a prelude to asking another related question of their own). Write this section with those readers in mind—telling them everything they need to know in order to do exactly what you did.

III. Results

Report the data that you collected. You are telling the reader what you observed—nothing more or less. As a result of your procedure did something get bigger, colder, explode, glow in the dark, turn blue, make the reading on a meter change? Whatever it was, tell the reader.

IV. Conclusions

So how do you explain the data that you collected? Did it answer your question? Explain to the reader how and why you arrived at your conclusion.

The End!
LAB 3: Carbohydrates

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

(strict)

Read and learn the section on carbohydrates in your text before coming to the lab.

Part 1: Testing for Carbohydrates

Two simple laboratory tests allow us to test solutions for the presence of either simple sugars (simple carbohydrates) or starch (a complex carbohydrate).

These kinds of tests often use chemicals called indicators (indicator solutions). The indicator used to test for the presence of simple sugars is called Benedict's reagent. The indicator used to test for the presence of starch is called Lugol's iodine (or Lugol’s solution, or iodine).

By performing these tests on “known” solutions, you will: a) learn how to do the tests, and b) see what positive and negative test results look like.

Your tray should have the following for your group:

- 10 test tubes
- test tube holder
- spot plate
- test tube rack
- wax pencil or Sharpie®

Other materials and supplies that you will need will be found in the supply area. Take them as you need them and return them when you are finished.

On your lab table there is a beaker of water (about ¾ full) on a hot plate. There are small ceramic boiling stones in the water.

- Turn on the hot plate to the high setting.
Part 1-A: Types of Tests (Assays) - Chemical tests (analyses) are often called assays.

Positive Control Assay

It is important for you to see if the chemicals, supplies, and equipment you will use are working properly. A positive control assay will accomplish that by showing the results of an assay of a solution that you know should contain the chemical of interest.

Negative Control Assay

It is also important that you know what a negative test result looks like. Negative test results are what you get if the substance for which you are testing is NOT present. You will test dH₂O for both starch and sugar.

Experimental Assay

An experimental assay is done when you test something because you don’t know whether it contains the chemical you are interested in, such as simple sugar or starch, in order to find out if it does or does not contain that material.

Chemicals You Will Use

- Glucose is a known monosaccharide (simple sugar). Karo® syrup contains glucose, and was used to make your glucose solution. The results of this assay will show you what a positive result for simple sugar looks like.
- A starch solution should contain just complex carbohydrate. Shake it before you use it.
- Pure deionized water (dH₂O) does not contain starch or sugar.

Part 1-B: Assay (Test) for Simple Sugar (simple carbohydrate)

1. If the water on your hot plate is boiling, turn the hot plate setting to level 3.
2. Fill a labeled test tube to a height of 1 cm with the solution to be tested for each of the two solutions listed in Table 3.1.
3. Add 5 drops of Benedict’s reagent (the indicator for this test) to each tube.
4. Mix each solution with a vortex mixer.
5. Using a test tube holder, place both test tubes in the boiling water bath and heat it. Watch it while the color changes and record all of those colors in Table 3-1. Each color other than blue represents a positive test for sugar. Remove the tubes after 2 minutes.
6. Save the tube for later reference.
### Table 3-1: Simple Sugar Assay

<table>
<thead>
<tr>
<th>Assay Done</th>
<th>Solution Tested</th>
<th>Indicator Used</th>
<th>Colors of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>glucose (10% Karo syrup solution)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>dH₂O</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Part 1-C: Assay (Test) for Starch (a complex carbohydrate)

1. For your positive control, place two drops of the solution to be tested (see Table 3-2) into a labeled well of your spot plate.

2. Add 2 drops of Lugol’s iodine (the indicator for this test) to the same well.

3. Test your negative control (dH₂O) in the same way.

4. Record the colors of your results in Table 3-2.

5. Save the spot plate for later reference.

### Table 3-2: Starch Assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>Solution Tested</th>
<th>Indicator Used</th>
<th>Color of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>starch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>dH₂O</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Part 2: Making Predictions**

Based on what you already know about the substances in Table 3-3, which ones do you predict will test positive for the presence of sugar and/or starch?

Mark each box with a + (plus) if you think it contains that organic compound or − (minus) if you think it does not.

*Each substance may contain one, both, or neither of these carbohydrates.*

**Table 3-3: Food Predictions**

<table>
<thead>
<tr>
<th>Food</th>
<th>Simple carbohydrate (sugar) Prediction</th>
<th>Complex carbohydrate (starch) Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>apple juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>potato juice (shake it well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>salt water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>baking powder solution (shake it well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>baking soda solution - sodium bicarbonate (shake it well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozarka® flavored water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>powdered sugar (sucrose) solution (shake it well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vinegar</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Part 3: Analyzing Solutions (Experimental Assays)**

Now test the substances in Table 3-4 to see if they contain simple carbohydrates (sugars) or complex carbohydrates (starch). The most efficient way to do this is to test all of your solutions for the presence of sugar and then test all of your solutions for the presence of starch. Be sure to label all tubes and spot plates.

Since all your tubes for testing for sugar will be labeled with the contents, you can test several in the boiling water at the same time. If a tube turns color indicating a positive test before the two minute time limit, you can remove it from the boiling water bath.
Enter the results (using + for positive results and using - for negative results) in Table 3-4. Keep the spot plate for later reference. Each substance may contain one, both, or neither of these carbohydrates.

### Table 3-4: Food Test Results

<table>
<thead>
<tr>
<th>Food</th>
<th>Simple carbohydrate (sugar) Results</th>
<th>Complex carbohydrate (starch) Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>apple juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>potato juice (shake it well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>salt water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>baking powder solution (shake it well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>baking soda solution (shake it well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozarka® flavored water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>powdered sugar solution (shake it well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearly Canadian® flavored water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vinegar</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Comparing your data with your predictions**

12. For which substances were your predictions accurate?

13. For which substances were your predictions inaccurate?

14. If some of your predictions were inaccurate, explain why you think you were wrong.
Part 4: Detective Work

The two bottles labeled “A” and “B” each contain a commercial soft drink. One contains a diet drink and the other contains a regular (not diet) drink.

Perform the proper tests and answer the following questions.

15. Which one is the diet soft drink?
16. What is your evidence?
17. Which one is the regular soft drink?
18. What is your evidence?

CAUTION

Pour all sugar and starch test liquids in the waste disposal beaker in the lab.

Rinse the spot plates with plenty of water in the sink.

Save the spot plates.

Turn off your hot plate and unplug it.

Leave the water beaker with the boiling stones on the hot plate.

Clean your lab table.

Throw away your gloves in the regular trash.
QUESTIONS for LAB 3 - These can be answered after lab class.

19. Starch molecules are quite large — too large to be transported into the cells lining our digestive tract. For our cells to be able to use this source of energy, starch must be broken down into smaller ___________________________ molecules that can be absorbed by the cells.

20. What type of chemical reaction produces the smaller molecules in the question above?

21. When monosaccharides bond together, what name is given to that chemical reaction?

22. To test for the presence of starch you added _________________________________.

23. How quickly are the test results of a starch test visible?

24. A positive test for starch gives a _________________________________ color.

25. To test for the presence of simple sugars you added a solution called:

You also did something else, what else did you do?

26. A positive test for simple sugar gives the colors:

27. How quickly are the test results of a test for simple sugar visible?

The End!

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LAB 4: Lipids and pH

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

Part 1: Acids and Bases

One characteristic of water solutions is that they are either acidic, alkaline (basic), or neutral. Acidic solutions are sour, like lemon juice. Strong acids can corrode metal and burn skin. Bases are bitter and have a slippery feel. Most soaps are bases. Solutions that are neither acids nor bases are neutral.

The pH scale provides a measurement of just how acidic an acid is, whether a solution is neutral, or just how basic (alkaline) a base is.

Water Falls Apart: As you know, a water molecule is an oxygen atom joined to two hydrogen atoms, which is why it is called H₂O. In absolutely pure water, a very small number of water molecules fall apart spontaneously. A hydrogen atom (H) comes off the water molecule and leaves behind an OH hydroxyl group.

The H that comes off of the water molecule leaves its only electron behind when it does this. Since a hydrogen atom is made of only one proton and one electron, a hydrogen atom with no electron is just a proton. A proton has a positive charge, so this single proton is written H⁺.

An electron has a negative charge. Since the OH that was left behind has an extra electron from the hydrogen which left, it now has a negative charge. The OH is written OH⁻.

Neutral Solutions: In pure water, whenever a water molecule falls apart, one H⁺ and one OH⁻ are produced. Hopefully you can see that when this happens, the number of free H⁺s in pure water will be exactly the same as the number of free OH⁻s. Any solution that has the same number of H⁺s and OH⁻s is neutral, with a pH of 7. Therefore, pure water is a neutral solution.

Acids: An acidic solution is a solution with more H⁺s than OH⁻s. Any pH less than 7 is acidic.

Bases: A basic (alkaline) solution has more OH⁻s than H⁺s. Any pH greater than 7 is basic (alkaline).

What is pH? pH values are used to measure the concentration of H⁺s in a solution.
How acidic is it? How basic is it? Some acids are more acidic than others. For example, which would you expect to be more acidic, a teaspoon of pure lemon juice or an 8 oz. glass of water with a teaspoon of lemon juice dissolved in it? ___________________________

Measuring pH: The pH scale is used to determine how acidic or how basic (alkaline) a solution is. Special pH paper can be used to measure the pH of a solution. Measure the pH of several common solutions. Record your results in Table 4.1.

1. Obtain a clean spot plate.

2. Label each well of the spot with the name to the solution to be tested, as listed below.
   - Ammonia
   - Detergent
   - Milk of Magnesia
   - Vegetable oil
   - Sodium bicarbonate (baking soda)
   - Apple juice
   - Lemon juice
   - Salt water (10% NaCl)
   - Sodium hydroxide (NaOH)
   - Bile
   - Lysol
   - Vinegar

3. Hold a strip of pH paper so that the bottom end is touching the bottom of a well in the spot plate.

4. Place 4 drops of one solution above the color bands of the paper so that the solution runs down the paper and into the well which is labeled for that solution.

   Caution! Be very careful that the different solutions do not mix or splash into one another.

5. Compare the color of the pH paper to the colors on the pH paper container in order to determine the pH of each solution. Record your results in Table 4.1 as you test each solution.

6. Lay the used pH papers on a paper towel as you use them.

When you are finished, throw the pH test papers and paper towel in the regular trash.
There may be more than one solution with the same pH value.

### Table 4.1

<table>
<thead>
<tr>
<th>pH Value</th>
<th>Substances With That pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>14</td>
</tr>
<tr>
<td>L</td>
<td>13</td>
</tr>
<tr>
<td>K</td>
<td>12</td>
</tr>
<tr>
<td>Al</td>
<td>11</td>
</tr>
<tr>
<td>Lime</td>
<td>10</td>
</tr>
<tr>
<td>NE</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Neutral</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
</tr>
<tr>
<td>I</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
</tr>
<tr>
<td>Ic</td>
<td>2</td>
</tr>
<tr>
<td>Dic</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Dispose of the materials on your spot plate by washing them down the drain with plenty of water.
Part 2: Triglycerides and Their Building Blocks

When we eat triglycerides, either as fats or oils, the triglyceride molecules are too large to be absorbed through the cells that line your digestive tract into our bloodstream. First they must be taken apart (digested) by enzymes into smaller components. Triglycerides are made up of three fatty acids joined to a glycerol molecule.

We will be digesting vegetable oil triglycerides into their building blocks. The enzymes that will take apart the triglycerides in mammals are found in pancreatin, which is produced by the pancreas. Pancreatin contains the enzyme lipase, which takes apart lipids, as shown below. Plants also produce such enzymes, including lipase. Remember that these molecules are too small for us to see. However, we can use indicators to tell if fatty acids (which really are acids) are released because that would make the solution more acidic.

![Diagram of triglyceride digestion](image)

Estimating pH with an Indicator Solution

Since these molecules are too small to see, we will have to use an indicator solution (something that turns color when it reacts in a predictable way) to help us know whether or not our vegetable oil breaks down. Phenol red is an indicator solution with the colors shown on Table 4.2 (also see the color reference photo at your lab table). Using phenol red allows us to detect changes in the pH of solutions.

<table>
<thead>
<tr>
<th>Table 4.2 - Phenol Red Colors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Color</strong></td>
</tr>
<tr>
<td>Yellow</td>
</tr>
<tr>
<td>Peach</td>
</tr>
<tr>
<td>Red or Pink</td>
</tr>
</tbody>
</table>

Phenol red has a red or pink color in a solution that is basic (alkaline), peach color in a solution that is approximately neutral, and yellow color in a solution that is acidic.
Remember that an acid contains excess H\(^+\) ions, and an alkaline (basic) material contains excess OH\(^-\) ions.

7. Label a test tube A. Add all the following materials to the tube. **Bile** is a chemical which is produced by the liver and stored and concentrated in the gall bladder until needed, like when we have just eaten some ice cream or a double bacon cheeseburger. It helps speed up the digestion of lipids.

   - 3 mL bile solution
   - 3 drops phenol red
   - 5 drops vegetable oil (Look in Table 4.1 for the pH.)

Mix using a **vortex mixer**.

Hold the tube above your head and look up through the edge of the thin layer at the top of the liquid.

   **What color is the upper layer of liquid in the tube?**

   **What color is the lower layer of liquid in the tube?**

8. Add one drop of 0.05% or 0.025% NaOH (sodium hydroxide), which is a base and will raise the pH, and mix well with a vortex mixer. The solution should turn **red, pink, peach, or yellow**.

   If the solution is red, add HCl (Hydrochloric acid, which is an acid and will lower the pH), one drop at a time. Mix well after each drop until it just turns pink.

   If the solution is peach or yellow, add NaOH, one drop at a time (mixing well after each drop) until it just turns pink or red color.

   **NOTE:** The liquid in the lower layer in the tube should be pink or red now.

9. **Consider the pH** of the lower layer of liquid, and look at Table 4.2. Without using pH paper, determine the approximate pH of the lower layer in this tube. **Circle one:** acidic, neutral, alkaline.

10. **What material** is in the top layer?

    Explain how you know that and why the material is at the top.
Digesting Triglycerides

You will now attempt to digest the triglyceride molecules in the tube.

11. Watch the tube carefully as you add 2 mL of plant enzyme solution to the tube.

What color is the lower layer of liquid in the tube?

12. Mix the tube with a vortex mixer.

Hold the tube above your head and look up through the edge of the thin layer at the top of the liquid. What color is the upper layer of liquid in the tube?

Were the triglycerides digested?

13. Consider the pH of the lower layer of liquid, and look at Table 4.2. Without using pH paper, determine the approximate pH of the lower layer in this tube. Circle one: acidic, neutral, alkaline.

14. Were any triglycerides broken down during the experiment?

Describe the evidence for your answer.

CAUTION
Hazardous Waste

Place the liquids in the waste disposal area in the lab.

Put the disposable pipettes in the regular trash.
Part 3: Surface-to-Volume Ratio

Observe how the surface area changes, as the size of an object changes, by doing the following.

*Volume* (cm$^3$) of a cube = length x width x height  
*Surface Area* (cm$^2$) of one side = length x width of that side.

15. Obtain a potato and cut it into a cube that is 2 cm in each direction, with no skin on any sides.

   What are the dimensions of each side? ________
   
   What is the total volume of the cube? __________________
   
   How many sides does the cube have? ________
   
   What is the total surface area of the cube? ________________

16. Pour enough food coloring into a glass petri dish or weigh boat to cover the bottom of the container. Now (wearing gloves) dip each side of the potato cube into the food coloring.

17. Use paper towels to blot the food coloring from all sides of the cube and from your gloves.

18. Place the cube on a clean paper towel.

19. Now cut the potato cube into eight smaller cubes, each measuring 1 cm on a side.

   What is the total volume of potato on the lab table? __________________
   
   Has the total volume of potato on the lab table changed? ________

20. How many total potato surfaces are now colored by food coloring? __________ Not colored? __________

   What is the total surface area of potato on the lab table? __________________
   
   Has the total surface area of potato on the lab table changed? ________

21. By what percentage was the surface area increased by cutting the potato? __________ %

22. What is the surface area-to-volume ratio of a 2 cm cube? ________ : ________

23. What is the surface area-to-volume ratio of all of the 1 cm cubes together? ____ : ____
Dispose of all weigh boats, potatoes and paper towels by placing them in the regular trash.
Food coloring can be washed down the drain with plenty of water.
Ask your professor what to do with any petri dishes.

Part 4: Observing Emulsification

Bile is not an enzyme; it is an emulsifier. The following two exercises will help you understand what an emulsifier is and why an emulsifier is important in the digestion of fats and oils (lipids). Detergent, like bile, acts as an emulsifier. To observe what an emulsifier does, do the following.

24. Put 1 cm of dH₂O (deionized water) in a test tube.
25. Add 1 drop of food color (not yellow) to the tube and mix with the vortex mixer.
26. Obtain two flat pieces of wax or laminated paper, each at least 4" square. On both, make a small puddle of three drops of colored water, and 2 cm away make another puddle of three drops of vegetable oil. Do not let the puddles touch each other.
27. On one piece of wax paper, place a drop of detergent in between the oil and the colored water. (It is okay if the detergent touches the oil.)
28. Use a clean toothpick to mix the puddles gently for 5 seconds on the wax paper with no detergent. Observe the results with a hand lens right away and again after 30 seconds. Draw the results in Table 4.3, and label both the water and the oil.
29. Use a clean toothpick to mix the puddles gently for 5 seconds on the wax paper with detergent. Observe the results with a hand lens right away and again after 30 seconds. Draw the results in Table 4.3, and label both the water and the oil.

<table>
<thead>
<tr>
<th>Table 4.3 Oil Emulsification Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Emulsifier</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
30. What difference did the emulsifier make?

31. How did the emulsifier affect the surface area of the lipid?

32. Considering everything you have done, what was the effect of the bile in the test tubes for Part 2 of this lab?

Dispose of the wax paper and liquids by placing them in the regular trash. Save any laminated paper.

The End!
LAB 5: Microscope Use

PPE Required - None -
You must have completed the Safety Lab before doing this lab!

Each student will set up their own microscope on the lab table. Study the photograph of a microscope below and identify the same parts on your microscope. Different brands of microscopes are designed differently so yours may not look exactly like the diagram. Ask your professor if you are unsure how to find any of the parts on your particular microscope.

Part 1: Microscope Information and Techniques
Parts of the Microscope

Be able to identify and describe the function of the following parts of the microscope.

Illustration by Terry Arzola
Carrying the microscope

When carrying the microscope back and forth from a cabinet to a lab bench, always grasp the arm of the microscope with one hand and rest the microscope base on your other hand.

Focusing the microscope

Inexperienced microscope users sometimes lower an objective lens onto a slide and break the slide. An expensive commercially-prepared slide will have to be replaced and the microscope may be damaged. Learning the proper way to focus the microscope will ensure that this never happens to you.

Complete steps 1 - 6 by looking at the microscope stage from the side of the microscope. **Do not** look through the ocular(s) (eyepieces) yet.

1.  Turn the **coarse focus knob** until the stage is as far away from the objective lenses as possible.

2.  Turn on the microscope **light**.

3.  Click the **lowest** power objective lens into place. This will be 4X or 10X, depending on the microscope.

4.  Obtain a slide of the letter “e” and place the slide on the microscope stage.

5.  Move the slide with the **stage manipulator knobs** so that the part of the slide you want to look at (the e) is in the **middle** of the circle of light shining upward from the light source.

6.  Turn the **coarse focus knob** until the objective lens is just above the slide. Notice that this lens does not touch the slide.

Now look through the oculars (eyepieces) for the next steps.

7.  Slowly raise the lowest power objective using the **coarse** focus adjustment knob until the e on the slide is in focus. Now use the **fine focus** adjustment knob to make the image as sharp as possible.

   **Note:** If you are using a **binocular** microscope, look through both of the ocular lenses (eyepieces). The distance between the two lenses is adjustable. Adjust that distance for your eyes each time you use a binocular microscope. There should be no dark areas or double images. This makes it easier to use the microscope and is less tiring for your eyes. It is worth it to invest a little time getting used to using these microscopes properly.

8.  These microscopes are **parfocal**, that is, if the object is in focus at one magnification it is very nearly in focus at the next higher power. Without moving the stage, view the object at the next higher magnification by clicking the next higher power objective lens into place.
9. Adjust the focus by using the **fine focus control knob only**. Also, adjust the light level.

**NEVER USE THE COARSE FOCUS KNOB WITH ANY OBJECTIVE LENS EXCEPT THE LOWEST POWER LENS ON YOUR MICROSCOPE!**

10. If you have 4X, 10X, and 40X objective lenses on your microscope, you should now be focused at 10X. Click the 40X lens into place and focus again using the fine focus knob. If you do not have a 4X objective on your microscope, you should already be focused at 40X. Be sure to adjust the amount of light.

Do **not** attempt to use the **100X** lens (if present) unless instructed to do so.

11. If you can’t find the e or have no luck at all focusing at higher magnification, click the **lowest** power lens back into place and start over.

**Learning more about the microscope**

12. Move the letter **e** slide from left to right using the stage manipulator knobs. As you do this, look at the slide from the side of the microscope and also through the eyepieces. How do the movements from the side and through the eyepieces compare?

13. Move the letter **e** slide toward you and away from you using the stage manipulator knobs. As you do this, look at the slide from the side of the microscope and also through the eyepieces. How do the movements from the side and through the eyepieces compare?

14. Use the 10X objective, look through the ocular lenses, and move the **e** to the **center** of your field of view. **Draw** the “e” as it appears to you at 10X.

15. Now change to the 40X objective and focus the microscope. **Draw** the “e” as it appears to you at 40X.
16. Explain why your two drawings of the “e” (at 10X and 40X) are different.

17. Considering your answer above, how should you position any slide before changing from a lower magnification objective to a higher magnification objective?

18. Use your microscope to observe three different commercially prepared slides provided to you.

19. **Choose the magnification that gives you the best view.**

20. Write the name of the slide and the magnification for each drawing on the lines below. Use a pencil and draw what you see on each slide in the spaces below. Ask your professor to look at each slide before you finish your drawing.

21. Please return all prepared slides to their proper location. Also, put the 4X objective in place of your microscope.

**Note** - on the slides, the abbreviation **w.m.** means ‘**whole mount**,’ which means that the specimen on the slide is complete or whole.

Slide ____________  Slide ____________  Slide ____________
Part 2: How to Make a Wet Mount

Wet mount #1: Elodea leaf
Make a wet mount of *Elodea* (not *Euglena*) as shown below.

22. Place one *Elodea* leaf in the center of the slide and put one drop of water on top of the leaf. Place the edge of a cover slip on the slide near the edge of the leaf. Now drop the cover slip gently down onto the slide.

23. Focus carefully using the lowest power objective lens on your microscope. Move up, one lens at a time, from 4X to the 40X objective and observe the leaf for several minutes. Look near an edge of the leaf for a clearer view.

The leaf should appear to be composed of stacked rectangular boxes. These boxes are the cell walls. Inside each cell are the plasma membrane, cytoplasm, and nucleus. When you focus up and down, you can probably see two different cell layers, which may look like overlapping black rectangles. Focus on just one layer of cells.

24. Observe one cell. Look for the green oval-shaped chloroplasts to drift slowly around in the cytoplasm. This phenomenon is the result of cytoplasmic streaming.

25. Draw what you observed. Label the cell wall and chloroplasts.
26. Find the lever or knob that adjusts how much light passes through the slide. Look at your slide under different magnifications and experiment with the light adjustment. What did you discover?

Dispose of the Elodea leaf and liquids by washing them down the drain with plenty of water. Follow the instructions in your lab concerning both slides and cover slips.

27. Obtain a new slide. Using the disposable pipette, squeeze the air out, hold it that way.

For a hay infusion, put the end of the pipette on the bottom of the culture, and slowly take up a small amount of liquid.

For a vinegar eel culture, put the end of the pipette in the middle of the culture and slowly take up a small amount of liquid. Vinegar eels are animals (in the roundworm, or nematode phylum) which feed on the acid-loving bacteria that make apple cider vinegar.

28. Add a cover slip.

29. Place the slide on the microscope stage and, starting with the lowest power objective, focus it, working your way up, until you have the best magnification for your specimen. Beginning at one corner of the slide, systematically move back and forth across and down the slide until you find organisms.

30. Look for green, tan/brown, or moving things. Dark black circles with clear centers are air bubbles (don’t draw those). Draw what you observe. Show your view to your professor.
Putting Away the Microscope

When you put away the microscope make sure that
- there is no slide left on the stage
- the light has been turned off
- the lowest power objective lens is in place and raised to its highest level
- the power cord should be wrapped around the cord holder on the microscope
- you put the microscope cover on the microscope.

Follow the instructions in your lab concerning disposal of the slides and cover slips.

Questions for Lab 5

31. Name the parts of the microscope that you would adjust in order to make the following changes:
   c. Moving the slide so that you can see a different part of it.
   d. Changing the objective lens.
   e. Adjusting the focus when using the 4X objective.
   f. Adjusting the focus when using the 40X objective.
   g. Adjusting the amount of light shining through the slide.
32. Under each of the headings below, list each of the specimens you viewed using the microscope.

| PREPARED SLIDES | WET MOUNTS |

33. Review your lists above. What characteristics could you see that were common to all living things?

34. Review your lists above. What characteristics could you see were common to all of the commercially prepared slides?

The End!
All the bits of matter that are too small to see, such as atoms and molecules, are constantly moving or vibrating due to the kinetic energy of atoms. This motion is called Brownian Motion, and it never stops in the temperature ranges in which living things survive, although it slows down as the temperature drops and speeds up as the temperature increases. The point at which the temperature is cold enough for molecular motion to stop completely is called absolute zero. Absolute zero is minus 459°F (minus 273°C). All of the above information applies to both living and nonliving things.

Imagine that you are looking at the room from above. Suppose there are 50 women wearing red hats on one side of the room and 50 men wearing yellow hats on the other side of the room. Each person moves in a straight line until they bump into someone or something, then ricochets off in another direction. It will not take long for the men and women to be distributed randomly throughout the room. This overall movement, or net movement is called diffusion.

This lab requires a clock watcher! Set up Parts 1, 2, and 3. Note the time when you begin each activity. As you are doing Parts 4 and 5, the clock watcher is responsible for reminding the group when to make observations or measurements for Parts 2 and 3. When you finish Parts 2, 3, 4, and 5, return to Part 1 and finish it.

Part 1: Diffusion and Its Limits

Caution! Methylene blue is a stain—that means it stains. It will eventually wear off of your hands but it will not come out of your clothes! Wear gloves and be very careful.

1. Obtain three 30 or 50 mL beakers. Fill each beaker half full of methylene blue solution.

2. Wear gloves so that the oils of your fingers do not contaminate the agar you will use. Get your container of agar with the thickest agar layer. Run a thin flat metal spatula around the edge of the agar in order to loosen it and turn the agar upside down onto a paper towel.
3. Using a scalpel and a ruler, cut three pieces of agar according to the length, width, and height dimensions listed below. Assume that these different types of shapes represent shapes of real cells in living things. Remember that some materials will diffuse across cellular membranes.

12 mm x 12 mm x 12 mm = a **Cube**
8 mm x 12 mm x 18 mm = your **Thicker Rectangular Box** shape
6 mm x 12 mm x 24 mm = your **Thinner Rectangular Box** shape

If your agar came in a paper cup or plastic disposable dish, you can throw the empty cup or dish in the trash. If it was in glassware, ask your professor how to deal with the glassware. Extra agar goes into the regular trash.

4. Place each of the three agar pieces in a separate beaker of methylene blue solution. The agar pieces should be completely submerged. If they are not, add more methylene blue solution. **Leave the agar pieces in the solution for 1½ hours.**

Start Time ____________________

5. Use the following information to help you fill in Table 6.1.

**Surface Area** (mm$^2$) of each side = \( \text{length (mm)} \times \text{width (mm)} \)

**Volume** (mm$^3$) of a piece of agar = \( \text{length (mm)} \times \text{width (mm)} \times \text{height (mm)} \)

**Surface-to-Volume Ratio** - Divide the surface area by itself, which will equal 1, and write 1 on the left side of the colon. Divide the volume by the surface area and write the result on the right side of the colon (:). Round your answer to the nearest tenth of a mm.

<table>
<thead>
<tr>
<th>Original Agar Piece (mm)</th>
<th>Total Surface Area (mm$^2$)</th>
<th>Total Volume (mm$^3$)</th>
<th>Surface-to-Volume Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 x 12 x 12</td>
<td></td>
<td></td>
<td>:</td>
</tr>
<tr>
<td>8 x 12 x 18</td>
<td></td>
<td></td>
<td>:</td>
</tr>
<tr>
<td>6 x 12 x 24</td>
<td></td>
<td></td>
<td>:</td>
</tr>
</tbody>
</table>

Now is the time to complete parts 2, 3, 4, and 5 of this lab before you return to finish Part 1.
1½ Hours Later

End Time___________________  Total elapsed time ________________________

6. **Wear gloves** whenever you handle the dye. Carefully pour the methylene blue out of the small beakers into a larger one. The agar pieces should stick to the bottom of the small beakers. Lay 3 paper towels on top of each other and tap the agar pieces out of the beakers onto the towels. **Very gently** blot the excess dye off the agar pieces. Move the agar pieces to a clean paper towel.

7. Using a scalpel, cut one of the agar pieces in half, then cut a **thin slice** from the cut end. Place the slice on its side on a clean paper towel. Cut a thin slice from each of the two other original agar pieces in the same way, and place the slices on the clean paper towel. **Ask your professor** to look at your results.

8. Make drawings of the three agar slices in the spaces below. Make the drawings **actual size**. Label the clear and dark blue areas of the slices. **Dark blue** areas are considered to be agar that absorbed the dye. **Pale blue and clear** areas are considered to be agar that did not absorb the dye. Yes, this is a bit subjective; do your best.

   12 x 12 x 12 mm   8 x 12 x 18 mm   6 x 12 x 24 mm

9. Measure how far the blue dye moved into the agar by measuring the thickness of the dark blue color on the edges of each cut piece. Sometimes one side of the agar block sticks to the bottom of the beaker and then shows little or no dye absorption. To take that into account, **assume that the methylene blue was absorbed the same distance inward on all six sides for all three agar blocks**. Record your results in Table 6.2.
Table 6.2

<table>
<thead>
<tr>
<th>Original Agar Piece (mm)</th>
<th>Width (mm) of Dark Blue at Each Edge of the Slice</th>
<th>Measurements (mm) of Pale/Clear Area of the Slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 x 12 x 12</td>
<td>l =</td>
<td>w = h =</td>
</tr>
<tr>
<td>8 x 12 x 18</td>
<td>l =</td>
<td>w = h =</td>
</tr>
<tr>
<td>6 x 12 x 24</td>
<td>l =</td>
<td>w = h =</td>
</tr>
</tbody>
</table>

10. Use the following formulas to help you fill in Table 6.3. You may do this later if you are pressed for time.

\[
\text{volume that absorbed the dye} = [\text{total volume of an agar piece}] - [\text{volume that is still clear}]
\]

\[
\% \text{ of the agar that absorbed the dye} = \frac{[\text{volume that absorbed dye}]}{[\text{total volume}]}
\]

Table 6.3

<table>
<thead>
<tr>
<th>Original Agar Piece (mm)</th>
<th>Total Volume (mm³)</th>
<th>Clear Volume (mm³)***</th>
<th>Dyed Volume (mm³)</th>
<th>Percentage of the Agar That Did NOT Absorb the Blue Dye</th>
<th>Percentage of the Agar That Absorbed the Blue Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 x 12 x 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 x 12 x 18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 x 12 x 24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** Remember that the dye is absorbed along each surface on all six sides! There is a three dimensional box-shaped clear region inside the agar block, and you can just see a slice of it. Assume that the height of clear agar is the same as the length and width (from Table 6.2) of the clear region inside the dyed slices.
11. Consider all of your data, and rank the effectiveness of each shape at absorbing the dye.

Most effective shape ____________________________________________

Least effective shape ____________________________________________

Shape that is in-between most and least effective ______________________

Place the methylene blue in the waste disposal area in the lab.

Dispose of the agar and paper towels in the regular trash.

Rinse and save the beakers.

**Part 2: Does Molecule Size Affect Diffusion?**

12. Obtain your agar plate with the thinnest layer of agar and place it on a white paper towel or piece of white paper. Note that the lid is larger than the bottom of the dish and the bottom contains the agar. With the lid on, turn the whole thing agar-side up. Now make two small dots with your marking pen on the plastic bottom of the dish. The dots should both be at least 2 cm away from the edge of the dish and also at least 4 cm from each other.

13. Also, on the bottom of the dish, near the edge, write “met blue” in small letters on the bottom of the dish near one dot and "pot perm" at the edge near the other dot. When you turn the dish back right side up, you should be able to see the dots through the plastic and the agar, but the writing should be off to the sides so that mostly you just see agar.

**Caution!** Potassium permanganate also is a stain. Wear gloves, and be very careful.

14. Use the following method to make a hole directly above the dot labeled "pot perm." Get a disposable pipette and cut off the narrow end at the 0.5mL mark. Squeeze the bulb and keep it squeezed while you insert it 2-3 mm deep into the agar. **Do not cut all the way through to the dish.** Twist the pipette, release the bulb, twist it some more, then pull it out of the agar. There should be a nice clean small hole in the agar.

15. Using the dropper in the bottle, carefully transfer one or two drops of potassium permanganate (KMNO₄) solution into the hole in the agar **Just enough to fill the hole.**
16. Use the same method to make a hole and add two drops of concentrated **methylene blue** solution into a hole directly above other dot labeled “met blue.”

17. Place a thin flat ruler underneath the agar dish and measure the **diameter** of each dye circle.
18. Cover the agar with the other half of the dish.

19. **Every 15 minutes**, uncover the dish and measure the **diameter** of each dye circle again. Record your data in Table 6.4.

20. For Table 6.4, keep in mind that **molecular weight** is an approximation of the size of a molecule. Larger molecules have larger molecular weights than do smaller molecules.

<table>
<thead>
<tr>
<th>Elapsed Time (min)</th>
<th>Methylene Blue Molecular weight = 320</th>
<th>Potassium Permanganate Molecular weight = 158</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter of Dye Spot (mm)</td>
<td>Diameter of Dye Spot (mm)</td>
</tr>
<tr>
<td>Start</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

21. Which dye moved faster? ____________________________

22. Agar forms a gel when mixed with water and allowed to set. If you could view the agar at high magnification, you would see a network of agar threads. Between the threads are channels of trapped water. Identify two factors that might affect the speed with which the dye traveled through the agar. Explain each one.

1. ____________________________

2. ____________________________
Tape the two halves of the petri dish together and dispose of these materials and the disposable pipettes by putting them in the regular trash.

**Part 3: How Temperature Effects Diffusion**

23. Fill the culture dish on your tray ¾ full of ice from the ice maker.

24. Get the two glass petri dishes on your lab table. Put one dish on your hot plate (turned on to level 2), and one in the bed of ice in the culture dish.

25. Fill each petri dish about ½ full with water. Let the dishes sit for 5 minutes. Do not move them.

26. Next, slowly and carefully add a drop of potassium permanganate (KMNO₄) solution to the water in the center of each dish without moving or even touching the dish. It is important not to disturb the water. You want to observe diffusion, not mixing, not splashing.

27. Leave the dishes undisturbed and complete Table 6.5 by recording your observations every 4 minutes.

<table>
<thead>
<tr>
<th>Table 6.5</th>
<th>Approximate % of Water that is Colored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elapsed Time (min)</td>
<td>Hot Water Dish</td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

28. Turn off the hot plate and unplug it.

29. Explain what causes the dye molecules and the water to become evenly mixed.
30. In which dish was diffusion faster?

   Explain why.

   Pour the liquids into the waste container. Rinse both parts of each petri dish and leave them on your tray. Pour the ice into the sink.

**Part 4: Simulating Diffusion**

Diffusion is the mixing of different types of molecules or ions in a gas or a liquid because of the random motion of molecules bumping into each other. Since molecules are too small to observe directly, we will use beads to represent molecules.

31. Place all the gold beads at the left end of the plastic box and all the silver beads at the right end. Assume that there are 50 of each color.

32. With the box resting flat on the table, slide it back and forth on the table for 30 seconds. What has happened to the distribution of the beads?

33. Again, place 50 gold beads at the left end of the plastic box and 50 silver beads at the right end.

   Which end has the higher concentration of gold beads? _________________________

   Which end has the lower concentration of gold beads? __________________________

34. With the box resting flat on the table, gently slide it back and forth for 30 seconds. But, as you slide the box, observe carefully what is happening to just the gold beads. Ignore the silver beads.

   What is the overall (or net) direction of the movement of the gold beads?

   Watch one gold bead for a few seconds. Describe its movement.
Part 5: Observing Molecular Motion (Brownian Motion)

35. Make a wet mount using a drop of whole milk. Observe the milk with a compound microscope. Start with the low power objective lens. Finding and focusing on these may be a challenge!

You are looking for tiny clear fat droplets in the milk - they are not bubbles. They may look like tiny bubbles or even very small rounded cells, but they are not cells. Take your time, adjust the light on the microscope (lower light increases contrast), focus as clearly as possible, and look for a zillion tiny clear fat droplets that are vibrating in place, as well as moving around.

Since this can be hard to see in the lab, the following You Tube video may help. In the video, the clear areas are water and the darker small blobs are droplets of a lipid. http://www.youtube.com/watch?v=2VdjIn734gE

36. Work your way up to the 40X objective lens (whichever one your microscope has). Adjust the light level for the best view.

37. Find one single fat droplet that is pretty much staying in one place and not moving across your field of view. Watch it for a few seconds. Describe the movements of that one droplet.

38. What is it that is invisible to your eye but is causing the vibration of the droplets?

Dispose of these liquids by washing them down the drain with plenty of water.

Rinse all cover slips and slides, and put them in the glass disposal.
Questions for Lab 6

39. Read the following statement.

“During the process of diffusion, the net movement of molecules is from a region of higher concentration of those molecules to a region of lower concentration of those molecules.”

Do you agree or disagree with the statement? ______________

Explain your reasoning.

40. Read the next statement.

“During the process of diffusion, each individual molecule in a region of higher concentration of those molecules will move in the direction of the region of lower concentration of those molecules until the molecules are distributed evenly.”

Do you agree or disagree with this statement? ______________

Explain your reasoning.

41. What is the energy source that powers diffusion?
42. How does increasing the temperature affect the random motion of molecules?

43. Under what circumstances would molecular motion stop?

44. How did molecular weight affect diffusion in your experiment?

45. Consider what happened with your agar blocks soaked in methylene blue. Imagine that the agar blocks represent cells, and the methylene blue represents the environment in which the cells live. Name several substances which would be found in this fluid that are needed inside the cell.

46. Consider what you have done in this lab, and give reasons why most cells are so small.

The End!
A chemical reaction is a process that changes something into something else — usually something completely different. For example, ordinary gasoline ($C_8H_{18}$) is smelly, nasty stuff. You would not think of drinking it, and if you did it would poison you. When you burn gasoline in your car, however, a chemical reaction occurs that combines oxygen in the air with the gasoline, changing the “gasoline plus oxygen” into carbon dioxide and water. It is not only possible to drink water, but water is essential to our survival.

During that chemical reaction, no atoms in the gasoline or the oxygen gas in the air disappear, and none simply appear out of nowhere. Instead, the atoms are rearranged. Gasoline is made of carbon and hydrogen atoms. The carbon atoms from the gasoline combine with some of the oxygen atoms from the oxygen gas in the air to make carbon dioxide ($CO_2$), and the hydrogen atoms from the gasoline combine with other oxygens in the air to form water ($H_2O$).

In other types of chemical reactions, two substances combine their atoms to make one new substance. For example, chlorine ($Cl$), a poisonous yellow gas, and sodium ($Na$), a silvery metal that explodes when placed in water, can combine to form ordinary table salt ($NaCl$). In another type of chemical reaction, one molecule can split into two new substances. For example, mercuric oxide ($HgO$), a red solid, can be heated, separating it into pure mercury ($Hg$), a silver liquid metal, and oxygen gas ($O_2$).

**Part 1: The Effects of Enzymes & Cofactors on Chemical Reactions**

Catalysts are substances that make chemical reactions happen faster. That is all they do, and they are not used up or destroyed in the reaction. They cannot make a chemical reaction happen that would not have happened anyway. Enzymes are proteins, made by living things, that act as catalysts in your cells.

Speeding up reactions may not seem all that important, but suppose our cells need the product of a certain reaction to be made dozens of times per second in order to have enough of it. Perhaps, on its own without enzymes, the reaction would only happen once a week. In this case, the presence of the enzyme is crucial for survival. You can assume that chemical reactions in your cells always have enzymes present.

Many enzymes will not work efficiently unless other substances, called cofactors, are present. Certain ions may act as cofactors, including calcium, copper, magnesium, manganese, zinc, and...
selenium. In this exercise, you will determine which metal ions are required by the enzyme polyphenol oxidase.

**Potato cells do contain these various cofactors in small amounts as well as polyphenol oxidase.**

You will use potato juice/catechol solutions and add chemicals that remove certain ions from the solutions. Observe the solutions and determine whether or not the enzyme in each tube still works after each type of ion is removed from the solution.

In **this** reaction a positive test results in a yellow/gold/reddish brown color, which means that a chemical called **benzoquinone** was formed. See Part 3 of this lab for the details of that reaction.

*Note the Health Warning label on the catechol. Use proper caution.*

1. This demonstration involves three tubes with the following contents.

   **Tube “EDTA”**
   1 mL catechol solution
   1 mL EDTA solution (EDTA reacts with calcium and magnesium ions and removes them from solutions)

   **Tube “PTU”**
   1 mL catechol solution
   1 mL PTU solution (PTU reacts with copper ions and removes them from solutions)

   **Tube “H₂O”**
   1 mL catechol solution
   1 mL water (Water removes no ions from the solution)

2. Record your “Before” observations of the color and clarity of each tube in Table 7.1.

<table>
<thead>
<tr>
<th>Solution added</th>
<th>Color and clarity before adding potato juice</th>
<th>Color and clarity after adding potato juice</th>
<th>Is the polyphenol oxidase working in this tube?</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. **Your professor will now add 1 mL of potato juice to each of the tubes and swirl each to mix them.**
4. How long did it take for any color changes to occur? ____________________________

5. Complete Table 7.1.

6. Which cofactor(s) is/are necessary for polyphenol oxidase to work efficiently?
   Explain

7. Look at the solutions again in one hour. Explain any changes which you see.

8. Iron ions in food can cause the food to discolor. EDTA is used commercially as a food preservative. From what you know from this lab, how could EDTA help to preserve food from spoiling?

---

Part 2: Another Chemical Indicator Test

Caution! Wear protective goggles.

In chemical reactions, new substances (products) were created when the atoms in the substances that you started with (the reactants) were rearranged to form new substances.

You are going to observe a chemical reaction. Watch for changes such as bubbling (which indicates the release of gas), color changes, the appearance of suspended solids (called precipitates) which settle to the bottom of the test tube, etc.

9. This reaction provides a good method to test for the presence of salt (NaCl). Fill a clean test tube (labeled Ag) half full of salt (NaCl) solution and add six drops of silver nitrate (AgNO₃) solution to it. What do you observe?

   Right away __________________________________________________________
The reaction you saw is
\[
\text{NaCl} + \text{AgNO}_3 \rightarrow \text{AgCl} + \text{NaNO}_3
\]
Sodium chloride + Silver nitrate \( \rightarrow \) Silver chloride + Sodium nitrate

From the results you see, how can you tell that a chemical reaction took place?

Keep these test tubes for reference until the end of the lab. When you are finished with them, place the liquids in the waste disposal area in the lab.

Part 3: Why Do Banana Skins Turn Brown?

Plant cells contain two important chemicals that interact with air.
- Catechol
- An enzyme called polyphenol oxidase

When plants are scraped or cut, air comes into contact with the damaged cells and the following reaction occurs.

\[
\text{air} + \text{catechol} \rightarrow \text{benzoquinone}
\]

Polyphenol oxidase will speed up this reaction.

The benzoquinone which forms in the reaction has a reddish-brown color, and it kills or inhibits the growth of microbes. This helps protect the plant from infection by disease-causing microorganisms. We see the same effect in the kitchen when peeled or chopped fruits and vegetables turn brown after a while.

10. Obtain a yellow banana. What is its predominant skin color? (This is not a trick question!)

11. Peel it just halfway, bending and crunching the peeled portion of the skin in order to damage the cells. Observe the outer skin of the banana and fill in table 7.2 every 10 minutes.
### Table 7.2

<table>
<thead>
<tr>
<th>Elapsed Time</th>
<th>Predominant color of undamaged banana skin</th>
<th>Predominant color(s) of damaged banana skin</th>
<th>Which areas show benzoquinone present?</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dispose of the banana in the regular trash.

### Part 4: Isolating the Effects of Catechol on a Chemical Reaction

Now you will be using potato juice, which is ground up potatoes (and potato cells). Remember that plant cells contain the enzyme polyphenol oxidase. Although plant cells also contain catechol, you will add extra catechol in order to be able to observe a stronger reaction in these tests.

**Note the Health Warning label on the catechol. Use proper caution.**

12. Label three test tubes 1, 2, and 3 and also add a unique mark for your group to each tube. Add the following to the test tubes.

   - Tube 1: 2 mL catechol solution
   - Tube 2: 2 mL potato juice
   - Tube 3: 1 mL potato juice
     1 mL catechol solution

13. Complete the “Contents” and “Beginning color” columns in Table 7.3

14. Place all three tubes in a 37°C water bath and set the timer for 10 minutes. Complete Table 7.3 after 10 minutes.
15. In this reaction a positive test for benzoquinone is usually a yellow/gold/reddish brown color.

<table>
<thead>
<tr>
<th>Table 7.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

16. Why was test tube 1 included? (What is it designed to illustrate?)

17. Why was test tube 2 included? (What is it designed to illustrate?)

18. Why was test tube 3 included? (What is it designed to illustrate?)

Dispose of all disposable pipettes in this lab in the regular trash.

Place the liquids in tubes 1 and 2 (containing catechol) in the waste disposal area in the lab.

Rinse tube 3 in the sink with plenty of water.
Part 5: The Effects of pH on Enzyme Activity

19. Get five clean test tubes and label them “Acid A,” “Acid B,” “Water,” “Base A,” and “Base B” and also add a unique mark for your group to each tube.

20. Add the following to the tubes.
   - **Acid A:**
     - 1 mL potato juice
     - 2.5 mL acetic acid (0.3N)
   - **Acid B:**
     - 1 mL potato juice
     - 0.25 mL acetic acid (0.3N)
     - 1 mL water
   - **Water:**
     - 1 mL potato juice
     - 1 mL water
   - **Base A:**
     - 1 mL potato juice
     - 0.75 mL sodium hydroxide (NaOH)
     - 1 mL water
   - **Base B:**
     - 1 mL potato juice
     - 2.5 mL sodium hydroxide (NaOH)

21. Vortex the tubes to mix the contents, and then determine the pH using pH paper. Record the “Beginning pH” and “Beginning color” for each tube in Table 7.4.

22. Add 1 mL catechol (*be careful!* to each tube and swirl them (by hand, not with the Vortex mixer) to mix. Immediately place all the tubes in the 37°C water bath.

23. After 10 minutes, remove the tubes from the 37°C water bath and complete Table 7.4.
<table>
<thead>
<tr>
<th>Tube</th>
<th>Beginning pH</th>
<th>Color after 10 min in the water bath</th>
<th>What does the final color indicate about how much benzoquinone is present in the tube after 10 min in the water bath? Use the following terms: none, just a little, some, lots.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dispose of the pH papers in the regular trash.

Place all the liquids in the waste disposal area in the lab.

24. a) In which pH range did the enzyme function best?

b) How could that information be applied to the environments in which living cells (in general) function best and maximize the effectiveness of their enzyme activity?
25. When preparing food, what are three common techniques for preventing fruits such as bananas, apples, or avocados from turning brown if they are not to be served immediately?

26. Explain why the methods you listed in the question above would work.

27. Explain why the cut surface of an apple left in the air at room temperature turns brown.

The End!
LAB 8: Osmosis & Dialysis

**PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves**

**Osmosis** is a type of diffusion in which water moves across a selectively permeable membrane. A selectively permeable membrane is one that has passages through it which allow some substances to pass through but not others. During osmosis, as in all diffusion, the net movement of water in a solution will be from a region of higher concentration of water to a region of lower concentration of water. This should remind you of what happened with the gold and silver beads in Lab 6.

One reason that people sometimes have difficulty remembering how water flows across a membrane, as a result of osmosis, is that we usually focus on the concentration of what is dissolved in the water (the solutes) but rarely do we remember that water molecules, too, are at different concentrations depending on how much of the solute is dissolved in it. The more solute there is, the less room there is for water in the same space.

**Timing note:** Observe the osmometer for Part 1 first, then set up Part 2. You can work on Part 3 while the dialysis bags sit in the beaker for an hour. Be sure to keep track of the time.

**Part 1: Osmosis in an Osmometer**

An apparatus called an osmometer has been set up for the entire class to observe. It consists of a glass thistle tube which is open at both ends, suspended upside down and immersed in a beaker of distilled water. A solution containing a 40% (or greater) colored glucose solution was placed in the larger end of the thistle tube. Dialysis tubing holds the glucose solution in the thistle tube.

1. Observe the osmometer at 30 min intervals and complete Table 8.1.
2. When your observations are finished, explain what caused these results.
Table 8.1

<table>
<thead>
<tr>
<th>ELAPSED TIME (min)</th>
<th>HEIGHT OF LIQUID COLUMN FROM STARTING POINT (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>START</td>
<td>xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx</td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

Part 2: Osmosis with Dialysis Bags

Like the plasma membrane of a cell, dialysis tubing is a type of selectively permeable membrane. There are microscopic holes, or pores, in the dialysis tubing which allow substances to move from one side of the tubing to the other side if their molecules are small enough to fit through the pores. Molecules which are smaller than the pores pass freely across the tubing, but larger molecules are not able to cross the membrane. This can result in different materials being separated, resulting in different concentrations on the two sides of the membrane.

Dialysis tubing can be ordered from scientific supply companies in a variety of pore sizes. Dialysis is routinely used in biochemistry and molecular biology laboratories to separate and purify substances in complex mixtures. In people whose kidneys no longer function (kidney failure), dialysis is also used to remove toxic waste products from the blood for disposal, which is one of the main functions of healthy kidneys.

In this part of the lab, you will determine which molecules in various solutions are small enough to pass through the dialysis tubing. In addition, you will determine how the relative concentrations of various solutes, and their ability to pass through the membrane, affects the process of osmosis and dialysis.

Part 2-A: Make Your Own Dialysis Bags

Always wear gloves when handling dialysis tubing! Oils from your fingers may plug up the pores and ruin the tubing.

3. **Submerge and soak** 5 flat precut lengths of dialysis tubing in a small beaker of distilled water for at least 3 minutes.

4. Label five folded paper towels 1, 2, 3, 4, and 5 and lay them on your lab table.

5. Label five 200 mL beakers 1-5.
Prepare the beakers as follows. Label the beakers in Table 8.2 with these contents.

- **Beaker 1** - 150 mL distilled water (dH₂O)
- **Beaker 2** - 150 mL 10% NaCl solution
- **Beaker 3** - 150 mL distilled water (dH₂O)
- **Beaker 4** - 150 mL distilled water (dH₂O)
- **Beaker 5** - 150 mL 40% glucose solution

6. Make five dialysis bags in the following way.

- Remove a section of tubing from the distilled water.
- Place a plastic clip across the bottom of the tubing. Leave about ½ cm of tubing on one side of the clip, with the rest of the tubing on the other side of the clip.
- Separate the sides of the tubing from one another, so that it forms a tube - this is the hard part! Add 10 mL of solution (see the list below) to the bag with a 5 or 10 mL graduated pipette. Fill the bags from the bottles of solutions.
- Label the drawings in Table 8.2 with the specific solutions and their percentages.
- Use a clean 5 or 10mL graduated pipette for each of the different solutions.
- Place a plastic clip across the top of the bag, leaving about ½ cm of tubing sticking out beyond the clip, leaving little or no air bubble in the bag.
- Place each bag on the proper paper towel, #1-5.

- **Bag 1** - 10 mL 40% glucose solution
- **Bag 2** - 10 mL 10% NaCl solution
- **Bag 3** - 10 mL 10% NaCl solution
- **Bag 4** - 10 mL 1% boiled starch solution (already boiled)
- **Bag 5** - 10 mL 1% boiled starch solution (already boiled)

7. After you have filled all of the bags, gently blot them completely dry, including the grooves in the clips. Place a plastic weigh boat on the electronic balance and tare the balance. Weigh each of the bags (in grams check your balance), round the weight to the nearest 10th of a gram, and record the weights in Table 8.2.

**CAUTION**

Hazardous Waste

Place the used graduated pipettes in the glass disposal area in the lab.

Part 2-B: Testing for Osmosis with Your Dialysis Bags

8. Place all the bags in their corresponding beakers *at the same time*. Be sure that each bag is covered with liquid . . . remember not to touch the bags with your fingers! Leave the bags in the beakers for 1 hour.
Consider which solutions are in each bag and each beaker. Predict what will happen to the weight of each bag during the 1 hour that it sits in the beaker solution. Complete Table 8.3 now.

<table>
<thead>
<tr>
<th>Bag 1</th>
<th>Initial weight</th>
<th>Weight after soaking in beaker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bag 2</th>
<th>Initial weight</th>
<th>Weight after soaking in beaker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bag 3</th>
<th>Initial weight</th>
<th>Weight after soaking in beaker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bag 4</th>
<th>Initial weight</th>
<th>Weight after soaking in beaker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bag 5</th>
<th>Initial weight</th>
<th>Weight after soaking in beaker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. Consider which solutions are in each bag and each beaker. Predict what will happen to the weight of each bag during the 1 hour that it sits in the beaker solution. Complete Table 8.3 now.
Table 8.3

<table>
<thead>
<tr>
<th>Bag Number</th>
<th>Predictions - Check one choice for each bag.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight will increase</td>
<td>Weight will decrease</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do Part 3 of this lab now, but set your timer for one hour so that you remember to return to the dialysis bags on time.

10. After one hour, remove all the bags at the same time and place them on the paper towels according to their numbers. Do not unclip the bags. Gently blot them dry, including the grooves in the bag clips, so that there is NO liquid in the clips or on the tubing.

11. DO NOT discard the beaker solutions yet.

12. Weigh each of the bags and record the weights in Table 8.2, and then complete Table 8.4.

Table 8.4 Weight Change Summary

<table>
<thead>
<tr>
<th>Bag</th>
<th>Check One Box for Each Bag</th>
<th>Amount of weight change (g) rounded to nearest 10th g *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight increased more than 0.4g*</td>
<td>Weight stayed within 0.4g of original weight*</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = Small weight changes, up to about 0.4 g, may be due to incomplete blotting of the bags and clips, or just to the random movement of materials across the tubing, and not due to an actual effect of concentration differences.

13. Have your professor check your data in Tables 8.2 and 8.4.
A Review of Indicator Tests Which You Have Already Learned

These are tests that you learned in previous labs. Use these as you need to now.

**Simple Sugar Test:** Place 2 mL of the solution to be tested in a test tube. Add 5 drops of Benedict’s solution. Place the test tube in a boiling water bath for 2 minutes. A color change to green, yellow, orange, red, or brown indicates presence of simple sugar, e.g., glucose.

**Starch Test:** Place 3 drops of the solution to be tested into the well of a spot plate. Add a drop of Lugol’s iodine. A blue-to-black color indicates the presence of starch.

**Salt (NaCl) Test:** Place 2 mL of the solution to be tested in a test tube. Add five drops of silver nitrate solution. Formation of a white precipitate indicates the presence of sodium chloride (NaCl).

Place the liquids from all of these tests in the waste disposal area in the lab.

Follow your professor’s instructions concerning what to do with the test tubes.

14. Do tests to see if glucose crossed the dialysis tubing of bag 1. *Add your new data to Table 8.5.*

15. Do tests to see if NaCl crossed the dialysis tubing of bag 3. *Add your new data to Table 8.5.*

16. Do tests to see if starch crossed the dialysis tubing of bag 4. *Add your new data to Table 8.5.*

When you are certain that you are finished, dispose of the liquids by washing them down the drain with plenty of water.

Place the empty used dialysis tubing in the regular trash.

SAVE THE PLASTIC CLIPS FROM THE BAGS!
### Table 8.5

<table>
<thead>
<tr>
<th>Bag</th>
<th>What happened to cause the weight change? Do not just say &quot;osmosis (or diffusion or dialysis) occurred.&quot; Explain which molecules moved through the tubing and why they moved.</th>
<th>What evidence (data) do you have to support your explanation?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For bag 5, focus on the water concentrations inside and outside of the bag.
Part 3: Observing Osmosis in Living Elodea Cells

The saturated NaCl solution on your table is **hypertonic** relative to the inside of a living cell, but the dH₂O is **hypotonic** relative to the inside of a living cell.

*See illustrations in your text concerning cells in hypertonic and hypotonic solutions.*

Look at the plant cell model in the lab as well during this lab.

17. **Each student** will use their own microscope and make a wet mount of an *Elodea* leaf (use water from the *Elodea* container if you need water - do not use dH₂O). Focus clearly on the leaf at **low power**, then work your way up to using the 40X objective. **Find one rectangular cell near the edge of the leaf** for a clearer view.

18. Observe **one cell** at 40X. You should be able to see the chloroplasts and cell wall clearly.

Watch the cell long enough and see if the **chloroplasts** drift slowly around inside the cell. This will help you become familiar with the cell’s **normal** overall appearance.

**Draw one rectangular *Elodea cell*** in the space below. Make it as large as possible. Label the following: cell wall, chloroplasts, and central vacuole.

19. Now you are going to flood the cells with a **hypertonic salt solution**.

   ▶ Remove the slide from the microscope.
   ▶ Remove the cover slip.
   ▶ Blot away any water from the slide, *Elodea*, and cover slip with a Kimwipe®.
   ▶ Place a drop of 10% NaCl solution on the slide with the *Elodea* and put the cover slip on it.
   ▶ Wait for **5 minutes**.
   ▶ Return the slide to the microscope and focus again, starting with the lowest power and working up to the 40, 43, or 45X objective.
   ▶ Find one rectangular cell near the edge of the leaf for a clearer view.

20. **ASK your professor to look at your slide.**
21. **Draw one cell** as it looks now with a 10% NaCl solution. Label the cell wall, plasma membrane, chloroplasts, and central vacuole.

22. What appears to have happened inside the cells after the 10% salt solution was added?

23. Explain **why** this happened.

   **Hint:** focus on the water concentration not the salt concentration.

Dispose of the *Elodea* leaf and liquids by washing them down the drain with plenty of water. Follow instructions in the lab concerning the slides and cover slips.
Questions for Lab 8

24. How would the response of the Elodea cells to a hypertonic solution compare to the response of animal cells to these solutions? Explain the reasons for the differences.

25. How would the response of plant cells to a hypotonic solution compare to the response of animal cells to these solutions? Explain the reasons for the differences.

26. How is the plasma membrane of a cell like dialysis tubing?

27. How is the plasma membrane of a cell different from dialysis tubing?

The End!
LAB 9: Photosynthesis & Aerobic Cellular Respiration

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

For most living things on this planet, energy for life requires carbohydrates, and carbohydrates are made by sunlight shining on plants and other green living things. Plants use the energy from sunlight to join the hydrogen atoms from water to carbon dioxide from the air and make carbohydrates. The rest of the water molecule, the leftover oxygen, escapes into the air we breathe. This process is called photosynthesis.

***** Set up Parts 3 (#1-4) & 4 (#1-8) one week in advance. *****

Part 1: Thin Layer Chromatography

In plants, the overall leaf color which you see is due to a mixture of several different pigments in the leaf cells. These different pigments are involved in the process of photosynthesis. Thin layer chromatography (TLC) is an easy way to separate the pigments from each another.

To perform thin layer chromatography, a concentrated solution of plant extract is placed on a white chromatogram strip. The strip is then placed in a liquid solvent which will spread up the strip by capillary action. As it spreads up, the different pigments will dissolve in the solvent and will be carried along with the solvent as the solvent moves up the chromatogram strip.

The solvent you will use is a mixture of acetone and ether. The pigments in plant extract dissolve in the solvent at different rates. The more easily a pigment dissolves, the more quickly that pigment will move up the strip, and the farther up the strip it will travel in a given amount of time. The result is that the different pigments move at different rates and become separated from each other into different bands of color. When you are done, each color band shows where a certain pigment, from the mixture of pigments, ended up.

Practicing Your TLC skills

1. While wearing gloves, each student should obtain a “practice” chromatogram strip, holding the strip only by its edges. Note that there is a dull side and a shiny side to the strip. If you touch the dull side, oils from your fingers will interfere with the chromatography process.
2. Lay a strip on the lab table. Use a pencil to make a very light line side-to-side across the strip (on the dull side). This is just a reference mark. Be careful not to scrape the coating off the strip.

3. Your goal is to add concentrated green spinach extract to the chromatogram strip so that it makes a stripe of pigment on the pencil line. Do NOT put the pigment all the way to the edges of the chromatogram strip.

This pigment is like SUPER grass stain if it gets on your clothes, so be careful.

4. Tip the vial and place the end of a capillary tube in the spinach extract (do not put your finger over the other end). Gently sweep the capillary tube along the pencil line. You should have a green stripe now. If you rub or scrape the coating off of the strip, this lab will not work!

5. Have everyone in your lab group try this over and over to see who has the best technique. Repeat this practice until you get the hang of it. Have your professor approve your practice strip before you proceed.

Put the used practice chromatogram strips in the regular trash.

TLC for Real

6. Obtain a chromatogram strip holding it only by its edges. The strips may be in a desiccator, which keeps them dry. They work better if they are very dry.

7. On the dull side of the strip, make small very light pencil marks on both the right and left edges of the strip 3 cm from bottom of one end of the strip. Then add a stripe of pigment from one pencil line to the other.

8. Repeat, adding more pigment on top of what you have already added to the chromatogram strip 20 times, until you have a very dark green stripe. This is tedious and takes a while, so be patient!

Put the used capillary tubes in the glass disposal.

9. This is a good time to set up Part 2 of this lab while you finish adding the pigment.

Caution! The solvents in the jar you are about to use are toxic and flammable. Do not put the solvents near a heat source or breathe the fumes. Keep the lid on the jar tightly except when adding or removing the chromatogram strip.
10. Place the chromatogram strip into the jar containing the solvent by quickly removing the lid, placing the strip (with the green stripe at the bottom) in the jar so that the pigment stripe is easily visible, and replacing the lid.

The green stripe should be just above the level of the solvent. If the stripe is submerged in the solvent, your pigments will simply float away into the liquid and you will have to start over!

11. Leave the strip in the jar for 10-20 minutes, and watch the reaction as it occurs. This is a very colorful reaction! Read the next instruction to know when to proceed.

12. Take your jar (developing chamber) to the fume hood and remove the strip from the jar before the fastest moving pigment reaches the top. Replace the lid on the jar and leave the jar under the fume hood. Allow the strip to dry in the fume hood for 3 minutes.

CAUTION

HAZARDOUS WASTE

Leave the solvents in the jar. The staff will dispose of them properly

Put the used capillary tubes in the glass disposal.

13. Now you can handle the strip safely with your bare hands and take it back to your lab table. Find the different color bands and compare them to the colors in Table 9.1.

<table>
<thead>
<tr>
<th>Table 9.1 Spinach Pigments and Colors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigment</td>
</tr>
<tr>
<td>chlorophyll a</td>
</tr>
<tr>
<td>chlorophyll b</td>
</tr>
<tr>
<td>beta-carotene</td>
</tr>
<tr>
<td>pheophytin</td>
</tr>
<tr>
<td>xanthophyll</td>
</tr>
<tr>
<td>Pigment Color</td>
</tr>
<tr>
<td>blue-green</td>
</tr>
<tr>
<td>yellow-green</td>
</tr>
<tr>
<td>orange/dark yellow</td>
</tr>
<tr>
<td>gray/olive green</td>
</tr>
<tr>
<td>light or bright yellow</td>
</tr>
</tbody>
</table>

14. Attach the chromatogram strip to your lab report in the space below, or cover the colored side with a piece of transparent tape and then cut it lengthwise so each member of your group has a piece with all of the color bands, or draw a life-size picture of it below. Label the pigments with their names (not their colors).
15. Which pigment dissolved most easily in the acetone/ether solvent? How can you tell?

Part 2: Producing Oxygen Gas During Photosynthesis

Remember that the equation for the process of photosynthesis is as follows.

\[ 6\text{CO}_2 + 12\text{H}_2\text{O} + \text{light energy} \rightarrow 6\text{O}_2 + \text{C}_6\text{H}_12\text{O}_6 + 6\text{H}_2\text{O} \]

16. Obtain a 250 mL beaker and add 200 mL of NaHCO\textsubscript{3} (sodium bicarbonate) solution, which is NaHCO\textsubscript{3} dissolved in H\textsubscript{2}O. When sodium bicarbonate dissolves in water, it produces CO\textsubscript{2}, which also will dissolve in water.

17. Place a freshly cut 10 cm long sprig of \textit{Elodea} into a large test tube, placing the stem side down. With two people working together over the sink, fill the tube carefully with NaHCO\textsubscript{3} solution from the beaker until the level of the liquid is higher than the top of the tube. (Yes, really.) Slide a 4" wide piece of plastic wrap (not Parafilm) across the top of the tube so that there is no air bubble bigger than 5 mm wide at the top, and hold it around the tube so no air can enter the tube.

18. Hold the tube over the beaker, with the plastic wrap over the mouth of the tube and carefully turn the tube upside down, placing the plastic wrap and mouth of the tube into the solution. Remove the plastic wrap and rest the tube, upside down, and lean it against the side of the beaker. Ideally, there should be no air in the test tube (or only a small bubble).

If there is a bubble, mark an outline of it with your marker, measure the diameter of the bubble, and record the measurement in Table 9.2.

19. Bright lights have been set up in your lab room. With the lights on, place your tube containing the \textit{Elodea} in front of one of the lights.

20. Note the time__________.

21. After \textbf{25 minutes} (or longer), go watch the bubbles in your tube without disturbing the tube. Where do bubbles appear \textbf{first}?

Where do most of the bubbles go after they form?

22. What do you \textbf{think} the bubbles contain? __________________________
23. After a total of **60 minutes**, note the time___________.

Measure the diameter of the bubble in the test tube and record your data in Table 9.2.

<table>
<thead>
<tr>
<th>Table 9.2 Bubble Size in <em>Elodea</em> Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME</td>
</tr>
<tr>
<td>START</td>
</tr>
<tr>
<td>END</td>
</tr>
</tbody>
</table>

24. Explain why the size of your bubble increased.

Dispose of the liquids by washing them down the drain with plenty of water. Put the *Elodea* in the *Elodea* recovery container.

**Part 3: Do Plant Leaves Make Carbohydrates?**

25. Obtain a piece of black paper 4 cm by 8 cm, which has a shape cut out in already. Fold it in half so that it is 4 cm x 4 cm square. Write your group name or symbol on the black paper with a gel pen.

26. Take your paper to the growing plant that has been provided for your class. Locate a good-sized, healthy-looking leaf on the plant near the end of the stem, but do not remove the leaf from the plant or damage the leaf in any way. Place the piece of paper on the leaf with the hole on the top side of the leaf and the solid half on the bottom of the leaf. Use only one paper per leaf.
27. Place paper clips or tape on each side of the paper. Your goal is to allow light to strike the leaf only through the hole that you cut in the paper and do not let light leak in around the edges of the paper.

28. When all the students using this plant have attached their paper, place the plant under bright lights. Leave it under the lights for at least one week.

29. What kind of plant are you using?

30. While the leaf is photosynthesizing, which food molecules will it make?

31. Which organic compound molecules do plants use for long-term storage of their unused food?

***** At Least One Week Later *****

32. Cut your entire leaf off of the plant, and remove the paper (and any clips or tape). Draw your leaf and label the areas of different colors (look carefully). Different colors may not be visible.

33. The green chlorophyll in leaves dissolves in alcohol. Boil the leaf very gently in a 250 mL beaker of alcohol until the leaf is white, with possibly green color still in the veins.

34. The leaf will be very brittle now. Use the large forceps to remove your leaf gently from the alcohol and place it in a dish. Ask your professor to place the beaker of alcohol in the fume hood.

35. Add enough Lugol's iodine to cover the leaf completely, and leave it for 5 minutes.
36. The leaf will no longer be brittle but it will tear easily. Remove the leaf from the dish, put it on a paper towel, and spread it out with your gloved hands. **Draw your leaf and label where starch is found and where it is not found.**

![CAUTION HAZARDOUS WASTE]

*Place the liquids in the waste disposal area in the lab.*

37. Compare your two leaf drawings and answer the following questions.

After treating the boiled leaf with Lugol’s solution, **the dark areas indicate** the presence of _____________________.

Explain what happened over the past week to cause this material to be present in the leaf.

**Lighter areas indicate** the absence of _____________________. Explain what happened over the past week to cause this material to be absent there. **Be careful,** this answer may be more involved than it seems to be. Don’t just say it is because of no photosynthesis.

![Dispose of the leaves and paper by throwing them in the regular trash in the lab.]

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Part 4: What Happens to CO₂ During Photosynthesis and Aerobic Cellular Respiration?

Remember that when cells use oxygen to break down carbohydrate food molecules during the process of aerobic cellular respiration the following reaction occurs within those cells.

\[ 6O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O \]

Also, when carbon dioxide dissolves in water, such as when it is released from cells into a watery environment, some of the CO₂ reacts with the water molecules to form carbonic acid (which is acidic), as illustrated below.

\[ CO_2 + H_2O \leftrightarrow H_2CO_3 \]

This reaction is reversible, which means the arrows could point in either direction. That means that carbon dioxide and water can form carbonic acid, and also that carbonic acid can break down into carbon dioxide and water.

You will be using Phenol red, which is an indicator solution that has a red or pink color in a solution that is neutral or basic/alkaline, and is yellow in a solution that is acidic.

38. Obtain two large test tubes. Label one L and the other D. Fill each one ⅔ full of tap water (not dH₂O). Add 6 drops of phenol red to the water in each tube, mixing with a vortex mixer (hold the tube at a slight angle for better mixing), to turn the solution a dark pink color.

If the water seems yellow or orange instead of pink, add dilute NaOH (sodium hydroxide), which is alkaline, to your tube, mixing it one drop at a time, until the solution in the tube turns pink. This is usually not necessary since Austin water tends to be alkaline, or basic.

39. Cut the top off of a disposable pipette, put the narrow end into the L test tube, and very gently blow bubbles into the phenol red solution. If you blow too hard, the liquid will splash out of the tube. Continue to do this until the solution turns yellow.

40. Explain why the color of the water changed.

41. Place a piece of Elodea, about 10 cm (4”) long, into each test tube.
42. Look at the color of the water in both tubes and **estimate** the pH of the water (acidic, neutral, or alkaline). Record your estimate in Table 9.3.

43. Seal the top of each tube with **Parafilm®**.

44. **Completely** cover the **D** tube with aluminum foil so that NO light can reach the *Elodea*. Label the foil with your group name or symbol. Place the foil-covered tube in a dark cabinet. Leave it in the dark for at least 2 days.

45. Label the **L** tube with your group name or symbol, and place it under a bright light. Leave it there for at least 2 days.

| Table 9.3  Changes in Light and Dark Tubes |
|-----------|------------------------------------------|
| **Tube**  | **Liquid on the day it is set up**         | **Liquid at least two days later** |
|           | **Color when being placed in the light or dark** | **pH estimate when being placed in the light or dark** | **Color when removed from the light or dark** | **pH estimate when removed from the light or dark** |
| **L**     | (in the light)                             |                                      |                                      |                                      |
| **D**     | (in the dark)                              |                                      |                                      |                                      |

**At Least Two Days Later**

46. Retrieve your two test tubes and complete Table 9.3.

47. State whether the pH of the water increased, decreased, or stayed the same in the

   **L** tube ________________________________.

   **D** tube ________________________________

48. Remember that the reaction shown at the beginning of Part 4 is a reversible reaction. Explain what took place in the **L tube** to cause the pH of the water to increase, decrease, or stay the same.

49. Explain what took place in the **D tube** to cause the pH of the water to increase, decrease, or stay the same.
Place the liquids in the waste disposal area in the lab.

Put the *Elodea* in the *Elodea* recovery container.

50. Rinse your D and L tubes in the sink and return the tubes to your tray.

51. In terms of the chemical reactions that release energy in living things, how are you and a plant the same?

52. What is different about the ways you and a plant store energy?

**Part 5: Survival of Aquatic Producers and Consumers**

*Producers* are living organisms which can produce (make) their own food by photosynthesis. *Consumers* are living organisms which cannot produce their own food by photosynthesis.

This part of Lab 9 is a *simulation* of what would occur if *Elodea* and *aquatic snails* were put into test tubes and left for ten (10) days under different conditions.

Which organism in this simulation is a

- Producer? __________________________
- Consumer? _________________________
The tubes have an indicator solution, bromthymol blue, added to them. Table 9.4 explains the different colors this indicator may show.

<table>
<thead>
<tr>
<th>pH of Solution</th>
<th>Bromthymol blue color</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 or less</td>
<td>yellow</td>
</tr>
<tr>
<td>6-7.5</td>
<td>clear</td>
</tr>
<tr>
<td>7.5 or greater</td>
<td>blue</td>
</tr>
</tbody>
</table>

53. **Assume the following.**

- There are NO other organisms in any of the tubes.
- All organisms were alive and well at the beginning.
- Tubes A1 - A4 were kept in the light for 10 days.
- Tubes B1 - B4 were kept in the dark for 10 days.
- Tubes A4 and B4 have remained the same color all the time.
- The water in all the tubes started out the same color that A4 and B4 are now.

54. **Use a pencil for the rest of this lab.**
### The Setup

<table>
<thead>
<tr>
<th>“A” Tubes --- In the Light</th>
<th>“B” Tubes --- In the Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>B1</td>
</tr>
<tr>
<td>A2</td>
<td>B2</td>
</tr>
<tr>
<td>A3</td>
<td>B3</td>
</tr>
<tr>
<td>A4</td>
<td>B4</td>
</tr>
</tbody>
</table>

55. Draw the eight tubes below. Draw *Elodea* and a snail in each tube as appropriate.

56. Would any of the snails or *Elodea* plants have been **dead on Day 1, one hour** after this was set up, if this had been an actual experiment?

If so, list what would have died, in which tube(s), after one hour.
57. Fill in Table 9.5 according to what **would have been occurring on Day 1, one hour** after this was set up if this had been an actual experiment.

*Write an X in a box to indicate YES, and leave a box blank to indicate NO.*

<table>
<thead>
<tr>
<th>Test Tube</th>
<th>Aerobic Respiration Was Occurring</th>
<th>Photosynthesis Was Occurring</th>
<th>( \text{O}_2 ) Was Being Produced</th>
<th>( \text{CO}_2 ) Was Being Produced</th>
<th>( \text{O}_2 ) Was Being Used</th>
<th>( \text{CO}_2 ) Was Being Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

58. *Have your professor approve your answers above before you continue.*
59. Fill in Table 9.6 according to how things look NOW, after 10 days.

<table>
<thead>
<tr>
<th>Test Tube Number</th>
<th>Water Color Now</th>
<th>Approximate pH (qualitative)</th>
<th>Condition of the Elodea (alive or dead)</th>
<th>Condition of the Snail (alive or dead)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>XXXXXXXXX</td>
<td>XXXXXXX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

60. Have your professor approve your previous answers before you continue.

Complete the following questions and check your answers before you leave class.

61. What do the colors of the water indicate about the pH of a tube?

Yellow = ____________________  Blue = __________________________

What do the colors of the water indicate about the amount of CO\text{2} present?

Yellow = ____________________  Blue = __________________________
62. All *Elodea* and snail deaths were due to the lack of ___________________________.

Because of that, in which tube would the organism(s) have died first?

Why THAT tube, and not a different one? Be Careful!

63. If this experiment was not a simulation and we let it continue, Tube A3 would continue to survive just fine. Explain why, given what you have learned in this lab.

64. Life on our planet depends on the process of ____________________________, which provides both ____________________ and ____________________, BOTH of which are necessary for aerobic respiration. However, organisms called ____________________________ cannot make either of these, so they are dependent on organisms called ____________________________ to make them.

The staff will dispose of the tubes in this demonstration properly, so just leave them where you found them.

The End!
LAB 10: Using a Micropipetter

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

Part 1: The Micropipetter

Your micropipetter is a delicate instrument which allows you to measure precise volumes of liquids and transfer those liquids from one container to another. How much liquid you can measure depends on the capacity of the micropipetter.

Look at the micropipetter(s) on your lab table. There is a colored button (possibly yellow or blue) on the top. The color corresponds to the capacity, and the maximum amount that your instrument will hold is printed on the side. It may say something like 20-200 μL or just 200, which means that you can measure from 20 μL to 200 μL with that micropipetter. Your lab may have three different sizes of micropipetters, which will allow you to make a wide range of possible measurements. If you have forgotten what a μL is, turn to page -iv- in this lab manual to refresh your memory.

♦ Always place a disposable plastic tip on the end of the micropipetter before you use it.
♦ Never let any liquid flow into the micropipetter itself.
♦ Hold the micropipetter upright at ALL times.

Part 2: Transferring Liquids

Your professor will show you how to set your micropipetter so that it takes up the volume of liquid you need. Start with a setting of 25 μL.

1. Have your professor check your micropipetter to be sure it is set correctly.

2. You will find an Eppendorf tube on your table with red water in it. You are going to transfer the red water into an empty Eppendorf tube. Put a tip on the micropipetter by pushing the end of the micropipetter into the top of one of the tips in the box of clean tips. Just pull the micropipetter up and the tip should come along with it. Close the lid on the box of tips.

3. Use your thumb to push down on the colored button on the top of your micropipetter. You should notice that you reach a point where it is harder to push. This is called the first stop. Keep pushing harder and the button will go down further until it really stops. This is called the second stop. Let go of the button now.
4. Open the Eppendorf tube with the red water. Hold the **micropipetter upright** at all times!
   - Before you put the tip into the red water, push the colored button on the micropipetter to the **first stop and hold it** there.
   - Now, insert the micropipetter tip into the Eppendorf tube, almost all the way to the bottom. **Gently and slowly release** the button. Red water will be drawn up into the tip.
   - Slowly raise the tip above the liquid, remove it from the Eppendorf tube.
   - Close the Eppendorf tube and put it away.

5. Look at the tip and you should see 25μL of red water in it. Pretty cool!

6. Now you will **transfer** the 25μL of red water into an empty Eppendorf tube. Hold the **micropipetter upright** all of the time!
   - Open an empty Eppendorf tube, place the tip of the micropipetter inside the Eppendorf tube, and **gently** push the button to the **second stop**, which will eject all of your 25μL into the Eppendorf tube. Keep holding the button down to the second stop.
   - Touch the tip to the side of the Eppendorf tube above the red water, which gets rid of any stray micro-drops at the end of the tip.
   - Remove the micropipetter from the Eppendorf tube, look at the tip to be sure that it is completely empty, and release the button. The 25μL of red water is now in the new Eppendorf tube.
   - Close the lid of the Eppendorf tube.

7. Remove the tip (into the tip disposal container) by pushing the ejector tab on the micropipetter. Your professor will demonstrate this. Note that **you never touched the tip**. This keeps you, the Eppendorf tube, and the liquids from becoming contaminated.

8. Each student in the group should practice this several times. You only need to get a new tip when you use a different liquid.

   *Dispose of the tips and Eppendorf tubes in the regular trash. Leave the liquids in their tubes.*

### Part 3: Loading an Agarose Gel

In some of the lab exercises for this course, you will do a process called gel electrophoresis, in which you will place small amounts of liquid into little rectangular holes (called wells) in an agarose gel (which looks like a piece of clear gelatin). Today you will practice this technique, called **loading a gel** (putting liquid into the wells).

9. Find the **practice gel** for your group. It is submerged in water in order to keep it from drying out, even though this will make your task more difficult. **Add dH₂O** until the container holding the gel is nearly full. You should be able to see rectangular wells near one end of the gel. **These wells do not go all the way through the gel.**

10. Place the dish with the gel on a dark surface, which makes it easier to see the wells.
11. Locate the Eppendorf tube labeled LS (for loading solution). This contains a blue or purple dye so that you can see it. It also contains glycerol, which is heavier than water, so it will sink in water (and in the buffer solution that you will use another time).

12. Set your micropipetter to 20μL and have your professor check the setting.

13. Using the micropipetter, push the button to just the first stop and remove 20 μL of loading solution from the LS Eppendorf tube with a clean tip. Hold the micropipetter upright at all times!

14. This next step requires a very steady hand. It helps if you brace the hand that is holding the micropipetter with your other hand.

15. Carefully place the micropipetter tip just above the leftmost well and lower the tip slightly into the well. If you go too far down you will punch a hole in the bottom of the well and your dye will leak out, which ruins your sample.

16. Gently push the button on the micropipetter to the first stop (going to the second stop creates bubbles which can blow the dye right out of the well, which is another way to ruin your sample). Keep holding the button at the first stop.

17. Raise the tip slowly, so that you do not swirl the dye out of the well (yes, one more way to ruin your sample!), until the tip is out of the liquid. Now you can release the button on the micropipetter.

18. Look at the well. It should look like a purple rectangle and there should be no purple swirls above or below the well. It gets easier with practice!

19. Each student should practice this several times. If you run out of wells, just flip the gel over and let the dye run out.

20. Ask your professor to approve your filled wells.

21. Keep practicing until you get comfortable with this technique. The next time you do this, you will need to do it right the first time!

22. Leave the agar in the dish and leave it on your lab table.

CAUTION HAZARDOUS WASTE

Place the liquids in the waste disposal area in the lab.

The End!
Lab 11: The ELISA Assay

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

The information in this lab is modified from EDVO-Kit #274: In Search of the Kissing Disease, produced by EDVOTEK, Inc. Diagrams used with permission.

Lab 10 must be completed before doing this lab.

Part 1: The ELISA Assay (or Test)

This type of test, or assay, involves a chain reaction (see Fig.11.2 in this lab) in which materials stick to one another in a specific sequence, resulting in a color change if what you are testing for is present. It is called an enzyme-linked immunosorbent assay, or ELISA.

The techniques you will learn in this lab exercise are used as clinical tests (or assays) for many different infections, including mononucleosis (mono), HIV, Japanese encephalitis, tuberculosis, and West Nile virus. An ELISA assay can determine if patients currently have, or have had, the disease. It is also used to test for food allergies, and for the presence of hormones and various other proteins.

Mononucleosis is caused by the Epstein Barr Virus (EBV), and today you will simulate an ELISA assay for mononucleosis. Blood serum (the liquid part of blood) from patients can be used to test for EBV antibodies because the antibodies that infected patients produce against EBV, can be found in human blood serum.

All cells and viruses have exterior recognition proteins called antigens (Ag), which give the cell its identity and make it recognizable by other cells in your body. When your body detects “foreign” antigens (exterior proteins on bacteria, viruses, pollen, cells from an organ transplant, etc.) it tries to destroy them. One step that your body takes is to make specialized proteins called antibodies (Ab) which attach to the foreign antigens. This causes the foreign cells, etc., to clump up and your body can destroy them more easily.

In this lab exercise you will be testing serum from four different patients to determine whether or not they test positive for EBV, which would mean that they have EBV antigens because they have mono now, or have had it or been exposed to it in the past. If the patient has ever produced antibodies to attack EBV, there will still be some of these antibodies in their blood serum. Also included are two controls. Well 1 is the negative control without EBV antibodies, and well 2 is the positive control with EBV antibodies.
Part 2: Testing Serum Samples

1. Find your small plastic vials called **microtiter wells** (Figure 11.1). The wells have been attached to a weigh boat to stabilize them. Label the weigh boat to identify it as yours, and number the wells 1-6 from left to right.

   ![Microtiter Wells](image)

   **Figure 11.1 - Microtiter Wells**

2. Use a **micropipetter** when adding reagents to the wells (except for PBS).

3. Set the micropipetter to **50 \( \mu L \)** and have your professor check the setting.

4. Put a **clean tip** on the micropipetter.

5. Add 50 \( \mu L \) of EBV to all six wells. The EBV solution contains EBV antigens, and is labeled “Antigen” in Fig. 11.2 in this lab handout. **Be very careful** how you add materials to the microtiter wells so that you do not splash, spill, or move materials from one well into a different well. That would contaminate the wells and your final results will be inaccurate. Patients and doctors get really upset when that happens!

6. What color is the EBV solution? _______________________________

7. Let it sit for **5 minutes**. Because of the nature of the plastic, the **EBV antigens** that you just added will stick to the plastic microtiter wells.

8. Set the micropipetter to **55 \( \mu L \)**, and use one tip to remove the EBV from all 6 wells. **Place the liquids in the waste disposal area in the lab.**

9. **PBS** (Phosphate Buffered Saline) will be in a small beaker or flask labeled “PBS.”

   What color is the PBS solution? _______________________________

10. Label a disposable transfer pipette “PBS.” You will use this for adding PBS to the wells as a way of rinsing out the wells.
11. Now you are going to rinse out each well and also remove any remaining unattached EBV antigens.

12. Wash each well once with PBS by adding enough PBS with the PBS disposable transfer pipette to fill each well slightly more than \( \frac{3}{4} \) full. Remember, do not let any liquids from one well get into another well!

Now remove the PBS with a micropipetter set at 150\( \mu \text{L} \). Use one tip for all 6 wells. Since there is probably more than 150\( \mu \text{L} \) of PBS in each well, just keep removing the PBS until the well is empty.

**Place the liquids in the waste disposal area in the lab.**

In the next steps you will be adding several materials to the microtiter wells, in a very specific sequence. Look at Figure 11.2, called “Schematic for ELISA” and read it from the bottom upward to see the sequence of materials you will use in the following instructions.

13. The liquids listed in Table 11.1 from each patient are also the primary antibodies from that patient (see Fig 11.2). Add the positive control and serum from the four patients to different wells as indicated in Table 11.1. Set the micropipetter to 50\( \mu \text{L} \). Use a new tip for each well. Do not add anything to well #1 in this step.

<table>
<thead>
<tr>
<th>Eppendorf Tube Label</th>
<th>Liquid in the Eppendorf Tube</th>
<th>Color of the liquid</th>
<th>Put Liquid Into Well Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>Positive Control</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>Patient A serum</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>Patient B serum</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>Patient C serum</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>Patient D serum</td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

14. Write the color of each liquid in Table 11.1.

15. Incubate the wells in a 37\( ^\circ \text{C} \) incubator (not the water bath!) for 15 minutes.

16. Remove the liquids from wells #2-6 with the micropipetters as before, with a clean tip for each well.
17. Why use a clean tip for each well?

18. Wash each well with PBS as before but use a clean tip to empty each well.

19. Next, add artificial secondary antibodies to all six wells from the Eppendorf tube labeled “2° Ab.” These secondary antibodies are made in a specific shape that allows one end of the molecules to stick only onto EBV antibodies (called primary antibodies in Figure 11.2). Add 50 μL of 2° Ab to each well using one tip. Be careful not to touch the tip to any of the wells.

20. Incubate the wells as before, in the incubator, for 15 minutes.

21. Remove the liquids from all six wells as before with a micropipette, with a clean tip for each well.

22. Wash each well with PBS as before, but use a clean tip to empty each well.

23. Lastly, add substrate from the Eppendorf tube labeled “SUB” to all six wells. Substrate is a mixture of chemicals that turn color, but only if there are secondary antibodies already present that the substrate can stick to. Everything in the chain reaction has to be correct or there is no color change! Look at Figure 11.2 again.

Add 50μL of SUB to each well. Use one tip. Do not touch the wells.

24. Incubate the wells as before, in the incubator, for 15 minutes.

25. Remove the wells from the incubator. Look at the color of each well. If there is no distinct color visible in any wells, incubate them for another 5 minutes, continuing until there is a distinct color in at least one well.

26. Show your results to your professor.

27. Remove the liquids as before but use the same tip for all six wells.
28. Record your results in Table 11.2 and complete all the information in Table 11.2.

29. Leave the weigh boat and microtiter wells with your other lab materials.

30. Explain why the **positive control** was necessary.

31. Explain why the **negative control** was necessary.

Watch the following YouTube video for a summary of what happens in an ELISA assay like the one you just completed. [http://www.youtube.com/watch?v=RRbuz3VQ100](http://www.youtube.com/watch?v=RRbuz3VQ100)
<table>
<thead>
<tr>
<th>Well Number</th>
<th>Unique Material Added to Just This Well</th>
<th>Final Color</th>
<th>Are EBV Antibodies Present?</th>
<th>What does this result indicate about the patient’s medical history / present condition?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>XXXXXXX XXXXXXX</td>
<td></td>
<td>XXXXXXXXXXXXXXXXXXXxxxxxxx</td>
<td>XXXXXXXXXXXXXXXXXXXxxxxxxx</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>XXXXXXXXXXXXXXXXXXXxxxxxxx</td>
<td>XXXXXXXXXXXXXXXXXXXxxxxxxx</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The End!
LAB 12: Making New Cells by Mitosis & Cytokinesis

Part 1: From One Nucleus to Two Nuclei

Every cell uses its own set of the genetic codes found in DNA to survive and function. In eukaryotic cells, every cell has a nucleus which contains the DNA.

The life span of a cell is marked by changes in structure and function described by the cell cycle.

Review the stages of the cell cycle in your text.

Stage G1 of the cell cycle is the stage during which a cell carries out its ordinary activities. At some point during the G1 stage, the cell may receive a chemical signal to divide into two cells. If so, it begins to collect and organize the raw materials needed for making a copy of its DNA. As the cell begins the actual DNA replication, it enters the S stage of the cell cycle. Copying DNA is called DNA duplication or replication.

Once all of a cell’s DNA has been copied, the cell shifts to stage G2. During G2, the cell is marshaling its resources so that it can distribute the duplicate chromosomes in such a way that each new nucleus has a copy of every chromosome.

The complicated choreography of moving chromosomes around to ensure that each new nucleus will have a copy of each chromosome, as well as disassembling the nuclear envelope and then reassembling two new nuclear envelopes, is called mitosis.

Mitosis is the process of making duplicate nuclei. Once there are two nuclei in a cell, the cell can divide into two cells, and each of the resulting cells will have its own nucleus. Note that the dividing of the cell is not mitosis. The process by which the cell actually divides into two cells is called cytokinesis.
Part 2: Chromosome Duplication

Review chromosome duplication in your text.

In this lab you will simulate what happens in the cells of a flowering plant called a Grass-Leaf Tansy-Aster (*Machaeranthera gracilis*). They are related to sunflowers and live in desert areas from far west Texas along the western USA into Canada, and grow to 25 cm tall (http://www.home.earthlink.net/~christrask/MacGra01.pdf). Their cells have a total of 4 chromosomes, which makes its diploid number 2n = 4.

1. Obtain 90 yellow pop beads, 90 red pop beads, 8 “magnets in tubing,” 8 small hollow plastic tubes, meter stick, string, and tape.

2. Join pop beads together to make a string of 20 red pop beads and a separate string of 20 yellow pop beads. Together, these represent the pair of unduplicated chromosomes called **Chromosome 1** of the Grass-Leaf Tansy-Aster. See Figure 12.1 and Table 12.1.

3. Make a string of 25 red pop beads and another string of 25 yellow pop beads. Together, these represent the pair of chromosomes called **Chromosome 2**. See Table 12.1.

Since there are two strings of beads for each type of chromosome, is the nucleus of a Grass-Leaf Tansy-Aster **haploid** or **diploid**?

What do the two different **colors** of each type of chromosome represent? Be very specific.

<table>
<thead>
<tr>
<th>Table 12.1</th>
<th>Lengths of the Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chromosome</strong></td>
<td><strong>Number of pop beads long</strong></td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
</tr>
</tbody>
</table>

4. For the sake of simplicity, you will not keep track of the **nuclear envelope** in this lab.

Which **stage** of the **cell cycle** is represented at this point?
5. Make an exact copy of yellow Chromosome 1. Break each Chromosome 1 apart in the middle (each piece 10 beads long) and rejoin them by inserting a “magnet in tubing” (magnet) into the small projections of the pop beads. **Place the two identical yellow** 20-bead strands together so that they are joined by the magnets, as shown in Figure 12.2.

What do the magnets represent? ________________________

What does the entire joined-together unit represent? ____________________________________________

What is each one of the two joined strands called? ____________________________________________

6. Repeat the chromosome duplication process but with the red Chromosome 1 and with both Chromosomes 2's. Separate each Chromosome 2 strand and add magnets so that there are 7 beads on one end and 18 beads on the other. See Figure 12.3.

Which stage of the cell cycle have you represented? ____________________________________________

7. Spread out the strands of replicated chromosomes in the middle area of the lab table so that they are not tangled up with each other. In a live cell they would no longer be tangled at this point because they would have coiled and recoiled into neat “packets” called condensed chromosomes.

Which stage of the cell cycle have you represented? _____________________________

How many duplicated chromosomes are present now? ______________________________

**Part 3: Moving Chromosomes and Making Nuclei**

Review the stages of mitosis and cytokinesis in your text.
8. Tape four hollow plastic tubes to your lab table, side by side, 1 cm apart, with an open end facing the middle of the lab table. Tape four more hollow plastic tubes one meter away in the same arrangement. All of your chromosomes should be somewhere between them.

9. Cut 8 pieces of string 80 cm long. Using your duplicated yellow Chromosome 1, tie one end of a string around the magnet of one sister chromatid and thread the other end of the string through one of the hollow plastic tubes that you taped to your table (see Figure 12.4). Repeat with the other sister chromatid, threading its string through one of the hollow tubes at the other end of the table.

10. Repeat the procedure with all your replicated chromosomes.

The strings represent __________________________ which are made of ___________________________.

What do the hollow tubes represent? ___________________________

11. Gently move the replicated chromosomes around until they are all lined up end-to-end in the midline of the space between the centrioles.

Which mitotic stage of the cell cycle have you represented? __________________

12. Tug gently on both sets of spindle fibers until the replicated chromosomes come apart at their centromeres. Continue pulling the spindle fibers just until all of the chromatids have separated.

Which mitotic stage of the cell cycle have you represented? __________________

What does each strand of pop beads represent now? __________________

13. Continue pulling the spindle fibers until the two groups of chromosomes are near the centrioles. Remove the strings.

Which mitotic stage of the cell cycle have you represented? __________________

Which part of the cell cycle will happen next? __________________

After it occurs, which stage of the cell cycle will each new cell enter? __________________

14. How does the number of chromosomes in each new cell compare to the number of chromosomes that were in the original cell?
15. How do the **lengths** of the chromosomes in the new cells compare with the lengths of the chromosomes that were in the original cell?

16. How do the **colors** of the chromosomes in the new cells compare with the colors of the chromosomes that were in the original cell?

17. What does this indicate about the **kind of genetic information** in the new cells compared to the original cell?

Now you have finished simulating mitosis and cytokinesis for one cell. The original cell is no longer there, but you have two new daughter cells.

**Part 4: Observing Mitosis and Cytokinesis in Real Cells**

**Part 4-A: Plant Cells from Onions**

The **root tips of plants** are good places to look for cells showing the different stages of mitosis, since rapid growth in these regions requires a great deal of mitosis and cell division. You will be looking at a **commercially prepared slide** of real onion root tips that have been killed, cut into thin slices, and stained, which allows you to see the individual boxlike cells and their chromosomes. The diploid chromosome number of this onion is $2n = 16$.

18. **Each student** should obtain a slide labeled “Onion root tip l.s.” (not c.s.) Observe the slide with your naked eye. Notice that there are three or more elongated items on the slide. Each one is a slice (section) of the tip of an onion root.

19. **Each student** should place the slide on their own microscope and focus on one of the onion root tip sections at low power, then work your way up to the 40X objective. Notice that there are many cells visible and most are rectangular. Since this is a slice of a real root rather than a textbook diagram, not all of the cells will be cut at just the perfect angle for viewing the cells and their chromosomes.

20. Move the specimen back and forth and up and down in a methodical way and look at the cells. Refocus as needed. Find an example of a cell at **each stage of mitosis**.

21. **Ask your professor to look at each stage on your own slide as you find it.**

   ★ **Prophase** is usually the most difficult stage to identify.
   ★ **Metaphase** is the easiest stage to find and identify, with **anaphase** a close second.
   ★ **Cytokinesis** is usually already beginning as **telophase** is occurring.
22. Use a pencil to sketch what you see in Table 12.2, including the chromosomes, spindle fibers, and centrioles. Move to another root section on the same slide if you need to look at more cells.

23. Complete the Onion Root Tip column of Table 12.2.

24. What color stain do you see on the onion chromosomes? ________________

25. Return the onion root tip slide to its proper place.

**Part 4-A: Animal Cells from Whitefish**

During early embryonic development of animals, such as the whitefish, each embryo produces many cells in a short time. In fact, so much of the embryo’s resources are devoted to mitosis and cytokinesis that the embryo does not grow in size. Rather, the cells simply get smaller and smaller until enough cells are formed to begin the process of differentiation and development. You will use slides of embryos at a stage of development known as the blastula stage.

Most of the cells of early embryos that you will observe are in one stage or the other of mitosis at any particular time. These animal specimens have been sliced very thinly and the stain added may be faint, so focusing may be more of a challenge than it was for the onion root tip slides. Adjusting the light level of your microscope properly is very important.

There are several kinds of whitefish in the world, and their diploid chromosome numbers range from $2n = 36$ to $2n = 96$. We don’t know which was used for the slides you will examine.

26. Repeat the process you used for the onion root tip slide above, but now use a “Whitefish Blastula sec.” slide instead of an onion slide. Notice that there are about a dozen or so small circles on the slide. Each circle is a slice (section) of a whitefish embryo.

27. Notice that there are no cell walls, since these are animal cells. Most of the cells will be sort of round or irregular in shape.

28. Complete the Whitefish Blastula column of Table 12.2.

29. What color stain do you see on the whitefish chromosomes? ________________

30. Ask your professor to look at each stage on your own slide as you find it.

31. Return the whitefish slide to its proper place.
<table>
<thead>
<tr>
<th>Table 12.2  Stages of Mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Make a LARGE pencil drawing of ONE CELL in each space below.</td>
</tr>
<tr>
<td>Onion Root Tip</td>
</tr>
<tr>
<td>Prophase</td>
</tr>
<tr>
<td>Metaphase</td>
</tr>
<tr>
<td>Anaphase</td>
</tr>
<tr>
<td>Telophase &amp; Cytokinesis</td>
</tr>
</tbody>
</table>
Questions for Lab 12

32. How is the process of mitosis and cytokinesis the **same** in animal cells and plant cells?

33. How is the process of mitosis and cytokinesis **different** in animal cells and plant cells?

The End!
Lab 12 should be completed before doing this lab.

**Meiosis in diploid (2n) animals** is the process by which the two chromosomes of every pair of chromosomes are separated into different cells, and sperm or eggs are produced. The resulting sperm or egg cells only have one of each type of chromosome, and therefore are haploid (n).

A human sperm or an egg cell has only one of each type of chromosome, rather than a pair, so each one has just 23 chromosomes. At human fertilization, the 23 chromosomes of the sperm and the 23 chromosomes of the egg end up together in the fertilized egg (zygote), which now has 23 pairs of chromosomes, for a total of 46 chromosomes.

Every somatic cell in the human body contains 23 pairs of chromosomes for a total of 46 chromosomes.

Review chromosome duplication in your text.

**Part 1: Preparing a Germ Cell for Meiosis (Pop beads again!)**

Animals have specialized cells called germ cells in the ovaries and testes. These germ cells go through meiosis, producing eggs in the ovaries or sperm in the testes. However, before the germ cells begin meiosis they must go through the G1, S, and G2 stages of the cell cycle, and then the cells begin Meiosis I, as shown below.

\[
\text{Germ Cell} \to G1 \to S \to G2 \to \text{Meiosis I} \to \text{two cells} \to \text{Meiosis II} \to 4 \text{ cells}
\]

In this lab you will simulate meiosis in a female mosquito, with a diploid number of \(2n = 6\).

1. Obtain the following.
   140 yellow pop beads, 140 red pop beads, 12 “magnets in tubing,” 6 small hollow plastic tubes, string, a meter stick, and transparent tape

2. Make a string of 24 red pop beads and a separate string of 24 yellow pop beads. Each of these represents one of the members of the pair of chromosomes called **Chromosome 3** of your female mosquito. **See Table 13.1 in this lab.**
Since these two chromosomes make up a pair, and are the same size and shape as one another they are called ______________________________ chromosomes.

3. Make a string of **18 red** pop beads and another string of **18 yellow** pop beads. These each represent the members of the pair of chromosomes called **Chromosome 2** of your female mosquito.
Since these two chromosomes make up a pair, and are the same size and shape as one another they are called ______________________________ chromosomes.

4. Make a string of **12 red** pop beads and another string of **12 yellow** pop beads. These each represent the members of the pair of chromosomes called **Chromosome X** of your female mosquito. See Table 13.1.
Since these two chromosomes make up a pair, and are the same size and shape as one another they are called ______________________________ chromosomes.

<table>
<thead>
<tr>
<th>Table 13.1</th>
<th>Lengths of the Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquito Chromosome</td>
<td>Length (number of pop beads)</td>
</tr>
<tr>
<td>X</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
</tr>
</tbody>
</table>

5. Place all of these chromosomes together in the middle of your lab table, assuming for the moment that the table represents a cell. For the sake of simplicity, you will not keep track of the **nuclear envelope** in this lab.

Is this cell **haploid** or **diploid**? ______________________________

What do the two different **colors** of the chromosomes represent?

_______________________________________________________________________

How many total chromosomes are found in this cell? __________

Which **stage of the cell cycle** is represented at this point? (Be specific.) __________
Part 2: Chromosome Duplication

6. Now you will simulate chromosome duplication beginning with red Chromosome 3. With more beads, make an exact copy of red Chromosome 3, then break each strand into sections that are 16 and 8 beads long and rejoin each one by inserting a “magnet in tubing” into the small projections of the pop beads. Place the two identical red strands together so that they are joined by the magnets.

   What do the magnets represent? __________________________________________

   What is each of the two joined strands called? ______________________________

   What is the entire joined-together unit called? ______________________________

7. Repeat the chromosome duplication process with all the other unduplicated chromosomes.
   - On each Chromosome 2, insert a magnet so that there are 7 beads on one end, and 11 beads on the other.
   - On each Chromosome X (the 12-bead chromosomes), insert a magnet so that there are 6 beads on each end.

8. Which stage of the cell cycle is being represented now? __________________________

9. Arrange all of the duplicated chromosomes on the lab table so that they are not tangled up with each other. In a cell they would no longer be tangled at this point because they already would have coiled and recoiled into neat “packets” called condensed chromosomes.

10. Which part of meiosis is beginning now, Meiosis I or Meiosis II? ________________

11. Align the magnets of the homologous replicated Chromosome 3’s so that the two chromosomes are close to each other at their centromeres. The red sister chromatids now are next to the yellow sister chromatids.

   What is this 4-strand combination called? ________________________________

12. Arrange the homologous Chromosome 2’s and the homologous X Chromosomes into the type of arrangement that you just made for the Chromosome 3’s.
Part 3: Moving Chromosomes in Meiosis I

Review the stages of meiosis in your text.

13. Tape three hollow plastic tubes to your lab table, side by side, 1 cm apart, with an open end facing the middle of the lab table. Tape three more hollow plastic tubes one meter away in the same arrangement. All of your chromosomes should be somewhere between them.

14. Cut 6 pieces of string 80 cm long. Using your duplicated yellow Chromosome 3, tie one end of a string around the magnets of one replicated chromosome and put the other end of the string near the hollow plastic tubes that you taped to your table (see Figure 13.1). Repeat with the homologous replicated chromosome, placing its string near the hollow tubes at the other end of the table.

![Figure 13.1 How to tie the strings](https://example.com/fig13_1.png)

15. Repeat the procedure with your two other tetrads (pairs of duplicated homologous chromosomes) of chromosomes 2 and X.

The strings represent ____________________________ , which are made of ____________________________ .

What do the hollow tubes represent? ____________________________

16. Gently move the tetrads around until they are all lined up end-to-end in the midline of the space between the centrioles. Compare this arrangement to the diagram in your text.

17. Have your professor check and approve your group’s arrangement.

18. Thread the spindle fibers of each duplicated chromosome through the centrioles closest to each chromosome.

Which stage of meiosis have you represented? ____________________________

19. Your group’s overall chromosome arrangement must be different from the arrangements of the other lab groups in terms of which colors are on which side of the midline of the cell. There are eight different arrangements possible. However, four of
them are the same as the other four in terms of the actual kinds of eggs that will be produced.

20. **If your arrangement is approved**, use colored chalk and color in the copy of Table 13.2 which your professor has put on the chalk board color with **your group’s tetrad arrangement**. Use the colored pens, pencils, or crayons provided in lab to complete the Tetrad Arrangement columns in Table 13.2.

21. Tug gently on all the spindle fibers until the replicated homologous chromosomes come apart into two groups.

   Which stage of meiosis have you represented? ______________________________

22. Continue pulling the spindle fibers until the replicated chromosomes are near the centrioles, and remove the strings.

23. Which stage of meiosis have you represented? ______________________________

24. What cellular process will happen next? _________________________________

25. Leave the chromosomes where they are. How many cells do you have now? ________
### Table 13.2  Independent Assortment In Mosquitoes

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TETRAD ARRANGEMENT in METAPHASE I</th>
<th>POSSIBLE MOSQUITO EGG CHROMOSOME ARRANGEMENTS AFTER TELOPHASE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Diagram" /></td>
<td><img src="image2" alt="Diagram" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="image3" alt="Diagram" /></td>
<td><img src="image4" alt="Diagram" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="Diagram" /></td>
<td><img src="image6" alt="Diagram" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image7" alt="Diagram" /></td>
<td><img src="image8" alt="Diagram" /></td>
</tr>
<tr>
<td>5</td>
<td><img src="image9" alt="Diagram" /></td>
<td><img src="image10" alt="Diagram" /></td>
</tr>
<tr>
<td>6</td>
<td><img src="image11" alt="Diagram" /></td>
<td><img src="image12" alt="Diagram" /></td>
</tr>
</tbody>
</table>

26. **Meiosis I is now complete.** Consider your results and answer the following.

   a. How does the number of chromosomes in each new cell compare to the number of chromosomes that were in the germ cell that you started with? Remember that the attached copies (sister chromatids) are still called one chromosome at this point.

   b. Are the new cells haploid or diploid? __________________________
c. How do the lengths of the chromosomes 2, 3, and X in each new cell compare to the lengths of chromosomes 2, 3, and X in the germ cell?

________________________________________________________________________

d. In the germ cell, half of the chromosomes were the color _________ and half were the color ____________. Is that true for your two new cells? _________

e. Consider what this indicates about the specific genetic information found in the new cells compared to the germ cell. Are they the same or are they different?

________________________________________________________________________

27. Which stage of meiosis happens next? _____________________________________

Part 4: Moving Chromosomes in Meiosis II

28. If you think that using string by this point is no longer needed for your learning, just pretend that you have string from this point on. If using the string is helpful to you, just continue to do so.

Place all the chromosomes from one cell between the centrioles on your lab table and move the other set of chromosomes to another side of the table. Cut 6 pieces of string 80 cm long. Tie one end of a string around the magnet of one sister chromatid of Chromosome 3 and thread the other end of the string through one of the centrioles (see Figure 13.2). Repeat with the attached sister chromatid, threading its string through a centriole on the other pole of the cell.

![Figure 13.2 How to tie the strings]

29. Repeat the procedure of forming and attaching the spindle fibers with chromosomes 2 and X of this cell.

30. Repeat the two steps above on another part of the lab table with the chromosomes of the other cell. You will have chromosomes in two different areas of your lab table now. Be sure that you have a set of centrioles taped at both ends of the table for each of the sets of chromosomes.
31. Gently move your chromosomes around (in both locations) on the table until your replicated chromosomes in each cell are **lined up end-to-end in the center** of the space between their centrioles.

Which stage of meiosis have you represented? ________________________________

32. Pull gently on **all** the sets of spindle fibers until the sister chromatids come apart at their centromeres and move into two distinct groups for **each** of the two cells on your table.

Which stage of meiosis have you represented? ________________________________

33. Continue pulling the spindle fibers until all the pop bead strands (each is now called a **chromosome**) are near the centrioles. Remove the strings and the magnets.

a. Which stage of meiosis is represented now? ________________________________

After that occurs, there will be will four newly-formed cells in the ovaries of your female mosquito, which are called _______________________.

In the testes (if this was a male mosquito) they would be called: ____________.

b. Draw the different chromosome arrangements of your possible eggs in Table 13.2, as well as the chart on the chalk board.

c. How does the **number** of chromosomes in each of your **new cells** compare to the number of chromosomes that were in the germ cell at the very beginning of this exercise?

d. Are these **new cells** haploid or diploid? Circle one.

e. How does this **current (haploid/diploid) condition** compare with the number of chromosomes in the nuclei formed **at the end of Meiosis I**?

f. How do the **lengths** of chromosomes 2, 3, and X in each new cell compare to the lengths of chromosomes 2, 3, and X in the germ cell?

Compared with the cells formed at the end of Meiosis I?
g. How does the distribution of maternal and paternal chromosomes in each new cell compare with that of the chromosomes in the germ cell - that is, are they the same or are they different? Compared with the cells formed at the end of Meiosis I?

h. What does this distribution of maternal and paternal chromosomes in each new cell indicate about the specific genetic information found in the new cells compared to the original cell - that is, are they the same or are they different? Compared with the cells formed at the end of Meiosis I?

34. The different arrangements of tetrads from Meiosis I illustrated in Table 13.2, from the various lab groups, represent Mendel’s Law/Theory of

35. The separation of the homologous chromosomes in Anaphase I represented Mendel’s Law/Theory of

36. How many different types of eggs were possible from all the lab groups combined?

37. Explain why the possible eggs in Table 13.2 show different results for different groups. Note: This answer requires more than just a few words.

Review the stages of meiosis in your text. Note how Meiosis I is different from Mitosis and how Meiosis II may appear to be similar to Mitosis (although it is different from mitosis).
Part 5: Karyotypes

Making a karyotype allows you to identify the physical characteristics of the chromosomes of an individual. It involves taking a photomicrograph of all the chromosomes from a single cell and then cutting up the photo into individual chromosomes and arranging the individual chromosomes into pairs (homologous pairs) in a specific sequence.

Part 5-A: Preparing a Karyotype

You will not make a karyotype from fresh cells in this lab, but if you were to prepare a karyotype of a person with their own cells you would need to do the following.

a. A drop of blood is added to a tube of phytohemagglutinin. This substance stimulates the white blood cells (WBC) to begin to divide. The tube is incubated at 37°C.

Why is 37°C used? ________________________________.

b. Colchicine is added to the tube. This substance disrupts microtubules. Cells can proceed to metaphase of mitosis, but the microtubules are unable to pull the two sister chromatids apart, and anaphase cannot occur.

c. The solution containing the blood cells is centrifuged to separate the blood cells from the blood plasma.

d. The blood cells are added to a hypotonic solution. This causes the red blood cells (RBC) to rupture, and the white blood cells to become fragile.

e. A mixture of methanol and glacial acetic acid is added to preserve the white blood cells.

f. The white blood cells are dropped onto a cold slide and air dried. This causes the white blood cells to rupture and spread their chromosomes on the slide.

g. Giemsa stain is added to make the chromosomes visible and to reveal banding patterns of the different chromosomes.

h. A photograph is taken through the microscope (a photomicrograph). The resulting print shows a random assortment of chromosomes. Because the cells were stopped in metaphase, the duplicated chromosomes look slightly X-shaped because sister chromatids are still joined at their centromeres.

i. The individual chromosome pictures are cut out and arranged on a karyotype form as pairs of homologous chromosomes.
**Fetal Karyotypes**

The procedure for making a karyotype of a fetus is similar to the above. However, the cells used may be cells of the fetus that have been shed into the **amniotic fluid** and withdrawn (with some of the fluid) by a long needle during amniocentesis. In addition, fetal cells can be obtained from chorionic villi, a part of the membranes that surround the growing embryo.

**Why Make Karyotypes?**

The final karyotype will reveal

- the **gender** of an individual (a Y chromosome is present in males, but not present in females)
- any variation from the normal **number** of chromosomes.
- any variation from the normal **lengths** (size and shape) of the chromosomes.

**Part 5-B: Prepare a Normal Human Female Karyotype**

38. You will be given a handout that shows a completed karyotype made from the cells of a normal human male. Study the grouping of the chromosomes. Note below the characteristics of each group of chromosomes that distinguish it from the other chromosome groups. **Focus on chromosome size and the location of the centromere.**

   - Group A _______________________________________________________
   - Group B ______________________________________________________
   - Group C ______________________________________________________
   - Group D ______________________________________________________
   - Group E ______________________________________________________
   - Group F ______________________________________________________
   - Group G ______________________________________________________
   - Sex chromosomes _____________________________________________

39. Work as a group and prepare one karyotype of a **normal human female**.
   a. Obtain one blank karyotype form and a copy of a photomicrograph of a set of normal human female chromosomes.
   b. Cut out the chromosomes from the photomicrograph and arrange them on the blank karyotype form. If the centromere is off-center, place the chromosomes with the shorter arms up.
   c. **Have your professor check your work.**
   d. Now glue or tape them into place.
Part 6: Selected Genetic Disorders

Autosomal Trisomy

If chromosome pairs fail to separate properly during meiosis (due to nondisjunction), the zygote (fertilized egg) may have three of one type of chromosome (a trisomy) instead of the normal two. An extra chromosome always causes serious abnormalities, and the majority of embryos with a trisomy fail to develop, resulting in a spontaneous abortion (miscarriage).

The severity of the abnormality is somewhat related to the size of the chromosome. Chromosome 21 is one of the smallest chromosomes. Trisomy 21, or Down syndrome, causes the least serious defects, although Down syndrome individuals always have mental disabilities and may have other problems such as heart defects and immune system problems.

The incidence of Down syndrome for the entire population is 0.125% of births. This percentage increases with the mother’s age. For 25-year-old mothers, the incidence of Down syndrome births is only 0.05%. This remains low until women reach their mid-30’s when it begins to rise, reaching 1% by age 40. Note, however, that even though that represents a twenty-fold increase, it is still only 1%.

Trisomy 13 and Trisomy 18 are much more serious and most babies born with these defects do not survive past infancy. Trisomies of other chromosomes are so serious that spontaneous abortion is almost always the result.

40. Assume that you have a karyotype that shows trisomy 18. Sketch the stages of meiosis in the space below to show how nondisjunction occurred in order to produce the abnormal karyotype you made. You do not have to draw all 23 pairs of chromosome—just two pairs, including the affected chromosomes. Nondisjunction may occur during either Meiosis I or Meiosis II. You may illustrate either situation in your sketch.

41. When you complete your sketch, circle the gamete(s) which, at fertilization, could give rise to an individual with the normal human female karyotype that you prepared.
Abnormal Numbers of Sex Chromosomes

Because the X chromosome carries genes necessary for all humans, every individual must have at least one X chromosome. A failure of the chromosomes to separate properly during meiosis which results in a person having only one sex chromosome, an X chromosome, causes a syndrome called Turner syndrome. This genotype is usually represented as XO (having one X and no Y chromosome). Individuals with Turner syndrome are female, but adult sexual development does not occur normally. The ovaries are underdeveloped and produce little estrogen. Pubic and underarm hair does not grow, breasts do not develop normally, and the person is infertile. Estrogen may be prescribed to cause breast development. In addition to the abnormalities of sexual development, people with Turner syndrome have webbed necks and are usually less than five feet tall.

There is no viable YO genotype. It is possible that an egg could be formed with no X and be fertilized by a sperm carrying a Y chromosome. However, without at least one X, the embryo would not survive.

It is also possible to have more than two X chromosomes, such as the XXX genotype. These individuals are usually fertile, but have mental disabilities. XXXX and XXXXX genotypes are also possible. However, each extra X chromosome increases the severity of mental disability.

Males may have extra X chromosomes also. An XXY genotype person shows Klinefelter syndrome. These individuals are usually infertile and may have mental disabilities. They are usually very tall, with disproportionately long arms and legs. XXXY males are even more severely affected.

Males may also have extra Y chromosomes. Many XYY individuals are normal in all respects, while others have mild mental disabilities.

Translocation

Sometimes parts of chromosomes break away from their normal location and may attach to a different location (are translocated), even on a different chromosome. If all of the chromosomal material is present, the individual may be completely normal and unaware of any problems. However, their chromosomes may not match up correctly with normal chromosomes, causing abnormalities in their descendants. These individuals are called translocation carriers. When a baby is born with a disorder that might be caused by a translocation, a karyotype of the parents is sometimes done to assess whether future children are also likely to have this disorder.

Often when chromosome parts are translocated, pieces of the chromosome may be lost or duplicated during the process. The loss of part of the short end of chromosome 5 causes cri-du-chat syndrome, so-named because of the kitten-like cry of babies born with this disorder. These individuals have severe mental disabilities and often have other abnormalities as well.

One type of translocation results when most of chromosome 21 breaks off and attaches to chromosome 14. The person in which this happens (called a translocation carrier) is normal because, in essence, he or she only has the material of two chromosome 21s, but part of one of them just happens to be attached to chromosome 14. However, their child can inherit three “doses” of chromosome 21 material because the child might receive both a normal chromosome
21 and the chromosome 14 that has the extra chromosome 21 material, then the child would have **Down syndrome**. About 3-5% of Down syndrome cases occur this way.

For many parents who have had a child with **Down syndrome due to a translocation**, there is a 3-12% chance of that couple having another child with Down syndrome because the parent could pass on both a normal chromosome 21 and the chromosome 14 that has the extra chromosome 21 material to any child of theirs. Being able to tell parents of a Down syndrome child their risk of having another child with Down syndrome is a common reason for having a karyotype done.

**Questions for Lab 13**

42. What is another name for Down syndrome?
   Explain what this means.

43. What type(s) of cells would be used to prepare a karyotype of a child with Down syndrome?

44. A 42-year-old woman becomes pregnant and is concerned because she may be at a higher risk of having a child with Down syndrome than when she was 20 years younger. What type(s) of cells would be used to prepare a karyotype to reveal whether or not the fetus she is carrying has Down syndrome?

45. A couple’s first child died when he was only four years old from a genetic disease caused by a mutation in a single gene. The disease, which affects only boys, causes degeneration of the nervous system and severe mental disability. Death almost always occurs before the age of twelve. This disease is fairly common in the wife’s family, afflicting her uncle, her younger brother, and two nephews. The wife is pregnant again and has had a chorionic villi sample performed. Since single genes cannot be seen in a karyotype, why were the parents so relieved when they saw the karyotype of the fetus?

46. Describe the difference in appearance between the X chromosome and the Y chromosome.
47. There are two couples in their 20s. They each had a first child with Down syndrome and received genetic counseling before conceiving their next children. This counseling included having karyotypes made of all the parents and children.

The first couple was told that their risk of having another child with Down syndrome was no greater than for any other couple their age.

The second couple was told that they had a 3-12% risk of having another Down syndrome child.

What is the probability that the first couple would have another child with Down syndrome?

Why were the two couples given such different answers?

The End!
LAB 14: Patterns of Inheritance

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves
(None needed if blood typing is not done by anyone.)

Part 1: Testing for Your Blood Type

Before you are allowed to participate in blood typing, you must have read, agreed to, and signed the Safety Training for Biology Hematology Labs form. You professor will provide this prior to lab class.

1. Follow all of the safety procedures and instructions provided by your instructor.

Place all materials which have blood on them (except sharps) in the BIOHAZARD bag in the lab. All sharps must be placed in the proper sharps disposal container in the lab.

2. When you write a genotype for blood type for the ABO and Rh systems, use the alleles listed in Table 14.1.

<table>
<thead>
<tr>
<th>Table 14.1</th>
<th>ABO and Rh Alleles and Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allele</strong></td>
<td><strong>Red Blood Cell Antigens</strong></td>
</tr>
<tr>
<td></td>
<td><em>(Proteins) Present</em></td>
</tr>
<tr>
<td><strong>I^A</strong></td>
<td>A</td>
</tr>
<tr>
<td><strong>I^B</strong></td>
<td>B</td>
</tr>
<tr>
<td><strong>I</strong></td>
<td>neither A nor B</td>
</tr>
<tr>
<td><strong>Rh</strong></td>
<td>Rh</td>
</tr>
<tr>
<td><strong>rh</strong></td>
<td>no Rh</td>
</tr>
</tbody>
</table>

Look in your text for a list of possible genotypes and their phenotypes. Also, see Part 5-D of this lab.
3. If you typed your own blood, what is your blood, what is your:

Genotype? _______________  Phenotype? _______________

4. Write a list of all the different blood types (phenotype) found in your lab class.

**Part 2: Which Alleles Do You Carry?**

Many of what we call “normal” traits and functions are due to dominant alleles and many are due to recessive alleles.

The phenotype of a person is the actual appearance or function that a person has because of the alleles on his or her chromosomes. For example, the phenotype of a person might be "have dimples" or "does not have dimples" and is written in words.

The genotype of the person is a listing of their actual alleles for that trait, and is written using letters. Possible genotypes for the alleles concerning the dimples trait might be written DD, dd, or Dd.

As it happens, dimples are dominant in people, so if you have dimples your genotype could be DD or Dd; you cannot tell just by looking at your face, so you would write it as D __ . But if you do not have dimples, you must have two "no-dimple-alleles" and your genotype is dd.

In Table 14.2 the column for characteristics lists 11 traits to find on each other. All of the ones listed are caused by dominant alleles, and the uppercase letter to use for each one is written in parentheses in the table. Use a lowercase letter for recessive alleles.

5. Examine yourself and the members of your lab group, and complete Table 14.2. There may be handouts available in the lab which illustrate the traits.

6. Look at your results when you finish Table 14.2. Of the 11 traits listed, how many:

Dominant phenotypes do you show? _______________

Recessive phenotypes do you show? _______________

7. Explain why you have a mix of both dominant and recessive phenotypes.

8. Write your gender phenotype _______________ genotype _________.

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-134-  
BIOL 1408  Lab 14
### Table 14.2 Your Own Phenotypes and Genotypes

<table>
<thead>
<tr>
<th>Characteristics which show simple (complete) dominance in humans</th>
<th>Your Phenotype (write words)</th>
<th>Your Genotype (write letters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interlocking fingers (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>When you clasp your hands together,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>having the left thumb on top.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>right thumb on top = I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTC taster (P)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-tasters = p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tongue rolling (T)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not able to roll tongue = t</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free earlobes (E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>attached earlobes = e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimples (D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no dimples = d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widow's peak (W)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>straight hair line = w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bent little finger (L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>straight little finger = l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double-jointed thumb (J)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>thumb not double-jointed = j</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-digital hair on fingers (H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no mid-digital hair on fingers = h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freckles (F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no freckles = f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blaze (B) of white hair on your head.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no blaze = b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. If you have not already done so, go back and complete items #7 and #8 on the previous page.

Place all materials which have saliva on them in the BIOHAZARD bag in the lab.
Part 3: Probability

10. If you toss a coin, what is the probability that it will come up heads? _________

11. If you toss the coin again, what is the probability that it will come up heads this time? __

   Does the result of your first toss make any difference in the probability that it will come up heads the second time? ______________

12. Toss a coin twice. What was the result of the first toss? ________ Second toss? ______

13. Toss two coins at the same time. What was the result? ____________________

14. Do you think that there is any difference in the probability that the two coins will come up heads if you toss them one after another or at the same time? ______________

   Explain. ________________________________________________________________
   ________________________________________________________________

15. As you have seen, if you toss a coin, there is a 50% chance that it will come up heads. If you toss it again, there is still a 50% chance that it will come up heads.

   However, if before you toss the coin, you decide that you would like to see it come up heads twice in a row (or two coins both coming up heads if they are tossed at the same time), you know that there is less than a 50% chance of this happening. In fact, there is only a 25% chance of getting two heads in a row. There are four possibilities: head-head, head-tail, tail-head, and tail-tail. Only one of those four is the one you wanted, and one possibility out of four (¼) is a 25% probability.

   To calculate a probability before you toss the coins, you multiply the probabilities of each coin landing a certain way. That is, 50% times 50% equals 25%. You can do this by converting the percentages to decimals: .50 x .50 = .25 (25%) or by converting them to fractions: $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$.

16. What is the probability of tossing three heads in a row? _____________ Toss your coin three times.

   What was the result? __________________________

   Feel free to use %, decimals, fractions, or ratios.

17. The Monroe family is expecting their fifth child. Their first four children are boys. What is the probability that this next child is a boy? _____________

18. One of the sons, Fred Monroe is engaged to Sara Clifford. They plan to have five children. What is the probability that all five children will be girls? _____________
Part 4: Determine Probabilities Using Both a Punnet Square and Probability Calculations

A Punnet square is a visual way to represent the probabilities of two or more events happening at the same time.

You can represent the probability of the results of two coins being tossed at the same time by using a Punnet square in the following way.

<table>
<thead>
<tr>
<th>Coin A may come up either</th>
<th>&quot;A&quot; Heads</th>
<th>&quot;A&quot; Tails</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coin B may come up either</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;B&quot; Heads</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One Result: &quot;A&quot; Heads</td>
<td>&quot;A&quot; Tails</td>
<td></td>
</tr>
<tr>
<td>&quot;B&quot; Heads</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One Result: &quot;A&quot; Tails</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As you can see, there are four possible results (shown in bold in the four lower right-hand boxes), which are the same results as when you calculated the probability mathematically. So, there are four equally likely possibilities, and each possible result has a 25% (or ¼) chance of occurring; a 25% chance of both coming up heads, a 25% chance of both coming up tails, and a 50% (25% chance heads-tails plus 25% chance tails-heads) of one coin heads and the other tails.

Part 4-A: Offspring Probabilities, With Simple (Complete) Dominance, Using a Punnet Square

Many genetic diseases are caused by recessive forms of genes (recessive alleles). We know that:

- **Recessive alleles** are not expressed if the cell also has a dominant form of the allele.
- **Dominant alleles** are expressed whenever they are in a cell.

To understand what is actually happening, remember that an allele is a code specifying how to make a certain protein. In the type of interaction called simple (or complete) dominance, if you have at least one dominant allele, which codes for making a normal protein, you will make the normal protein.
For example, we each carry two alleles that specify the code for how to make a cell membrane protein that allows chloride ions to cross cell membranes. Without this protein, mucus builds up in the respiratory and digestive tracts, which results in a life-threatening condition known as **cystic fibrosis**.

Most of us have two normal **dominant** alleles of this gene. About one in 20 Caucasians, however, has one dominant allele and one **recessive** allele (that cannot code for the protein). With those mixed alleles we can make the correct protein and will have functional chloride channels. But if someone has **two recessive alleles**, there is no way to make the proper chloride channels and the person will develop cystic fibrosis.

Although everyone carries two alleles for this protein, because of the effects of the Law of Segregation, only one of the alleles—randomly distributed—will end up in each **sperm or egg cell** that we make. Which sperm meets a particular egg is also a random event. In that sense, which alleles are inherited by a child is like a coin toss. Heads or tails?

If we know which alleles the parents carry, using a **Punnet square** can show the likelihood of their children being born with cystic fibrosis. For example, suppose **Jeremy** carries one dominant allele (which codes for the protein) and one recessive allele (which does not code for the protein), while his wife **Michele** has two dominant alleles. A Punnet square will let us see the probabilities for their possible offspring at a glance.

<table>
<thead>
<tr>
<th>The allele in Michele's egg could be</th>
<th>The dominant allele that codes for chloride channel proteins</th>
<th>The dominant allele that codes for chloride channel proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeremy's sperm could be</td>
<td>Michele's allele for proteins and Jeremy's allele for proteins</td>
<td>Michele's allele for proteins</td>
</tr>
<tr>
<td></td>
<td>The recessive allele that does not code chloride channel proteins</td>
<td>Michele’s allele for proteins and Jeremy’s allele that does not code for chloride channel proteins</td>
</tr>
</tbody>
</table>

In this case, their children have 0% chance of having cystic fibrosis.

19. Someone who has one defective allele, but who is not affected by a genetic disease is called a **carrier**. What is the probability that Jeremy and Michele could have a child who is a carrier?

_______________________________
There is, of course, a much simpler way of diagraming this. In genetics, **capital letters** are used to represent dominant alleles and **lowercase letters** are used to represent recessive alleles. Because of what research has told us, use "C" for the allele that codes for the proteins, and "c" for the allele that does not code for the proteins.

A combination of letters which represents alleles (CC, Cc, or cc) is called a **genotype**. That makes **Michelle CC and Jeremy Cc**, just like before. Such a cross is represented as **CC x Cc** and the Punnet square is shown below.

![Punnett Square](image)

20. Notice that in the four boxes representing possible offspring there are only two different genotypes that Michelle and Jeremy could produce.

They are ___________ and ___________.

It turns out that Jeremy and Michele's son, **Jason, is a cystic fibrosis carrier**. His **genotype** is Cc, which is possible from the Punnet square above.

21. Assume that Jason (from above) marries Jennifer, who is also a carrier.

The genotype for:  Jason is ________________  Jennifer is ________________.

**Make a Punnett square** and determine the probability of Jason and Jennifer having a child **with** cystic fibrosis.

The probability of them having a child **with** cystic fibrosis = ________________
Part 4-B: What are the odds?

You may use fractions, percentages, or decimals to show probability.

For the following questions, assume that both you and your spouse (or a future spouse) are Caucasian. Solve these without using a Punnet square - use probabilities instead.

22. What is the probability that you carry the cystic fibrosis allele? ________
23. What is the probability that your husband/wife is (will be) a carrier? __________
24. What is the probability that both you and your spouse will be carriers? __________
25. If both parents are carriers, what is the probability that a child born to them will have cystic fibrosis? ________
26. What is the probability that both you and the person you decide to marry with will be carriers and that your child will have cystic fibrosis? __________
27. In real life, cystic fibrosis occurs in about 1 in 2,000 births to Caucasian couples. Does this agree, more or less, with your calculations? ________
   Explain.

Part 4-C: Offspring Probabilities, With Simple (Complete) Dominance, Using Probabilities

Instead of using a Punnet Square to predict possible offspring you can also use probabilities, like you did with the coin tosses.

Now you will use Jason (Cc) and Jennifer (Cc) (the same couple from Part 4A of this lab) and predict their possible offspring using probabilities instead of using a Punnett square.

28. Of all the types of sperm he can make,
   what is the probability of Jason making sperm with the C allele? _________________
   what is the probability of Jason making sperm with the c allele? _________________
29. Of all the types of eggs she can make,
   what is the probability of Jennifer making eggs with the C allele? _________________
   what is the probability of Jennifer making eggs with the c allele? _________________
Remember, if you want to know the probability of two things happening together you multiply the probability of one times the probability of the other.

Therefore, if you want to know the probability of Jason and Jennifer having a child with the genotype CC you **multiply his** probability of making sperm with the C allele **times her** probability of making eggs with the C allele, since the zygote (fertilized egg) would need to receive a C allele from each parent.

30. **In the space below, multiply those two probabilities and calculate the probability of Jason and Jennifer making a CC baby.**

What is the probability of them having a **CC** child? ________________________

31. **Look back at your answer for Part 4-A #3.**

   How many of the four boxes in your Punnett square have **CC** as a possible child? _____

   How does that compare to the answer in the question above regarding the possibility of Jason and Jennifer having a CC child?

**Now, you will solve #32 from this lab using probabilities instead of using a Punnet square.**

When solving problems this way, start with one of the possible offspring and calculate the probability of getting that type of offspring. Then do the same thing for each possible types of offspring.

32. **Use this method to complete Table 14.3 for the possible offspring that Jason and Jennifer could produce.**
Table 14.3  Using Probability to Predict Offspring

<table>
<thead>
<tr>
<th>Possible Offspring Genotypes</th>
<th>Write Your Calculations</th>
<th>Probability of Getting That Type of Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cc*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cc</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Add together all the Offspring Probabilities and write the total here  
Your total in Table 14.3 must equal 1, or 100%. If it doesn’t, ask your professor for help.

* There are two different ways for Jason and Jennifer to produce a Cc offspring.  
  First, calculate the probability of Jason giving a C allele and Jennifer giving a c allele.  
  Second, calculate the probability of Jason giving a c allele, and Jennifer giving a C allele.  
Then, add those two probabilities together to get the probability of Jason and Jennifer producing a Cc offspring.

33. How do your offspring probabilities in Table 14.3 compare with all of your answers in Part 4-A, #32?

34. This method of solving problems is also useful if you are keeping track of two or more traits at the same time. Your professor may demonstrate this some time.

**Part 4-D: Genetic Diseases Caused by Dominant Alleles**

Sometimes having just one copy of a mutated gene can cause serious health or developmental problems because the resulting presence of an abnormal protein causes something to go wrong in cells.

**Huntington's Disease** is a genetic disease caused by a dominant allele and causes brain cells to degenerate gradually, which affects thinking and reasoning skills, as well as causing loss of muscle control. Currently there is no cure. Use the letters "H" and "h" in this Punnett square.

35. Does "H" represent the allele that causes Huntington’s Disease or the healthy allele? (Circle one)

36. Does "h" represent the allele that causes Huntington’s Disease or the healthy allele? (Circle one)
37. Show your work, and determine the probability of inheriting Huntington's Disease if the father has the disease (with one abnormal allele and one normal one) and the mother is normal. Write the parents’ genotypes first.

\[
\text{\textcopyright parent genotype: } \text{_________} \quad \text{\textcopyright parent genotype: } \text{_________}
\]

38. The probability that this couple will have a child with Huntington disease = ________%

**Part 5: Other Patterns of Inheritance**

You have already learned one pattern of inheritance known as Simple (or Complete) Dominance. This part of the lab will give you practice with other types of inheritance.

**Part 5-A: Codominance**

Two different forms of the same gene are called alleles. Sometimes both of the different alleles code for functional, but different, proteins and both forms of the protein are produced by the cell. This interaction is called codominance.

**Hemoglobin (Hb),** a protein made of four polypeptide chains, carries oxygen in our blood. Hb is inside your red blood cells (RBCs). For our consideration, we will use two different Hb alleles: HbA (which codes for normal Hb) and HbS (which codes for abnormal Hb). Yes, each allele is represented by three letters, but there are only 26 letters in the English alphabet, and there are thousands of alleles, so not all alleles are represented by just single letters.

The interaction of HbA and HbS alleles is an example of codominance.

Hb molecules have a certain shape, like all proteins. In **homozygous** HbA HbA people, all the Hb is normal. In **heterozygous** HbA HbS individuals, half of the Hb molecules are the normal shapes and half are abnormal. This may cause some RBCs to collapse to some extent under low oxygen conditions, but generally the RBCs are normal shapes, even though they have two types
of Hb. The result is **sickle-cell trait**, a genetic disease which causes a person little problem unless they are under extreme oxygen stress.

**Homozygous HbS HbS** individuals, of course, make only abnormal Hb, and the RBCs may collapse into sickle shapes, which form blockages in small blood vessels. These episodes are extremely painful and cause damage to tissues and organs. The result is called **sickle cell anemia** which is often fatal.

39. Fill in the correct phenotypes in Table 14.4.

<table>
<thead>
<tr>
<th>Table 14.4 Hemoglobin Genotypes and Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>HbA HbA</td>
</tr>
<tr>
<td>HbA HbS</td>
</tr>
<tr>
<td>HbS HbS</td>
</tr>
</tbody>
</table>

40. Imagine that two people with sickle-cell trait marry and have children. Show your work, and determine the probabilities of their possible children. Write the parents’ genotypes first.

♂ parent genotype _______________ ♀ parent genotype _______________

**Probability of children**
sickle-cell anemia = ________ sickle-cell trait = ________ healthy = ________

**Phenotypic Ratio**
sickle-cell anemia : sickle-cell trait : healthy

_____________ : ___________ : ___________
Part 5-B: Incomplete Dominance

For some traits which have two different alleles, the **heterozygous genotype** shows a completely different phenotype than either of the homozygous phenotypes. Often its **phenotype appears to be a mixture or in-between version** of the parental phenotypes. This interaction of alleles is called **incomplete dominance**.

You text probably gives an example of incomplete dominance using red snapdragons crossed with white snapdragons and producing pink offspring. Another example concerns one aspect of human hair. **Curly hair** (C) appears in a CC person, and **straight hair** (S) occurs in an SS person. Note that both are represented by capital letters in examples of incomplete dominance. Heterozygous CS people have wavy hair.

### 41.
Assume that two wavy-haired people marry and have children. Show your work, and determine the probability of the phenotype of their first child. Write the parents’ genotypes first.

\[
\text{♂ parent genotype } \quad \text{♀ parent genotype }
\]

Probability of the first child having hair that is

- straight = __________
- wavy = __________
- curly = __________

**Phenotypic Ratio**

\[
\text{straight} : \quad \text{wavy} : \quad \text{curly}
\]

\[
\text{___________} : \quad \text{___________} : \quad \text{___________}
\]
Part 5-C: Sex-Linked Traits

One exception to the rule that a person has two copies of each gene occurs on the human sex chromosomes, X and Y. Men are male because they have both an X and a Y for their #23 chromosomes, while women have two Xs. For genes found only on the X chromosome (and not the Y chromosome), men have only one copy of these genes, while women have two copies of those genes. Traits controlled by genes found on the X chromosomes are called sex-linked traits.

The Y chromosome is a small chromosome that carries primarily genes that are necessary for maleness. On the other hand, X chromosomes are large chromosomes that have many genes which have nothing to do with gender and are needed by both men and women. That works because everyone has at least one X chromosome. And as we have seen, sometimes you only need one gene that works in order to make a necessary protein.

Due to simple dominance, when a woman has an allele that codes for an abnormal protein on just one of her X chromosomes, she can use the corresponding normal allele on the other X chromosome to make the protein, develop normally and be healthy. Only if the alleles are abnormal on both her X chromosomes will she have the genetic disease. However, any time a man has an allele that codes for an abnormal protein on his X chromosome, he has no "spare" to code for the protein on his Y chromosome. Since he has only one X chromosome, he will develop the genetic disease.

One gene found on the X chromosome codes for certain proteins (clotting factors) which are required for blood to clot. Individuals who cannot make these proteins have a genetic disease called hemophilia. This means that their blood does not clot properly and they bleed for longer periods of time than do people with normal blood clotting factors. In this case, the H allele codes for the blood factors and the h allele does not. Since we know that these alleles are on the X chromosome, they are written as X^H and X^h and the Y chromosome is just written as Y, with no H or h attached.

42. Fill in the following Punnett square which shows the probability of a child having hemophilia if the father has hemophilia and the mother has two normal alleles.

```
<table>
<thead>
<tr>
<th></th>
<th>X^H</th>
<th>X^h</th>
</tr>
</thead>
<tbody>
<tr>
<td>X^hY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X^H</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```
What percentage of the offspring are likely to be female? ________________

What percentage of the offspring are likely to have hemophilia? ________________

What percentage of the offspring are likely to be normal females? ________________

43. Dominant alleles which code for the normal proteins needed by the retina are located on the X chromosome. If these proteins cannot be made, color-blindness results. Color blindness is a sex-linked recessive trait.

A woman (married to a man with normal color vision) has the allele for normal color vision on one of her X chromosomes and a recessive allele on the other X.

Use $X^N$ to represent an X chromosome carrying the allele for normal color vision, and $X^n$ to represent an X chromosome carrying the allele that causes color-blindness. Write the parents’ genotypes first, then determine the answers to the items below.

$\sigma$ parent genotype ______________ $\varphi$ parent genotype ______________

Probability of: a color-blind son = _______ a color-blind daughter = _______

Part 5-D: When There Are More Than Two Possible Alleles

Even though a single allele pair determines a trait, it does not necessarily mean that only two alleles exist for the trait. ABO blood types are a prime example. We each carry only two alleles (one from Mama, one from Daddy) for this characteristic of our red blood cells, but there are three different alleles in the human population that determine this trait.

- one allele ($I^A$) codes for an RBC (red blood cell) membrane glycoprotein called “protein A”
- one ($I^B$) codes for an RBC membrane glycoprotein called “protein B”
- one ($I$) does not work as a protein code, so there is no protein A and no protein B made

If you have protein A on the surface of your RBCs, you are said to have type A blood. If you have protein B on your RBCs, you have type B blood.
If you have one $I^A$ allele and one $I^B$ allele, you make **both** proteins A and B and you have **type AB** blood, because $I^A$ and $I^B$ are codominant with each other (see the capital letters).

Note that $I$ is a recessive allele (a lowercase letter). If both your alleles are $ii$, the ones that **don’t** make proteins A or B, you have **type O** blood.

Since $i$ is recessive to both $I^A$ and $I^B$, a person who has type A blood carries alleles $I^A I^A$ or $I^A I$.

Since $I$ is recessive to both $I^A$ and $I^B$, a person who has type B blood carries alleles $I^B I^B$ or $I^B I$.

44. Show your work, and determine the possible blood types of the children when Momma is type AB and Daddy is type O. Write the parents’ genotypes first.

   $\sigma$ parent genotype ______________   $\Omega$ parent genotype ______________

45. If the parents above were your parents, and you are type B, **your** genotype is ______

46. Then, if **you** marry someone who has type A blood, could **your** children be type O? ___

Draw a Punnett square to illustrate your answer.

   Your genotype ______________   Your spouse’s genotype ______________
Part 6: Pedigrees

Sometimes you will already know the phenotypes of the organisms but you want to determine their individual genotypes. In that case, use a pedigree to solve the problem.

47. This problem is an example of using a pedigree to solve for an answer. You do not use any Punnett Squares, just fill in the blanks instead.

Assume that Cecile is a color-blind woman. Write the possible/correct Xs and Ys for everyone in the blanks below.

Her father _______________ Her mother _______________

Cecile _______________

e. Is it certain that her father was color-blind? ________

Explain.

f. Is it certain that her mother was color-blind? ________

Explain.

The End!
LAB 15: Cancer Gene Pedigree

PPE Required - None

The information in this lab is modified from EDVO-Kit #115: Cancer Gene Detection, produced by EDVOTEK, Inc.

Making a Pedigree

A family pedigree is a valuable tool to use in order to track the occurrence of genes and their expression from one generation to another. Look in your text for an example of a pedigree so that you can see the basic layout. Use the following symbols for this lab exercise.

○ = female without cancer • = female with cancer
□ = male without cancer ■ = male with cancer

Instructions

1. Use the family information below to draw a draft copy of the pedigree for Valerie Brown’s family.

2. Follow these guidelines.
   ♦ When you add offspring, write the offspring in sequence from oldest to youngest, going left to right across the page.
   ♦ Label each symbol with the person’s name and age (current age if alive, or age at time of death if deceased).

Valerie’s Family Information

Several members of this family have shown various forms of cancer due to Li-Fraumeni syndrome. This syndrome results from the mutation of a gene called p53, which is on Chromosome 17. The mutated gene leads to a predisposition toward certain types of cancers. It is common for these cancers to appear early in life. For information on how the p53 gene works, see http://www.biology.arizona.edu/cell_bio/tutorials/cell_cycle/cells2.html.

During her monthly breast self-exam, Valerie Brown found a small irregular mass. Of course she called her doctor. As part of her clinical exam, the doctor asked about her family history of cancer. She said that her father’s family was free of cancer but that several cases of cancer had appeared in her mother’s family. Her complete information follows.
Valerie’s maternal grandmother, Elsie, died at age 42 from bilateral breast cancer.
Elsie’s husband, Bernard, was free of cancer and died at the age of 88.
They had three children: James, Mabel, and Diane.

Valerie’s mother, Diane (age 60), was diagnosed and treated for breast cancer at the age of 39. Valerie’s father, Ray Earl (61), is alive and well.
Diane’s sister, Mabel, died of a malignant brain tumor at the age of 2, the year Diane was born.
Diane’s older brother, James (64), underwent surgery recently, followed by chemotherapy for colon cancer, and was comforted by his healthy wife Fiona (61).

James’ first child, his son, Patrick, died of brain cancer at age 14.
Patrick’s sister, Jane, died at age 2 of childhood leukemia, 40 years ago.
James has two other sons, Luke (30) and Curtis (28), who are in good health and free of cancer.

Both Valerie’s older sister, Nancy (38), and Nancy’s husband Raul (40), are free of cancer.
Nancy’s first son, Omar, was diagnosed with sarcoma at age 3 and with osteosarcoma this year. He is 18.
Nancy’s second child, Jake (12), and his younger sister, Maria (8), are free of cancer.

Valerie is 36 years old and has five children, Justin (16), Sheila (14), Robert (10), Angela (8), and Anthony (6). They are all alive and show no signs of cancer.
Valerie’s husband Zeke (37) is cancer free.

3. **Have your professor approve your draft copy.**

4. Make a final copy on graph paper.

5. Write your name and Lab 15 on the final copy of the pedigree that you draw.

In **Lab 19** you will do a DNA analysis of Valerie to find out whether or not her irregular breast mass is cancerous. That information will help her learn more about the chances of her children developing cancer. Stay tuned!

*The End!*
LAB 16: DNA

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

What we inherited from our parents and what we pass on to our children are the instructions for how to make the right proteins at the right time and in the right cells. These proteins control the formation of all our parts and organs from our earliest moments as an embryo. Throughout our lives, the appearance of the right proteins at the right time in our cells maintains the structure and function of our bodies.

We call DNA the “master molecule” because DNA is the code for how to make all the proteins an organism requires. Proteins are polymers of dozens to hundreds of amino acids joined together. There are 20 different amino acids used to make proteins, and DNA carries thousands of codes, or genes, that say exactly which amino acids should be linked together and in exactly which sequence they should be joined so that the correct proteins are made.

DNA itself is a polymer and is made from two strands of linked nucleotides twisted together, forming a double helix. There are only four different nucleotides used to make the DNA polymer, and figuring out how a molecule with such a simple structure could control making the enormous diversity of proteins in a cell was one of the great challenges of 20th century biology.

Part 1: Working With Real DNA

DNA is composed of two long chains (polymers) of nucleotides (monomers) twisted together to form a double helix. This arrangement is very stable because strong covalent bonds join the nucleotides together to form the polymers. However, weaker hydrogen bonds link the two strands together. Other chemical interactions maintain the overall shape. As a double helix, DNA forms long fibers that cause a solution containing DNA to look and feel very viscous, or thick.

During this part of the lab, you will observe the look and feel of each of the following.

- DNA in purified form
- DNA after being treated with the enzyme DNase (pronounced “DNA ace”) 
- DNA in its natural form taken directly from strawberries

Note that several of your materials for this lab are in ice. Leave them on ice unless you are actually using them, then replace them into the ice. This keeps them cold, which slows down all chemical reactions which might harm the materials.

Keep the ice bucket on your tray, not on the lab table.
Part 1-A: Observing Purified DNA

You will work with DNA which was extracted from calf thymus glands and then purified.

**Good lab technique gives great results and poor technique gives results that are not useable.**

1. Put on gloves and eye protection.

2. Obtain the following for your lab group.

   - 3 glass vials
   - 3 wooden stir sticks
   - ice bucket
   - cold ethanol (ETOH)/ethyl alcohol
   - 1 large and 1 small transfer pipette
   - Sharpie®
   - 4 strawberries (frozen then thawed)

   - two 250 mL beakers
   - 1 coffee filter
   - detergent
   - salt
   - dH₂O
   - 1 zip loc bag
   - 1 teaspoon

   Your professor will have the DNAse that you need.

3. Label the three vials “Pure DNA,” “DNAse,” and “Strawberry.”

4. Describe the **purified DNA** and fill in the following.

   Color/clarity ___________________________________________________

   Viscosity (thickness) ____________________________________________

5. Using the large transfer pipette, place 3mL of purified DNA into the glass vial labeled “Pure DNA.”

6. **Carefully** pour cold ethanol (ETOH) into the vial. Pour it gently down the side of the vial so that the alcohol forms a layer on top of the DNA. You want layering, not mixing. Add enough alcohol so that the vial is ¾ full.

7. Dip one end of a wooden stir stick into the vial through the cold alcohol and into the DNA. **Slowly twirl (not stirring)** the rod clockwise and then raise it back into the alcohol. **Clusters** of DNA molecules should attach to the wooden stir stick. Now, more vigorous twirling may help to collect even more DNA onto the wooden stir stick. Remember that even though DNA molecules are long molecules, **individual DNA molecules are too thin (small in diameter) for you to see.**
8. Continue twirling the wooden stir stick and moving it up and down between the DNA and the alcohol to capture more DNA fibers on the wooden stir stick. There are many polar water molecules attached to the polar (charged) regions of the DNA, and **ETOH removes most of the water**.

9. After no more DNA clings to the wooden stir stick, remove the wooden stir stick (while holding it over the vial). Observe the results and complete the Purified DNA column of Table 16.1.

10. Put the wooden stir stick back into the vial and put them back on ice for comparison with the rest of the DNA experiments.

**Part 1-B: The Effect of Adding DNase to Purified DNA**

Another way to disrupt the long stable DNA molecules is to **cut** the double-stranded DNA into short pieces. This is exactly what happens to DNA during digestion of the food we eat, because intact DNA is much too large to be absorbed by intestinal cells. The **enzyme deoxyribonuclease (DNase)** is produced by the pancreas; it **breaks DNA into short lengths of double helix**, and then, over time, into individual nucleotides. Following that, other enzymes produced by the intestinal cells break apart the nucleotides, and the small sugars, phosphates, and bases that are left can be absorbed by cells.

11. Place 3mL of purified DNA into the glass vial labeled “DNase.”

12. **CAUTION:** For this step, **do not allow the liquid DNase to be sucked all the way up into the bulb of the transfer pipette**. Using the small transfer pipette, add 50\(\mu\)L (about 4 drops) of DNase to the vial and gently swirl the contents. **You only have a tiny amount of DNase, so use it carefully.** Let the vial sit for **10 minutes** at room temperature.

13. After **10 minutes**, add cold ETOH to the DNA, layering it as you did before, then spooling the DNA onto the wooden stir stick as you did before.

14. After no more DNA clings to the wooden stir stick, remove the wooden stir stick (while holding it over the vial). Observe the results and complete the Purified DNA Treated with DNase column of Table 16.1.

15. Put the wooden stir stick back into the vial and put them back on ice for comparison with the rest of the DNA experiments.

**Part 1-A: Extracting DNA from Strawberries**

Modified from The National Human Genome Research Institute (NHGRI)
This DNA extraction activity results in a large quantity of DNA that can be seen with the naked eye. If you follow the instructions, there is almost no way to make a mistake that would affect the results. This method is much more effective than extracting DNA from any other source because strawberries are soft and easy to smash. In addition, ripe strawberries produce enzymes, including pectinase (a group of enzymes which take apart pectin) and cellulase (a group of enzymes which take apart cellulose), which help in breaking down plant cell walls.

Strawberries have an enormous genome. Human cells have two copies of each kind of chromosome (a diploid genome). Some commercially-grown strawberry hybrids have cells with eight copies of each chromosome (an octoploid genome).

16. Pull off any green leaves from the strawberries.

17. Put the strawberries into the plastic bag, seal it, and thoroughly smash the strawberries for two minutes. Completely crush the strawberries. This starts the process of releasing the plant enzymes mentioned above which break open the cell walls of the strawberries.

18. Now you will make some DNA extraction liquid. Add the following to a 250 mL beaker.
   - 100 mL of dH₂O
   - 2 teaspoons of detergent
   - 1 teaspoon of salt
   Gently mix them together with the spoon.

19. Add 2 teaspoons of the DNA extraction liquid into the bag with the strawberries.

   The detergent will break apart the nuclear envelopes of the strawberry cells, which releases the DNA (and the proteins that are attached to it) from inside the nuclear envelope

   The salt will remove the proteins from the DNA. Then the proteins will dissolve in the watery strawberry liquid and stay separated from the DNA.

20. Reseal the bag and gently mash the mixture for another minute. Avoid making too many soap bubbles.

21. Place the coffee filter inside an empty 250 mL beaker.

22. Open the bag and pour the strawberry liquid into the filter. Twist the top of the filter enough to close it, just above the liquid, and very gently squeeze the remaining liquid into the beaker.

23. Pour enough of the liquid from the beaker into the vial labeled “Strawberry” to make the vial about ⅔ full.
24. Next, (down the inside of the vial) pour an amount of cold alcohol equal to the amount of strawberry liquid which is already in the vial. **Do not mix or stir.** This will isolate the DNA from the rest of the material contained in the cells of the strawberries.

25. Within a few seconds, watch for the development of a white cloudy substance (DNA) in the top layer above the strawberry extract layer.

26. Tilt the vial and pick up the DNA using a wooden stir stick.

27. Use your results to fill in the appropriate parts of Table 16.1.

### Table 16.1 Comparison of DNA Extracted from Different Sources

<table>
<thead>
<tr>
<th>Measurement / Observation</th>
<th>Purified DNA</th>
<th>Purified DNA treated with DNAse</th>
<th>DNA Extracted from Strawberries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of DNA on the stir stick (measure the glob)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Amount of DNA still visible in the vial (circle one choice)</td>
<td>Small amount</td>
<td>Small amount</td>
<td>Small amount</td>
</tr>
<tr>
<td>Color/Clarity of DNA on stir stick</td>
<td>Large Amount</td>
<td>Large Amount</td>
<td>Large Amount</td>
</tr>
<tr>
<td>Viscosity (thickness) of DNA on stir stick - touch it with a gloved finger</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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28. Compare all 3 of your vials of DNA, examine the data in Table 16.1, and answer the following questions.

Which treatment/vial resulted in the **greatest amount** of material being attached to the wooden stir stick?

Why?

Which treatment/vial resulted in the **least amount** of material being attached to the wooden stir stick?

Why?

Which treatment/vial resulted in an amount of material being attached to the wooden stir stick which was **neither the most nor the least amount**?

Explain.

Be sure that you are completely finished comparing all of your results before you clean up with Lysol®.

Pour the DNA from all 3 vials down the sink drain with plenty of H₂O.
Rinse all vials and wooden stir sticks in the sink with plenty of H₂O.
Save the vials.
Throw the wooden stir sticks in the regular trash.
Part 2: Making a DNA Model of One Gene

In the lab you will find colored sheets of paper, each with four rectangular drawings that represent nucleotides. Your group will need eight sheets of each color labeled “DNA Nucleotides.”

29. Remove all of the materials used for Part 1 of this lab from your lab table and clean your table.

30. Find DNA Nucleotide 1, DNA Nucleotide 2, DNA Nucleotide 3, and DNA Nucleotide 4, in the lab. Your group should use 8 pages of each one and cut out the rectangular nucleotides from each page. Put all paper scraps in the paper recycling container.

31. Now your group should have a total of 32 nucleotides (rectangles) of each color.

32. Study the diagrams of the structure of the four types of DNA nucleotides in your text. Compare these diagrams to your cutouts. Determine what the dotted lines, the straight lines, the circle, the large pentagon, and the hexagon or hexagon-pentagon represent.

33. Label the phosphate, sugar, specific base (A, T, C, or G), and hydrogen bonds of the nucleotide on just one nucleotide of each different color of paper. Tape these four labeled nucleotides to a sheet of blank paper.

34. Have your professor check your labels.

35. Write the letter (A, T, C, or G) which represents the nucleotide in the base ring of all your cut-out nucleotides.

36. Table 16.2 shows six different DNA base sequences.

- Your professor will assign your group a number.
- Each of the six DNA sequences, reading from left to right, shows the order of nucleotides (called “bases” for short) for only one strand (the coding strand) of a section of a DNA molecule. Each section of DNA listed for Groups 1-6 represents one (small) gene.
- Notice that each group has a different DNA sequence, which means that the six genes are different. This means that each one is a code for a different protein.

37. Now arrange only the first two nucleotides in the sequence assigned to your group, reading from left to right, to begin building your model of one DNA gene.

Lay just the first nucleotide on the lab table, then lay the second nucleotide next to the bottom edge of the first nucleotide. The sugar-phosphate edges (the left edge) of the two nucleotides will line up with one another, but the bases will form a ragged (right side) edge. Tape the nucleotides together with transparent tape.
You are acting as the enzyme DNA polymerase. DNA polymerase can only add a nucleotide to the sugar side of another DNA nucleotide.

Which type of chemical bond does the tape represent? _________________________

38. **Draw** in the missing covalent bond from the bottom left carbon of the sugar of your first nucleotide to the phosphate of your second nucleotide.

39. **Check with your professor now to be sure you have done this correctly.**

40. Continue adding nucleotides, **one at a time**, until you finish building your coding strand. You will use about half of your nucleotides for this part of the exercise.

41. **Draw** in all the missing bonds as indicated above.

When you are finished, your group has built the **coding strand** (one side) of a DNA section representing one gene.

### Table 16.2 Group Assignments for the DNA Coding Strand

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Nucleotide Sequence for One Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ATGGGACTATGCTTTGACCAGGTTGAAATTGACCAGTCCACAGAGTGA</td>
</tr>
<tr>
<td>2</td>
<td>ATGCCGGGTAACAGCCTGCTTTGGCGAGACGTCGTTGCTCTACCTACATAA</td>
</tr>
<tr>
<td>3</td>
<td>ATGCCGGGGTGGAACCTAAAAGCCTTGACAAGATGTTTTGACTAG</td>
</tr>
<tr>
<td>4</td>
<td>ATGCCCGGTGTTGGATGAACCTAAAAGCCTTGACAAGATGTTTTGACTAG</td>
</tr>
<tr>
<td>5</td>
<td>ATGGGACTACCGGACTTTTTGGGCAAATTGACCGTTGCCACAGAGTGA</td>
</tr>
<tr>
<td>6</td>
<td>ATGGCTCTAACAAGAAACCTTGTGCTATTGTGGTGCGGTCGTCCGTAA</td>
</tr>
</tbody>
</table>

42. How many bp (base pairs) long is your DNA gene? ________

43. **Write the following on the front side of both ends of the completed strand.**
   - Your group number
   - Names of your group members
   - “DNA coding strand.”

44. Group 1 should then give their DNA strand to Group 2, Group 2 will give theirs to Group 3, etc., and the last group will give theirs to Group 1.

45. **Proofread** the strand that your group has been given to make sure that the bases are in the correct sequence. If there are any errors (mutations), return the strand to the group which built it so that they can repair it and return it to your group.

46. **Keep** this coding strand made by the other group and use it for the next steps.
Note: Do the following steps exactly as they are written in the instructions!

Why?
This is how the cell matches up nucleotides. The purpose of this exercise is not just the creation of a colorful strip of paper. It is to help you understand how nucleotides fit together to form a DNA molecule in a cell. Taking shortcuts to get your group's strip of paper made more quickly is pointless and defeats the purpose. One shortcut, that many students are tempted to take, results in you learning incorrect information.

47. Review the rules governing how DNA bases in the two strands match up by complementary base pairing (look in your text for help).

Beginning with the last nucleotide of the coding strand (AKA the 3’ end), place the complementary nucleotide across from it, face up, with the H-bonds touching. You are beginning to build the transcribed strand. Be sure that you are starting at the correct end!

48. Tape the two complementary nucleotides together at the dotted lines.

Which type of chemical bond does the tape represent? __________________________

49. Check with your professor now to be sure you have done this correctly.

50. Add the next nucleotide to the new transcribed DNA strand. Notice that you will tape it to the sugar side (AKA the 3’ end) of the first nucleotide of the new strand, like you did before.

What type of chemical bond does this tape represent? __________________________

51. Continue taping nucleotides to the new strand in sequence, one nucleotide at a time, not skipping around on the strand. Draw in the missing bonds as before.

Now you are acting the part of the enzyme __________________________.

What patterns do you see in the base pairing of each pair of complementary nucleotides?

52. How many hexagonal or pentagonal rings (look at only the bases) are found in each complementary base pair? ____________
53. Write the following on the front side of both ends of the completed strand.

- your group number
- names of your group members
- "DNA transcribed strand"

54. Roll up your DNA model. Your professor will tell you where to store it.

More Questions for Lab 16

55. Complete Table 16.3 with the features that helped you determine the correct name of each nucleotide when you did Part 2 of this lab.

Table 16.3 Features of the DNA Bases

<table>
<thead>
<tr>
<th>Base</th>
<th>Number of rings in the base</th>
<th>Number of hydrogen bonds it forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytosine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

56. Assume that you have a growing strand of DNA nucleotides and it is time to add another DNA nucleotide to it.

Which enzyme would add the new nucleotide? ________________________________

Which end of the growing strand would the new nucleotide bond to? _______________

57. Why are the two strands of the DNA double helix said to run "antiparallel?"

The End!
LAB 17: Genetic Engineering
Using pGLO Plasmids

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

This lab uses the BIO Rad pGLO™ Bacterial Transformation Kit #166-0003EDU.

Complete Parts 1 & 2 of this lab before coming to class.

Part 1: Making Recombinant DNA

Recombinant DNA technology includes a set of techniques for taking one or more genes from one organism and inserting it/them into the genome of another organism. This technology is often referred to as **genetic engineering**.

Note that there are two parts to the process.

» **First** one must **cut out** a specific sequence of DNA (the **gene of interest**), from the **donor** organism.

» **Second** one must **insert** the gene of interest into the DNA of the **recipient** organism.

Identifying a specific gene and removing it from the rest of the DNA is a time-consuming and labor-intensive process. Remember that gooey stuff from the DNA lab? There were not any ACTG-type labels on it—just goo. That’s what molecular biologists have to work with. Once they identify the locus of a gene of interest they will mix the DNA with enzymes called **restriction enzymes** which cut the DNA into small fragments, including the fragment with the gene of interest. Those DNA fragments can be separated from the rest of the DNA by gel electrophoresis, which you will do in a different lab exercise.

In order to insert a gene into another organism’s genome there must be a way to get the gene into the recipient cell. Molecular biologists generally use either **plasmids** (small sort-of-circular pieces of DNA that are normally found in bacteria), or **viruses**, that have the genes placed inside them. Then the recipient organism is exposed to the plasmids or viruses and if all goes well the new genes are inserted into the cells.

The **recipient** organism is often a **bacterium**, such as *E. coli*, but genes from various species may be inserted into the genome of a plant or animal also. The recipient cells are said to have been **transformed** once that they have new genes. After they have new genes, they are said to contain **recombinant DNA**.
Once the gene of interest is inserted into the DNA of another organism it is necessary to get that organism to **use the foreign gene** to make the protein for which the new gene codes. Since all DNA in all organisms is made of the same four nucleotides, it generally can be read and used by the recipient organism.

**Part 2: Plasmids Called pGLO**

Complete Part 2 before you come to class.

Fortunately for you, the first part of the process of genetic recombination has been done for you. The plasmids (called pGLO) already have the genes of interest inserted into them and they are waiting for you in the lab.

Each **pGLO plasmid contains several genes of interest** which are used in biotechnology. Those genes are listed below.

A) Included in this plasmid is a **bla gene**, which has the DNA code to make the **enzyme beta-lactamase**. This enzyme makes cells **resistant to the antibiotic ampicillin**. Normally, harmless strains of **E. coli** like those growing on your starter plates, are readily killed by ampicillin.

B) Cells in the jellyfish **Aequorea victoria** contain the **GFP gene**, which allows the jellyfish to glow in the dark. The pGLO plasmids have a copy of this GFP gene.

C) The **araC gene** turns on the GFP gene, if the sugar called **arabinose** is present.

1. Plasmids are drawn as circles. Use the map at [http://department.monm.edu/chemistry/chemistry330/fall2002/naulclair/Biochem/Determining_structure/blast_procedures.htm](http://department.monm.edu/chemistry/chemistry330/fall2002/naulclair/Biochem/Determining_structure/blast_procedures.htm) to make a simple drawing of one pGLO plasmid in the space below, and **label just** the regions where the **bla**, **araC**, and **GFP** genes are found.

Soon you will transfer the pGLO into cultures of live **E. coli** cells. Read the instructions carefully.
Caution! Important safety instructions.

Any student actually transferring bacteria must wear gloves.

All non-sharp disposable items such as transfer pipettes, transfer loops, gloves, Eppendorf tubes, petri dishes, pipette tips, and paper towels used in this lab should be placed in the BIOHAZARD bag.

Part 3: Inserting the Plasmids into E. coli cells

In this section you will treat harmless live E. coli cells with the chemical CaCl₂ (calcium chloride) and put them on ice, which alters their plasma membranes so that they will easily allow plasmids to enter the cells. This makes them competent cells.

The following instructions are modified from the instructions available in the BIO Rad pGLO™ Bacterial Transformation Kit #166-0003EDU.

Use a micropipetter for transferring liquids for this lab.

2. Your group should have a starter plate of nutrient agar with E. coli growing. The E. coli grows in colonies which look like off-white round bumps on the agar, or if the colonies grow on top of one another, it may look like an off-white smear on the agar.

3. Label one closed Eppendorf tube (+)pGLO, and label another (-)pGLO.

4. Label both tubes with your group’s name, and place them in the foam tube rack.

5. Use a micropipetter with a sterile tip to transfer 250 μL of transformation solution (TS), which contains CaCl₂, to each of your two Eppendorf tubes.

6. Close the two Eppendorf tubes and put them into ice. It is important to keep the DNA cold so that it does not degrade.
7. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Don’t use all of the colonies now because you need more for the next step. Pick up the (+)pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Close the tube and put it back in the tube rack in the ice.

8. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the (-)pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Close the tube and put it back in the tube rack in the ice.

9. Your professor has a vial of a solution of pGLO plasmids. While wearing UV protective eye protection, examine the pGLO plasmid DNA solution with the UV lamp. Write your observations of the color and clarity of the solution with the UV light shining on it.

   Color of the pGLO solution in the vial ___________________________

   Clarity of the pGLO solution in the vial ___________________________

10. Immerse a new sterile loop into the plasmid DNA solution. Withdraw a loopful of the solution. There should be a film of plasmid solution across the ring.

11. Mix the loopful into the cell suspension of the (+)pGLO tube. Close the tube and return it to your professor.

12. Do not add plasmid DNA to the (-)pGLO tube. Why not?

13. Leave the Eppendorf tubes on ice for 10 more minutes.

14. While the tubes are sitting on ice, find your four agar plates. Do not open them. They all contain LB agar (abbreviated LB), a type of agar on which E. coli grows very well.
   Some contain the antibiotic ampicillin (abbreviated as amp).
   Some contain the sugar arabinose (abbreviated as ara).

Label your four nutrient agar plates on the bottom (agar side) as follows.

   Label one LB/amp plate: (+)pGLO.
   Label the other LB/amp plate: (-)pGLO.
   Label the LB/amp/ara plate: (+)pGLO.
   Label the LB plate: (-)pGLO.
15. In the following instructions you will transform your *E. coli* bacteria. 

First you will **heat shock them briefly**, and then you will **cool them rapidly**.

*Be very precise about your timing!*

16. Make sure to push the tubes all the way down in the rack so that the bottoms of the tubes stick out.

Put the foam rack with your (+)pGLO and (-)pGLO tubes **into the 42 °C, water bath for exactly 50 seconds**.

17. **When the 50 seconds are done, place both tubes back on ice for 2 minutes.**

For the best transformation results, the change from the ice (0°C) to 42°C and then back to the ice must be rapid.

After the 2 minutes on ice, place the tubes on the lab table. They are now cold again.

18. Using a new sterile tip, add 250 μL of LB nutrient broth to the (+)pGLO tube.

19. Using another new sterile tip, add 250 μL of LB nutrient broth to the (-)pGLO tube. Close the tubes and leave them for **10 minutes** at room temperature on your lab table.

During this 10 minute time period the cells will recover from the heat shock and **begin using their new genes**, which are in the pGLO plasmids.

20. Tap the closed tubes with your finger to mix their contents, then put them in the foam rack.

21. Turn your 4 small agar plates over, so that each is now agar-side-down.

22. Using a new sterile tip, transfer 100 μL from the (+)pGLO tube to each of the 2 small agar plates which you labeled with (+)pGLO. If needed, spread the liquid with a new sterile loop.

23. Using another new sterile tip, transfer 100 μL from the (-)pGLO tube to each of the 2 small agar plates which you labeled with (-)pGLO. If needed, spread the liquid with a new sterile loop.

24. Let all 4 of the agar plates sit agar-side down for **2 minutes**. This allows time for the materials to soak into the agar.

25. Stack your plates on top of one another and tape them together. Write your group name and class on the tape on the bottom of the stack, and place the stack agar-side-up in the **37°C incubator** for two days. During this time the bacterial cells will grow and multiply.
Place all used loops, tips, gloves, used micropipette tips, paper towels, and Eppendorf tubes and their foam racks in the BIOHAZARD bag in the lab.

When finished, clean your table top with the appropriate cleaning solution and paper towels. Wash your hands thoroughly with soap and water after this lab.

**Part 4: Observe the Results**

1-2 Days Later

26. After the plates have been incubated, examine the plates that your group made.

Which plates show the growth of bacterial colonies?

   Explain why.

Which plates do not show the growth of bacterial colonies?

   Explain why.
27. While wearing UV protective eye protection, shine the UV light onto each of the plates that your group made and determine whether or not the colonies on the plate glow under UV light.

Which plate(s) glow?

Explain why for each one.

Which plate(s) did not glow?

Explain why for each one.

Leave all agar plates (petri dishes) in the proper disposal area in the lab.

Questions for Lab 17

28. What is recombinant DNA technology (or genetic engineering)?

29. What is the function of a plasmid or virus in cell transformation?
30. Explain why the pGLO plasmids in the vial did not glow when the UV light was shined on them.

31. What is GFP?

32. Why were various tubes / vials kept on ice?

33. Why was it necessary to include the araC gene in the pGLO plasmids?

The End!
Lab 18: Making Proteins: Transcription and Translation

Lab 16 must be completed before doing this lab.

- DNA carries the codes for how to make all the different proteins that a cell might need.
- Ribosomes are small structures made of rRNA and proteins.
- Ribosomes are the place where the amino acids actually join together to make polypeptides.

Because ribosomes allow the cell to read and use the code, the right amino acids are able to join in the correct sequence and make proteins. In eukaryotic cells, DNA is stored in the nucleus of the cell, but the ribosomes that actually construct proteins are found outside the nucleus in the cytoplasm. Therefore, before a ribosome can make a protein, it must have a copy of the code. Messenger RNA (mRNA) is the copy of the code.

By making many copies of the original DNA code and sending all the copies (many mRNA molecules) out into the cytoplasm, all the copies can be read by different ribosomes at the same time and proteins can be made faster, and in larger quantities, than if each protein had to be made directly from the DNA in the nucleus.

In this lab your group is going to make a paper model of one molecule of mRNA. The process of making an mRNA copy from the original DNA code (a gene) is called transcription. For this exercise, work in the same groups as when you did Lab 16.

Part 1: RNA Nucleotides

Sheets of colored paper with drawings representing the four RNA nucleotides will be available in the lab. Each group will need four sheets of each color labeled “RNA Nucleotides 1-4.”

1. Find diagrams of the structure of the four types of RNA nucleotides in your text. Compare these diagrams to the RNA nucleotide drawings on your colored sheets. Determine what the dotted lines, the straight lines, the circle, the large pentagon, and the hexagon or hexagon-pentagon represent on your paper RNA nucleotides.
2. Label the phosphate, sugar, specific base (A, U, C, or G) and hydrogen bonds on just one nucleotide of each different color of paper. Tape these four labeled nucleotides to a piece of blank paper.

3. Have your professor check your labels.

4. Write the name of the nucleotide (A, U, C, or G) on the base ring(s) of each nucleotide of every page.

5. Cut out the RNA nucleotides. Put all paper scraps in the paper recycling container.

Part 2: Transcribe One DNA Gene into mRNA

6. Retrieve the paper DNA model for which your group made the original coding strand and lay it on your lab table. Remember that your DNA strand represents just one DNA gene.

   Which part of a cell does your lab table represent now? _________________________

7. Begin at the same end (end with the phosphate group, A.K.A. the 5' end) of the DNA coding strand where you started when you were making the DNA model. Separate the two DNA strands by breaking the hydrogen bonds (cut the tape with scissors) that hold them together. Separate the strands to a point about halfway down the DNA molecule for right now.

   Which enzyme are you acting like when you cut the hydrogen bonds?
   _______________________________________________________________________

8. Start at the free end of the transcribed DNA strand. Yes, the transcribed strand! You are about to transcribe the DNA into mRNA, and for that your cells use the transcribed strand. Match three new RNA nucleotides to the first three DNA nucleotides.

9. A group of three RNA nucleotides is called a codon. What is your first codon? ______

10. Have your professor check your results.

11. Tape the RNA nucleotides together, and also tape the RNA nucleotides to their complimentary DNA nucleotides at the hydrogen bonds.

   Which enzyme are you acting like when you tape the RNA nucleotides together?
   _______________________________________________________________________

12. Add more RNA nucleotides, in sequence, one at a time, to the sugar end (A.K.A. the 3' end) of the previous RNA nucleotide of your growing mRNA strand. Stop when you get close to where the DNA strands are still bonded together.
13. Separate (cut apart) the rest of the two DNA strands and add more RNA nucleotides, one at a time, to the sugar end (A.K.A. the 3’ end) of the previous RNA nucleotide until you run out of DNA.

14. Cut the hydrogen bonds that hold the mRNA and the DNA together, move the mRNA out of the way, and rejoin the two strands of the DNA molecule.

Which enzyme are you acting like when you tape the DNA strands back together?

15. Now your DNA model should be in its original condition, and you also have one new, separate, single-stranded molecule of mRNA.

16. Compare your new mRNA molecule and the DNA coding strand. Remember that the sequence of DNA bases of the coding strand is the genetic information of the gene.

List several ways in which they are alike.

In what way are they different?

17. Write the following on the front side of both ends of the completed mRNA molecule.

- Your group number
- The names of your group members
- “mRNA Molecule”

18. Your professor will tell you where to store your DNA and mRNA molecules.

Part 3: Amino Acids

Proteins are polymers made of amino acids. In other words, to make a protein you must join together many amino acids. Ribosomes provide a place for this to happen.

What is really amazing about ribosomes is that they regulate which amino acids will be joined and in precisely which sequence. They do this by “reading” the mRNA code. In this way, the genetic code of mRNA is translated into a sequence of amino acids, and an actual protein is made. This is the process of translation.

19. Look at the structure of the various amino acids in your text. Compare these to the diagrams of the named amino acids in Fig. 18-1.
20. Your group needs two sheets of Figs. 18-2 and two sheets of 18-3. Label each amino acid on those pages with its correct name.

21. Cut out the newly labeled amino acids of Figs. 18-2 and 18-3 for your group. Put all paper scraps in the paper recycling container.
Fig. 18-1  Amino Acids and the Characteristics of their 
"R" Groups

- Aspartate
- Glutamate
- Lysine
- Histidine
- Arginine
- Serine
- Cysteine
- Asparagine
- Partial charge (polar)
- Glycine
- Threonine
- Isoleucine
- Phenylalanine
- Methionine
- Valine
- Leucine
- Proline
- Tryptophan

Negative charge
Positive charge
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Write the Correct Name on These Unnamed Amino Acids

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**Fig. 18-3**

Write the Correct Name on These Unnamed Amino Acids

<table>
<thead>
<tr>
<th>OH</th>
<th>H-C-H</th>
<th>H-C-Hg</th>
<th>CH3-CH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH2</td>
<td>H-C-OH</td>
<td>H-C-OH</td>
<td>CH2</td>
</tr>
<tr>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NH3+</th>
<th>C-NH</th>
<th>+NH3</th>
<th>CH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH</td>
<td>NH</td>
<td>NH</td>
<td>CH2</td>
</tr>
<tr>
<td>CH2</td>
<td>CH2</td>
<td>CH2</td>
<td>CH2</td>
</tr>
<tr>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C=CH</th>
<th>C-N=C-NH</th>
<th>C=O-O</th>
<th>C-N=CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH2</td>
<td>CH2</td>
<td>CH2</td>
<td>CH2</td>
</tr>
<tr>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
<td>H2N-C-C-OH</td>
</tr>
</tbody>
</table>
Yes, this page is blank! You will cut up the previous page.
Part 4: Translate Your mRNA Molecule

Your group should have a packet with a large paper ribosome model and many cardstock tRNA (transfer RNA) molecule models. You also need your newly made mRNA molecule.

Please do not put tape on the ribosome or the tRNA molecules.

22. Now your lab table represents the cytoplasm of a eukaryotic cell. Lay the large ribosome model near one end of your lab table. Find the end of the RNA strand with the phosphate group at the end, and place the entire mRNA molecule on the lab table so that the first six mRNA nucleotides of that end of the mRNA strand are covering the RNA Codon 1 and Codon 2 positions on the ribosome.

What is your first codon? _________________ This is known as the START codon.

How many mRNA codons are “inside” your ribosome now? _______________

23. Look at your tRNA molecules and compare them to drawings of tRNA molecules in your text. Notice that they all have three letters written along the bottom. These represent three of the RNA nucleotides that make up the tRNA molecule, and are called the anticodon. Find a tRNA with UAC as its anticodon.

Since tRNAs are very specific, your tRNA, with the anticodon UAC, always carries (transfers) the amino acid methionine. In the upper right corner of that tRNA, either Methionine or its abbreviation MET is already printed.

24. Find one methionine amino acid and lay it on top of its printed name on the tRNA (do not tape it).

25. Place the tRNA and its amino acid (methionine) on the proper tRNA space in the ribosome labeled Anticodon Position 1.

Do the codon and the anticodon fit together? ______________

Are the complimentary RNA bases correct? ______________

26. Read the next mRNA codon and place the tRNA with the proper anticodon, and its associated amino acid, on Anticodon Position 2 of the ribosome.

27. Have your professor check your results.

28. Now this simulation gets a bit messy, so be patient!

Pick up just the white MET amino acid (not the tRNA) and lay it on the left side of your second amino acid and tape the two amino acids together.

Which kind of bond does the tape represent? ___________________________
29. Slide the ribosome, underneath the mRNA and the amino acids, so that your second tRNA is now in Anticodon Position 1.

30. The first tRNA will no longer have a place on the ribosome so it gets kicked out of the ribosome and goes back onto your lab table but its amino acid has been left behind.

   How many RNA codons are in the ribosome now? ______________________

31. Determine which tRNA matches the third codon (by complimentary base pairing). Place that tRNA, with its proper amino acid, into Anticodon Position 2. Join the second and third amino acids with tape as before. You now have the beginning of a polypeptide chain made of three amino acids so far.

32. Move the ribosome as you did before, and repeat the process until you finish the first 21 mRNA nucleotides

   How many mRNA codons have been “read” so far? ______________________

   How many amino acids have been joined so far? ______________________.

33. By now you should understand the roles played by the ribosome, mRNA, and tRNAs in this process of making a polypeptide chain. You may also be getting frustrated with the paper model, so, you can switch to a shortcut, even though a real cell does NOT use this shortcut.

   In this shortcut you will stop using the ribosome and the tRNA molecules. Instead you will read the mRNA directly and determine which amino acid is coded for by each mRNA codon.

34. Find the Genetic Code table in your text. Remember that this genetic code is used by scientists to read mRNA. It was put together to help humans read the code found in mRNA.

   Practice using the code, and write which amino acid is called for by the mRNA codon

   AGC _____________ . Have your professor check your answer before you continue.

35. Now, read the rest of the mRNA codons of your mRNA molecule, in sequence, one at a time, and add the correct amino acids, in sequence, to your growing chain of amino acids.

36. The last codon does not code for any amino acid. In a real cell, when the mRNA molecule moves the last codon into Codon Position 2 of the ribosome, there is no additional amino acid to connect. The ribosome would move again, and the chain of amino acids would fall away from the ribosome. For this reason, in the Genetic Code table, this last codon is called a “stop” codon. No more amino acids are added to the chain.
37. Put the ribosome and all of the tRNAs back in the packet. Either give your extra amino acids to your professor or put them in the paper recycle container.

Write the following on the front side of the first amino acid of your polypeptide chain.

- Your group number
- The names of your group members

38. Give your polypeptide chain to your professor to be checked. If it is correct, place your chain in the designated location, since you will need to look at all the chains in order to answer the questions in this lab exercise.

39. Roll up your DNA and mRNA models. Your professor will tell you where to store them.

40. Compare the polypeptide that your group made with the polypeptides on display that all the other groups made. Look back in Lab 16, Table 16.2 as a reminder of the sequences of DNA with which each group began.

a. How are they alike?

b. How are they different?

c. Explain why there are both similarities and differences.

**Part 5: Levels of Protein Structure**

The primary (first level) structure of a protein is the specific sequence of amino acids joined, in the correct order, to begin making that particular protein. *That is what you made in Part 4 of this lab.* The stretch of DNA that makes up a gene, and its mRNA copy, contain the code of genetic instructions for building the primary structure of a protein.

After the amino acids are joined, interactions between adjacent amino acids cause the amino acid chain (the polypeptide) to coil or fold. This coiling or folding forms the secondary (second level) structure of the protein. It would be difficult to do this with your paper polypeptide, so imagine that it has already happened.

Interactions between different amino acids and between the amino acids and the environment inside the cell, cause the polypeptide to fold into even more-convoluted shapes. The final shape, the tertiary (third level) structure, is unique to each protein. This all acts together to form the 3-dimensional third level structure of the protein.
Some proteins fold **two or more** third level structure polypeptide **chains** together into a specific shape. The same forces that shape the folding of a single polypeptide chain also work to join these two or more chains together. This results in forming the **quaternary (fourth level) structure** of a protein, which is present only in those proteins made from two or more polypeptide chains.

The tertiary (and quaternary, if present) structure of a protein, make up its **overall shape**, which determines the **function** of the protein. *A protein “works” because it has a certain characteristic shape. Life itself depends on the thousands of unique shapes of proteins.*

![Note: How proteins fold is a mysterious and challenging process.](image)

Figuring out exactly how proteins do this keeps many research biologists busy these days. Determining the primary sequence of a protein is fairly easy using modern technology. However, given the primary sequence, a powerful computer, and a lot of time, scientists cannot always predict accurately how the polypeptide chain will fold, **so you will not need to fold your short polypeptide.**

**Questions for Lab 18**

41. What is transcription?

42. Write two reasons why **eukaryotic** cells make proteins using mRNA rather than making proteins directly from DNA?

   a.

   b.

43. Which of the two reasons you listed in #42 would be irrelevant in **bacteria**?

   Explain why.
44. Which organelle is the location where amino acids actually join together when a protein is being made?

45. What is a codon?

46. What happens when the ribosome reaches an mRNA stop codon?

47. List and describe all the **levels of organization, also called the levels of protein structure**, (think back to when you first learned about proteins) of what you made in Part 5. Be careful here!

48. Why are the third and fourth levels of protein structure important?

The End!
LAB 19: Cancer Gene Detection

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

Part 1: The p53 Gene

Bring to class the pedigree that your group made during Lab 15.

This lab concerns a gene called p53, which is found on chromosome 17 in humans.

- Gene p53 is an example of a proto-oncogene, which codes for a protein that regulates cell growth.
- Specifically it codes for a tumor suppressor protein which protects you from tumor growth.
- Sometimes a proto-oncogene will mutate which, as you might expect, causes it to code for either an altered protein or no protein at all.
- Such a mutated proto-oncogene is then called an oncogene.
- The result is that any unregulated cell growth that occurs is not halted, and the result is a cancerous growth.

You know from Lab 15 that Valerie had found a lump in her breast. Since then, tests have shown that the tumor is malignant (cancerous). Her disease could be due to either one of the following.

a) mutated p53 alleles which she inherited

or

b) random somatic mutations of p53 (mutations in body cells) which occurred only in Valerie’s breast tissue cells but not in her other body cells.

Diagram of a Small Portion of Human Chromosome 17

Note: Chromosome 17 (the solid line above) has ~1,914 genes, so it would extend way beyond the edges of this page. The map above shows the p53 gene locus as ---------.
The **region** in a proto-oncogene where such mutations are most likely to occur is called a **hot spot**. Sometimes a normal p53 gene has a sequence of **DNA** nucleotides in its hot spot that **mutates and becomes**

...CAGCTG... even though it **was** a different sequence **before** the mutation.

...GTCGAC...

A **restriction enzyme** called *PvuII* will **cut that mutated sequence into two pieces with ends of**

\[ \ldots \text{CAG} \quad \& \quad \text{CTG} \ldots \]

\[ \ldots \text{GTC} \quad \text{GAC} \ldots \]

Notice that this restriction enzyme makes a **straight (blunt) cut, across both strands of the DNA molecule** which means that the cut site does not become **offset** sticky ends.

The five DNA samples you will use are listed in Table 19.1. For this test, your samples for wells 2-5 have only Valerie’s **chromosome 17s**, and not any of her other chromosomes.

<table>
<thead>
<tr>
<th>DNA Sample</th>
<th>Put into Well</th>
<th>Contents of DNA Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Standard DNA fragments</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>Control DNA (complete, whole chromosome 17s)</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td><em>PvuII</em> and Chromosome 17s from Valerie’s blood cells in a tumor-free area of her body</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td><em>PvuII</em> and Chromosome 17s from Valerie’s tumor</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td><em>PvuII</em> and Chromosome 17s from Valerie’s normal breast tissue</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>LANE 6 WILL REMAIN EMPTY</td>
</tr>
</tbody>
</table>

The **results of the gel** from this lab will allow your group to determine the following.

◊ Which of the above possibilities occurred in Valerie (*see bullet points on p. 1*)
◊ Valerie’s overall genotype
◊ The genotype of her affected breast tissue
◊ How likely it is that she has already **passed on** mutated p53 genes to her children

**Part 2: Make Valerie’s DNA Fingerprint**

⚠️ **Caution! Electrical hazard!** Make sure that the power supply is **OFF and UNPLUGGED**.
1. Place the electrophoresis chamber where it will not be bumped or disturbed. Pour all of the electrophoresis buffer provided for your group into the electrophoresis chamber.

2. Remove the comb from the gel by gently and evenly pulling it straight up. A very gentle wiggle of the comb may aid in releasing it from the gel. Be very careful not tear or damage the wells.

3. Caution! You now have a slice of slippery gel resting on a piece of plastic in a liquid!

Place your fingers at both ends of the gel bed (to keep the gel from sliding off) and lay the gel bed with the gel into the electrophoresis chamber with the wells (holes in the gel) closest to the black (negative) electrode. The gel should be submerged in the buffer. Moving or bumping the apparatus could cause the gel to slide off of the gel bed!

4. If your DNA samples are in labeled Eppendorf tubes, you know how to open those. If the DNA samples are in small sealed wells instead, you will need to push the micropipetter tip through the sealed tops of the samples to get to the DNA inside.

Set your micropipetter to 35\(\mu\)L, add a clean micropipetter tip, and extract 35\(\mu\)L of the DNA Standards mixture from Sample A.

5. Load (place) that DNA into the left-most well of your gel, which is in Lane 1 (see Fig. 19.1). Push the micropipetter to only the FIRST stop and hold it there until you lift the tip completely out of the buffer solution.

Discard all used tips into the proper waste container.

6. Place a clean tip on your micropipetter and load 35\(\mu\)L of Sample B, the Control DNA (chromosome 17s) into the adjacent well (Lane 2). Discard the tip.

7. Using a clean tip each time, load:
   - Sample C, \textit{PvuII} and Valerie’s blood cell chromosome 17s, into the third well (Lane 3)
   - Sample D, \textit{PvuII} and Valerie’s tumor cell chromosome 17s, into the fourth well (Lane 4)
   - Sample E, \textit{PvuII} and Valerie’s normal breast tissue chromosome 17s, into the fifth well (Lane 5).

Leave the sixth well empty.

8. Snap the cover firmly onto the electrophoresis chamber. Make sure that the red connector of the cover is linked to the red (+) electrode of the chamber and the black (-) connector of the cover is linked to the black electrode of the chamber.

9. Insert the plug of the black wire into the black input of the power supply and the plug of the red wire into the red input.

10. Ask your professor to check your apparatus to make sure that it is set up and connected correctly.
11. Plug in the power supply and turn it on. Set the voltage to 75 V or less. If the electrical current is flowing properly, small bubbles should form at both of the electrodes. Record the “start” time.

START TIME ______________ STOP TIME ______________

TOTAL RUN TIME ______________

12. DNA molecules have an overall negative charge, so they will be pushed away from the negative black electrode and attracted to the positive red electrode when the power is turned on.

13. Remember from the DNA Lab (Lab 16) that DNA is transparent. However, very short DNA fragments with purple tracking dye were included in each sample. Those small dyed fragments will move toward the positive terminal more quickly than any of the other DNA fragments in your samples, so watch the progress of the tracking dye as it moves through the gel in all five lanes.

Set your timer for 30 minutes.

Look at your gel now.

Is the purple tracking dye moving away from the wells? ______________

Which electrode is it heading toward? ___________________________

While you are waiting, you can finish any other labs that are on the schedule for today, and answer questions #1 and #2 at the end of this lab.

14. When the dye has moved to ¼ inch from the bottom (far end) of the gel, which should take about 40 minutes, turn off the power supply and unplug it.

Record the stop time in the blank above, and calculate the total time of the run.

15. Unplug the red and black wires from the power supply. Carefully, so as not to cause the gel to slide off of the gel bed, remove the cover of the apparatus.

16. The buffer solution may be hot! Wearing gloves, test the temperature of the buffer to make sure that you can reach into it without burning yourself. When it is cool enough, remove the gel by placing your fingers at both ends of the gel bed (to keep the gel from sliding off) and lift the gel in its bed out of the chamber.

17. Put the plastic gel bed with the gel into an empty weigh boat (or glass dish) and gently slide the gel off of the plastic gel bed, leaving just the gel in the weigh boat/glass dish.

18. Cut off a small (roughly the size of a green pea) triangle of the gel from the bottom (farthest from the wells) right corner of the gel. This will allow you to orient the gel...
properly even if it should get flipped upside down at some point. Throw the cut-off piece into the regular trash.

19. Label your weigh boat/glass dish with your group name.

20. Add enough buffer solution from the electrophoresis chamber (or 75mL dH₂O) to submerge the gel under 1 cm of liquid.

**Part 3: Stain Your Gel**

21. Float a blue InstaStain® sheet on the water above the gel, purple side down. Have your professor inspect your materials.

22. Cover the weigh boat/glass dish with plastic wrap and put it in the designated place. The stain will diffuse into the gel and stain the DNA fragments. **You will look at the results in your next lab class.**

**Part 4: Are the Mutations Inherited or Somatic?**

- **Do this during the next lab class.**

23. On Figure 19.1, write the following.

   DNA Standards - above lane 1  
   Control Chromosome 17s - above lane 2  
   Valerie’s Blood - above lane 3  
   Valerie’s Tumor - above lane 4  
   Valerie’s Normal Tissue - above lane 5  
   Remember, **nothing** was loaded into the sixth well.

24. Find your gel.
   - Remove the plastic wrap.
   - Pour the liquid into the proper waste disposal container.
   - Dry the bottom of the weigh boat/glass dish.
   - Have your professor check your gel to be sure that it useable.

**CAUTION**

Place the liquids in the waste disposal area in the lab.

25. Place the gel on a **light box**, with the wells at the top and the cut-out corner at the lower right, and turn on the light. You should see many **blue/purple bands** in the gel. Each band is a pile of stained DNA fragments.

26. The locations of the **DNA Standards** bands have already been added to Fig. 19.1.
Use a pencil and draw the locations of all the DNA bands onto Fig. 19.1 so that the pattern of bands on your drawing matches the pattern on your gel, using the Standards as reference points.

27. Wrap your gel in plastic wrap and leave it on your lab tray.

28. Clean the light box surface.

**Figure 19.1**

<table>
<thead>
<tr>
<th>Lanes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23,130 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9415 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6557 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4361 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3000 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2322 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2027 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>725 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>570 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dispose of any weigh boats and plastic wrap in the regular trash in the lab.
Questions for Lab 19

NOTE: These questions require more than the normal amount of thought and understanding. Be careful and complete.

29. What are the differences between tumor suppressor genes and oncogenes?

30. Let \( B \) represent the normal p53 allele and \( b \) represent the mutated p53 allele.

\[ \text{Remember that everyone has 2 chromosome 17s in every cell.} \]

The types of cancers which are found in Valerie’s family (from Lab 15) are due to mutated p53 genes in which B alleles mutated into b alleles at some time.

Which phenotype (cancerous or cancer-free) would result from each of the following genotypes?

- BB
- Bb
- bb

31. Explain why (in terms of the p53 gene) the number of bands in Lane 3 is different from the number of bands in Lane 2.

Be careful here and be specific. Remember that everyone has 2 chromosome 17s in every cell.
32. Explain why (in terms of the p53 gene) the number of bands in Lane 4 is different from the number of bands in Lane 3. **Be careful here and be specific.**

33. What is the **genotype** of Valerie’s **normal** cells? ____________

   Explain your answer **based on information from your gel results.**

34. Are the alleles in Valerie’s normal cells **inherited** or are they due to **somatic mutations**?

   __________________________________________________________________________

   Explain.

35. What is the **genotype** of Valerie’s **tumor** cells? ________________

   Explain your answer **based on information from your gel results.**
36. Are the alleles in Valerie’s tumor cells **inherited** or they due to **somatic mutations**?

Explain.

37. From just the evidence found in your gel, **how many bp long** is chromosome 17?

38. Based on the evidence found in your gel, draw one chromosome #17 and label the specific correct locus of gene p53.

39. How likely is it that Valerie’s **children** inherited a mutated gene from **her**? I know, you do not know anything about her husband, but this is just about what Valerie can pass on. **Be quantitative.**

Explain your answer.

**The End!**
Lab 20: Paternity Testing

PPE Required - Shoes, long pants or skirt or lab apron, eye protection, gloves

Part 1: Gel Electrophoresis Review

It has been a while since you learned how to load the wells of an agarose gel, so refresh your memory by loading the wells of the practice gel now, using 30 µL of practice gel Loading Solution.

- Remember that the purple liquid is heavier than the solution around the gel, so do not put the tip of the micropipetter deep into the well.
- DO NOT puncture the bottom of the well.
- When loading the well, push the micropipetter button to just the FIRST stop.
- Do NOT let go of the button of the micropipetter until you have lifted the tip completely out of the gel solution.

Part 2: DNA Fingerprinting

In this lab you will determine if either of two men is the father of a woman’s child. The available DNA samples are listed in Table 20.1. These samples have already been cut with the same restriction enzymes and therefore contain various DNA fragments as a result (Part 5 of this lab explains how that works). Your results will be a DNA fingerprint of these five people. Read about DNA fingerprinting in your text before class and learn how and why this technique works.

<table>
<thead>
<tr>
<th>DNA Sample</th>
<th>DNA Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard DNA fragments (see Fig. 20.2). This is a mixture of DNA fragments of known lengths, measured in number of base pairs (bp).</td>
</tr>
<tr>
<td>B</td>
<td>Mother’s DNA</td>
</tr>
<tr>
<td>C</td>
<td>Child’s DNA</td>
</tr>
<tr>
<td>D</td>
<td>Possible Father #1's DNA</td>
</tr>
<tr>
<td>E</td>
<td>Possible Father #2's DNA</td>
</tr>
</tbody>
</table>
Caution! Electrical hazard! Make sure that the power supply is OFF and UNPLUGGED.

1. Place the electrophoresis chamber next to the power supply where it will not be bumped or disturbed. Pour all of the electrophoresis buffer provided for your group into the electrophoresis chamber.

2. Remove the comb from the gel by gently and evenly pulling it straight up. A very gentle wiggle of the comb may aid in releasing it from the gel. Be very careful not tear or damage the wells.

3. Caution! You now have a slice of slippery gel resting on a piece of plastic in a liquid! Place your fingers at both ends of the gel bed (to keep the gel from sliding off) and lay the gel bed into the electrophoresis chamber with the wells (holes in the gel) closest to the black (negative) electrode. The gel should be submerged in the buffer. Moving or bumping the apparatus could cause the gel to slide off of the gel bed!

4. [If your DNA samples are in labeled Eppendorf tubes, you know how to open those.] If the DNA samples are in small sealed wells, you will need to push the micropipetter tip through the sealed tops of the samples to get to the DNA inside. Set your micropipetter to 35μL add a clean micropipetter tip, and extract 35μL of the DNA Standards mixture from Sample A. Push the micropipetter to only the FIRST stop.

5. Load (place) that DNA into the left-most well at the top of your gel, which is in Lane 1 (see Fig. 20.2 in this lab). Push the micropipetter to only the FIRST stop and hold it there until you lift the tip completely out of the buffer solution. Discard all used tips into the proper waste container.

6. Place a clean tip on your micropipetter and load 35μL of the Mother’s DNA into the adjacent well (Lane 2). Discard the tip.

7. Using a clean tip each time, load the Child’s DNA into the third well (Lane 3), Possible Father 1’s DNA into the fourth well (Lane 4), and Possible Father’s 2’s DNA into the fifth well (Lane 5). Leave the sixth well empty.

8. Snap the cover firmly onto the electrophoresis chamber. Make sure that the red connector of the cover is linked to the red (+) electrode of the chamber and the black (-) connector of the cover is linked to the black electrode of the chamber.

9. Insert the plug of the black wire into the black input of the power supply and the plug of the red wire into the red input.
10. **Ask your professor to check your apparatus** to make sure that it is set up and connected correctly.

11. Plug in the power supply and turn it on. Set the voltage to **75 V or less**. If the electrical current is flowing properly, **small bubbles** should form at both of the electrodes. Record the “start” time.

   START TIME _____________   STOP TIME ________________

   TOTAL RUN TIME ________________

12. **DNA molecules have an overall negative charge**, so they will be pushed away from the negative black electrode and attracted to the positive red electrode when the power is turned on.

13. Remember from the **DNA Lab** (Lab 16) that DNA is transparent, however, very short DNA fragments with **purple tracking dye** have been put in each sample. Those small dyed fragments will move toward the positive terminal more quickly than the other DNA fragments in your samples, so watch the progress of the dye as it moves through the gel in all five lanes.

14. Set your timer for **30 minutes**. Have your professor check your gel when the timer rings. You will probably have to let it run longer than just 30 minutes.

![Tip icon] While you are waiting during this and any other down times, you can be working on Parts 4-7 of this lab exercise.

15. When the dye has moved to ¼ inch from the bottom (far end) of the gel, which should take about 40 minutes, turn off the power supply and unplug it. Have your professor check your gel again.

16. Record the stop time in the blank above, and calculate the total time of the run.

17. Unplug the red and black wires from the power supply. Carefully, so as not to cause the gel to slide off of the gel bed, remove the cover of the apparatus.

18. **The buffer solution may be hot!** Wearing gloves, test the temperature of the buffer to make sure that you can reach into it without burning yourself. When it is cool enough, remove the gel by placing your fingers at both ends of the gel bed (to keep the gel from sliding off) and lift the gel in its bed out of the chamber.

19. Put the plastic gel bed with the gel into an **empty culture dish** and gently slide the gel off of the plastic gel bed, leaving just the gel in the culture dish.
20. Cut off a small (roughly the size of a green pea) triangle of the gel from the bottom (farthest from the wells) right corner of the gel. This will allow you to orient the gel properly even if it should get flipped upside down at some point. Throw the cut-off piece into the regular trash.

21. Label your culture dish with your group name.

22. Put the culture dish in the designated location for staining.

23. Add enough dH₂O to submerge the top of the gel under about 1cm of water.

**Part 3: Stain your gel**

24. Float a blue InstaStain® sheet on the water above the gel, purple side down. Have your professor inspect your materials.

25. Cover the culture dish with plastic wrap and put it in the designated place. The stain will diffuse into the gel and stain the DNA fragments. **You will look at the results in your next lab class, in Part 8 of this lab handout.**

**Part 4: Short Tandem repeats (STRs)**

Throughout the human genome there are segments of DNA which are repeated, one after another. If these repeats are short, just a few nucleotides long, they are called **Short Tandem Repeats (STRs)**. One example is “TGTTTA.”

Such a repeated segment may be found on several different chromosomes.

The **number of times** the sequence is repeated varies from location to location in any person’s DNA, and from person to person.

These **STRs** (repeats in the DNA molecules) are **not genes**, and have no known genetic function. They are just part of the random variation found in the “non-gene” regions of the human genome.

26. **For example**, a person might have TGTTTATGTTTATGTTTATGTTTA in one location in their DNA and TGTTTATGTTTATGTTTATGTTTATGTTTA in another location.

How many times is TGTTTA repeated in that person’s

First location? __________________ Second location? __________________

27. **But, someone else might** have TGTTTATGTTTA in the first location in their DNA and TGTTTATGTTTATGTTTATGTTTATGTTTATGTTTATGTTTA in the second location in their DNA.
How many times is TGTTTA repeated in this other person’s

First location? __________________  Second location? __________________

28. The point is that different people have different numbers of STRs in different locations. Identical twins are an exception.

DNA fingerprinting uses these differences in STR numbers to allow us to identify someone if we have a sample of their DNA.

Example: Sara and Carlos Espinosa

Now you will consider the family of Sara and Carlos Espinosa. Tables 20.2 and 20.3 show the DNA nucleotide sequences of a very small portion of Chromosome 20 of both Sara and Carlos. This is the region of Chromosome 20 that happens to contain STRs of “TGTTTA.”

Remember that Sara has one Chromosome 20 which she got from her mother (a maternal Chromosome 20), labeled S-20M. She also got one Chromosome 20 from her father (a paternal Chromosome 20) labeled S-20P.

Likewise, Carlos has two Chromosome 20s. One he got from his mother, labeled C-20M, and one he got from his father, labeled C-20P.

29. Look at the DNA sequences from Sara and Carlos in Tables 20.2 and 20.3. Find all of the TGTTTA repeats in each of their chromosome 20s. Draw a pencil circle around each TGTTTA repeat.

Table 20.2  A Very Small Portion of Sara’s Two Chromosome 20s

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Commentary</th>
</tr>
</thead>
<tbody>
<tr>
<td>- - - - ATGGATCCTGTTTATGTTTAGGATCCGG - - - -</td>
<td>Sara’s maternal chromosome (S-20M)</td>
</tr>
<tr>
<td>- - - - TACCTAGGACAAATACAAATCCTAGGC - - - -</td>
<td>Sara’s maternal chromosome (S-20M)</td>
</tr>
<tr>
<td>- - - - AAGGATCCTGTGTTATGTTTAGGATCCGA- - - -</td>
<td>Sara’s paternal chromosome (S-20P)</td>
</tr>
<tr>
<td>- - - - TTCCTAGGACAAATACAAATACAAATCCTAGGCT - - - -</td>
<td>Sara’s paternal chromosome (S-20P)</td>
</tr>
</tbody>
</table>

30. How many TGTTTA repeats (STRs) are found on Sara’s S-20M chromosome? ______

31. How many TGTTTA repeats (STRs) are found on Sara’s S-20P chromosome? ______
Table 20.3  A Very Small Portion of Carlos’ Two Chromosome 20s

<table>
<thead>
<tr>
<th>Carlos’ maternal chrom. (C-20M)</th>
<th>Carlos’ paternal chrom. (C-20P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- - - - CTGGATCCTGTTTATGTTTATGTTTATGTTTAGGATCCTT - - - -</td>
<td>- - - - GACCTAGGACAAAAATACAAAATACACAAATACACAAATCCTAGGAA - - - -</td>
</tr>
<tr>
<td>- - - - GAGGATCCTGTTTATGTTTAGGATCCGC - - - -</td>
<td>- - - - CTCCTAGGACAAAAATACACAAATCCTAGGCG - - - -</td>
</tr>
</tbody>
</table>

32. How many TGTTTA repeats (STRs) are found on Carlos’ C-20M chromosome? ______

33. How many TGTTTA repeats (STRs) are found on Carlos’ C-20P chromosome? ______

Part 5: Restriction Enzymes, Chromosome Fragments, and RFLPs

Bacteria make enzymes that cut DNA into pieces (fragments), probably to destroy the DNA of other organisms. This protects them against “genetic corruption” by any foreign DNA that might enter the bacterial cell. Such enzymes, called restriction enzymes, only cut DNA at certain specific nucleotide sequences, called recognition sites. Different restriction enzymes recognize and cut different DNA recognition sites.

Overall, different bacteria make more than 1,500 kinds of restriction enzymes that cut DNA molecules at specific nucleotide sequences, and these enzymes have become valuable tools for molecular biologists.

For example, the DNA sequence where the restriction enzyme called BamHI (pronounced BAM-H-one) cuts, is GGATCC:

\[ \begin{align*}
\text{GGATCC} & : : : : :
\end{align*} \]

\[ \begin{align*}
\text{CCTAGG}
\end{align*} \]

BamHI cuts that DNA recognition site as shown below. Arrows show where the cuts occur.

\[ \begin{align*}
\text{G} & \downarrow \text{GATCC} \\
\text{G} & \text{GATCC}
\end{align*} \]

\[ \begin{align*}
\text{CCTAG} & \downarrow \text{G} \\
\text{CCTAG} & \text{G}
\end{align*} \]
34. When the top strand was cut between the first G and the second G, which kind of chemical bonds did the restriction enzyme have to cut ________________?

Which kind of chemical bonds did the restriction enzyme have to cut between the two strands ________________?

**RFLPs**

When a DNA molecule is cut by a restriction enzyme, the resulting DNA fragments (which contain the STR sections) that are cut out are known as DNA fragments called **RFLPs** (pronounced RIFF-lips). RFLP stands for Restriction Fragment Length Polymorphisms, so you can see why we use RFLPs!

35. Go back to Tables 20.2 and 20.3 and draw **red lines** showing where *BamHI* would cut each Chromosome #20 of both Sara and Carlos.

36. Use a highlighter to highlight the “cutout” regions of DNA, which is everything between the cuts. Notice the different lengths of the cutout regions.

Those cut-out regions are called ____________________________________________________________________________.

37. Now look at Tables 20.4 and 20.5, which show all of the “TGTTTA” STR regions that are cut out by *BamHI* in Sara’s and Carlos’ DNA in a simplified form. For simplicity, each STR is shown as two ☀ symbols, one above the other.

38. Count the number of TGTTTA repeats (STRs) in each of Sara’s and Carlos’ Chromosomes #3 16 and 20, and write the numbers in the “Number of STRs present” column in Tables 20.4 and 20.5. Read just the top line of each DNA sequence.

You have just written the **fragment size** (by length) that would be cut from each chromosome if it were exposed to *BamHI*. For example, Sara’s Chromosome S-3M has a 7- repeat fragment length, so write a 7 to the left of that part of the diagram.
### Table 20.4  A Summary of Sara’s TGTTTA Repeats on Chromosomes 3, 16, & 20

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Nucleotide Sequence - Each single STR is represented as two ◦ symbols, one above the other</th>
<th>Number of STRs present</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-3M</td>
<td>- - - CTGGATCC...............GGATCCGA - - - GACCTAGG...............CCTAGGCT - - -</td>
<td></td>
</tr>
<tr>
<td>S-3P</td>
<td>- - - ATGGATCC................GGATCCTT- - - TACCTAGG...............CCTAGGAA - - -</td>
<td></td>
</tr>
<tr>
<td>S-16M</td>
<td>- - - GTGGATCC...............GGATCCAG - - - CACCTAGG...............CCTAGGTC - - -</td>
<td></td>
</tr>
<tr>
<td>S-16P</td>
<td>- - - CGGGATCC................GGATCCCG - - - GCCCTAGG...............CCTAGGGG - - -</td>
<td></td>
</tr>
<tr>
<td>S-20M</td>
<td>- - - AAGGATCC.................GGATCCCG - - - TTCCTAGG...............CCTAGGCC - - -</td>
<td></td>
</tr>
<tr>
<td>S-20P</td>
<td>- - - AAGGATCC.................GGATCCGA - - - TTCCTAGG...............CCTAGGCT - - -</td>
<td></td>
</tr>
</tbody>
</table>

### Table 20.5  A Summary of Carlos’ TGTTTA Repeats on Chromosomes 3, 16, & 20

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Nucleotide Sequence - Each single STR is represented as two ◦ symbols, one above the other</th>
<th>Number of STRs present</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-3M</td>
<td>- - - AAGGATCC.................GGATCCAG - - - TTCCTAGG...............CCTAGGTC - - -</td>
<td></td>
</tr>
<tr>
<td>C-3P</td>
<td>- - - GGGGATCC................GGATCCCG - - - CCCCTAGG...............CCTAGGCC - - -</td>
<td></td>
</tr>
<tr>
<td>C-16M</td>
<td>- - - CCGGATCC...............GGATCCGA - - - GGCCTAGG...............CCTAGGCT - - -</td>
<td></td>
</tr>
<tr>
<td>C-16P</td>
<td>- - - TTGGATCC................GGATCCCG - - - AACCTAGG...............CCTAGGCC - - -</td>
<td></td>
</tr>
<tr>
<td>C-20M</td>
<td>- - - CTGGATCC...............GGATCCTT - - - GACCTAGG...............CCTAGGAA - - -</td>
<td></td>
</tr>
<tr>
<td>C-20P</td>
<td>- - - GAGGATCC.................GGATCCCG - - - CTCCTAGG...............CCTAGGCG - - -</td>
<td></td>
</tr>
</tbody>
</table>
Part 6: Interpreting DNA Fingerprints

Figure 20.1 shows the DNA fingerprints of various members of the Espinosa family. For now, look at Sara’s and Carlos’ in lanes 2 and 3. Each dark line on the gel—called a band—shows where the DNA fragments of a certain length, or number of repeats, ended up.

Lanes 1 and 8 contain the DNA standards, which is a mixture of DNA fragments of specific lengths which were purchased from a DNA lab. This sample of standards ranges from one TGTTTA repeat to twelve TGTTTA repeats long. The bands in these two lanes show where fragments of these known lengths landed, and you can use them as a “ruler” to determine the lengths of the fragments in other bands in the other lanes.

39. Above only the bands from Sara and Carlos, write the original chromosome from which each band came. For examples see the four lines where the original chromosome abbreviations have already been written in for you—each has an asterisk.

40. The only bands that are shown in these results are the bands showing where the STRs landed.

Have your professor check your results before you continue.

41. Why is it that two different DNA fragments with their STRs can land in the same place in the gel to make one band?

Part 7: Whose baby is she?

Part 7-A: The Children

Sara and Carlos have a son, Carlos, Jr. and a daughter, Elena. Sara is expecting their third child.

42. DNA fingerprints for Carlos, Jr. and Elena are shown in Figure 20.1. For each band on their DNA fingerprints, write which parent gave them each STR and on which chromosome it is located.

For example - Carlos, Jr.’s 6-repeat STR and Elena’s 4-repeat STR have been done for you, where S-16M means it came from Sara’s 16M chromosome, and C-20M means it came from Carlos’ C-20M chromosome.
**Figure 20.1** DNA Fingerprints of the Espinosa Family and the Two Babies

<table>
<thead>
<tr>
<th>Lane 1</th>
<th>Lane 2</th>
<th>Lane 3</th>
<th>Lane 4</th>
<th>Lane 5</th>
<th>Lane 6</th>
<th>Lane 7</th>
<th>Lane 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Standards (Number of STRs)</td>
<td>Sara</td>
<td>Carlos</td>
<td>Carlos Jr.</td>
<td>Elena</td>
<td>Rachel?</td>
<td>Molly?</td>
<td>DNA Standards (Number of STRs)</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>10</td>
<td></td>
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<tr>
<td>9</td>
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<td>8</td>
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<td>7</td>
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<td>6</td>
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<td>3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* These answers have already been written for you. They are correct.
Part 7-B: The Mystery

Sara goes into labor on a Tuesday afternoon. Lucky for her, it’s Carlos’ day off and Sara’s parents are visiting. Since the contractions are strong and surprisingly close together, Carlos, Sara, and Sara’s mother jump into the minivan and rush off to the hospital. Her father stays home with Carlos Jr. and Elena.

This baby’s not waiting for anyone, though, and by the time Carlos races into the driveway of the emergency room, the baby has been born. It’s a girl. They’d already chosen Rachel as the name if the baby was a girl.

By a weird coincidence, Mrs. Fuentes also went into labor that afternoon. She, however, was not so lucky. Her husband was out of town on business, her parents live in Oregon, and she was home alone. She called a cab to go to the hospital.

The Fuentes baby was also in a hurry. Halfway to the hospital, the cab driver (Molly Gaines, age 22, who had never had a baby, never seen one born, and didn’t know anything, really, about birthing babies) realized she was going to have to pull off the freeway and help Mrs. Fuentes. Although she’d radioed for help, she’d already helped Mrs. Fuentes deliver her baby (a girl, who, of course, would be named Molly) by the time a police car pulled up.

With the police car leading the way, and Mrs. Fuentes and little Molly apparently doing okay in the back seat, Molly raced off to the hospital. Guess who else is also pulling into the ER driveway.

Imagine the scene: The minivan, the police car, the taxi cab, two new baby girls, two women who have just given birth in motor vehicles, a father, a mother-in-law, a cab driver, police officers, doctors, nurses, orderlies. (Imagine the story in the local newspaper the next day!)

Later that evening, things have calmed down. Sara’s father has brought Carlos, Jr. and Elena to meet their new sister, Mr. Fuentes made it back from his business trip. But the Fuentes and Espinosa families start to wonder a little. Both new babies were hurried off to the nursery at the same time. The Espinosas are just about positive that the baby girl they’re oohing and aahing over is Rachel, but what if . . . ?

The same thought has occurred to the Fuentes family. Just to make absolutely sure, they ask for DNA tests to be done. The DNA fingerprint of the baby the Espinosas were given (Rachel?) is shown in Figure 20.1.

The DNA fingerprint of the other baby (Molly?) was done on the same gel. Her DNA showed STRs of 12 repeats, 10 repeats, 8 repeats, 7 repeats, 5 repeats, and 2 repeats.

43. Draw lines (the bands) on the gel diagram to represent the DNA fingerprint of the other baby (Molly?).
Part 7-C: The Solution

Just in case you want to know, Sara is definitely the mother and Carlos is definitely the father of Carlos Jr., Elena, and their new daughter.

44. Why does Sara’s DNA fingerprint show six bands, while Carlos’ shows only five?

45. Since Sara and Carlos both have 2-repeat STRs, why doesn’t Elena have one?

46. Is the baby being called Rachel really Sara and Carlos’ child? _________________

Were the two families given the right babies? ________________________________

Explain how you know for sure.

Part 8: Who is the Father?

[do this during the second lab class]

47. On Figure 20.2, write the following.

DNA Standards above lane 1
Mother’s DNA above lane 2
Child’s DNA above lane 3
Possible Father #1 above lane 4
Possible Father #2 above lane 5
Remember, nothing was loaded into the sixth well.
48. Wearing gloves, retrieve your gel.
   - Remove the plastic wrap.
   - Pour the liquid into the proper waste disposal container.
   - Rinse the gel with dH₂O and pour the liquid into the proper waste disposal container.
   - Blot the gel dry.

**CAUTION**

**HAZARDOUS WASTE**

**Place the liquids in the waste disposal area in the lab.**

49. Place your gel on a **light box**, with the wells at the top and the cut-out corner at the lower right, and turn on the light. You should see many **blue/purple bands** in the gel. Each band is a pile of stained DNA fragments.

**Have your professor check your gel to be sure that it is useable.**

50. **The probable locations of the Standards bands have already been added to Fig. 20.2.**

   Use a pencil and draw the locations of all of the DNA bands from your gel onto Fig. 20.2, using the Standards as reference points, so that the pattern of bands on your drawing matches the pattern on your gel.

51. **Who was the father – Possible Father #1 or Possible Father #2”?**

   Explain how you know.

52. **Leave your weigh boat with your gel on your lab tray**

   **Dispose of any weigh boats and plastic wrap in the regular trash in the lab.**
Table 20.4

<table>
<thead>
<tr>
<th>Lanes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23,130 bp</td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9415 bp</td>
<td></td>
<td>=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6557 bp</td>
<td></td>
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<td>=</td>
<td></td>
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<td></td>
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<tr>
<td>4361 bp</td>
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<td></td>
<td>=</td>
<td></td>
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<tr>
<td>3000 bp</td>
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<td>=</td>
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<tr>
<td>2322 bp</td>
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<td></td>
<td></td>
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<tr>
<td>2027 bp</td>
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<tr>
<td>725 bp</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>570 bp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DNA Standards**

[red positive electrode]

**The End - Really!!!**