INTRODUCTION TO MOLECULAR BIOLOGY LAB TECHNIQUES

Molecular biology is the branch of biology that studies the composition, structure, and interactions of cellular structures such as DNA. This lab introduces you to equipment and practices that are commonly used in the field of molecular biology. The knowledge and proficiency gained in this lab will be critical to your success in future labs in which you will be working with DNA

Upon completion of this lab, you will be able to:

- Apply aseptic technique
- Make an agarose gel
- Properly use micropipettes
- Create mixtures and use a microcentrifuge
- Load a gel and run gel electrophoresis

Procedure 1. Creating a sterile environment using aseptic technique

We begin with an exercise that will give you a better understanding of aseptic technique and how to apply it. Aseptic technique refers to laboratory practices that reduce sample contamination by microorganisms such as bacteria and fungi. This semester we will use aseptic technique to prevent contamination when working with DNA samples.

DID YOU KNOW? The word "aseptic" means "without microorganisms". This is derived by combining the prefix "a-"meaning "not", and "septic", from Greek "septikos", meaning purifying.

Using aseptic technique in BSC2010 Lab will encompass the following practices that you should now read and follow before starting Procedure 2:

1. Obtain and wear gloves for the duration of the lab.

2. Clean lab bench and any other working surfaces with a 10% Lysol solution.

3. Upon completion of the lab you are expected to clean and re-organize your work area before leaving. Please tidy up your lab stations by putting everything back the way it was when you entered the room. Once everything is back in its proper place, please wipe down the lab bench and any other working surface with the Lysol solution. Failure to follow these steps will result in reduced participation points for the day's lab for your entire group.

4. Finally, before leaving the lab it is always a good idea to wash your hands thoroughly with soap and water.

5. You may now proceed to Procedure 2, but do not forget to return to steps 3 & 4 once you have completed all of today's procedures.

Procedure 2. Making an agarose gel

Gel electrophoresis is a technique for separating nucleic acids or proteins on the basis of their size and electrical charge, both of which affect their rate of movement through an electrical field in a gel made of agarose or another polymer. Agarose gel is a matrix structure with channels and pores through which biomolecules can pass. In the following experiment you will learn how to prepare and load an agarose gel. Your finished gel will be used later in Procedure 5, where you will use an electrophoresis system to separate several dyes that are of different molecular sizes and carry different electrical charges.

1. The procedure should be performed in groups with no more than 4 students.
2. In this step you will make 40 mL of a 0.8% agarose gel solution. Obtain the 125 mL flask with a pre-measured amount (0.32 g) of agarose powder. Add 40 mL of 1X TBE (Tris-Borate-EDTA) buffer to the flask.

FUN FACT: Agarose powder is derived from seaweed and forms a gel when dissolved in liquid.

3. Place a magnetic stir bar in the flask.
4. Dissolve the agarose powder by heating the solution on a stirring hot plate while continuously stirring with the stir bar. The solution should appear clear like water once the powder is completely dissolved.
5. While the agarose is heating, seal the ends of the gel casting tray with the rubber end caps.
6. Wearing a heat resistant glove, remove the flask from the hot plate. Monitor the temperature of the gel until it cools to 55ºC.
7. While the gel is cooling, obtain the well comb. Locate the side with the smallest teeth. Place this side downward into the middle notch of the gel casting tray.
8. Once cooled to 55ºC, pour the agarose gel solution into the gel casting tray. Quickly inspect the surface of the gel for the presence of bubbles. If bubbles are present, use a clean micropipette tip to pop them before the gel solidifies.

NOTE: In future labs involving DNA, the comb will be placed in the notch closest to the negative electrode. This is because DNA has a negative charge and will move towards the positive electrode.

9. The gel should solidify within 20 minutes. As the gel solidifies, it should stiffen and become less transparent. Proceed to Procedure 3 while gel is setting.

Procedure 3. Micropipetting practice

Measuring and dispensing small volumes of liquids is a critical skill in molecular biology labs. The smaller the volumes, the more difficult this is, and the more important it is to do so accurately and precisely. Micropipettes are sophisticated instruments designed specifically to measure and dispense small volumes. In this procedure, you will learn and practice proper micropipetting techniques.

1. Each person in the group should obtain a micropipette. Look at the micropipette to identify its measuring range. The lowest and highest values listed are the smallest and largest respective volumes that can be measured with that pipet. For example, on a 100 to 1000 µL micropipette, the smallest measurable volume is 100 µL and the largest measurable volume is 1000 µL. **What is the volume range for your micropipette, including units?**

2. Discuss your answer to #1 with the other members of your group. Be sure that everyone in the group is in agreeance of the volume ranges for each person's micropipette.

3. Identify the micropipette tip that corresponds to your micropipette.

4. Each person in the group should obtain a 1.5 mL microcentrifuge tube and label the lid with their initials.

5. Use an electronic balance to determine the mass of the empty tube. **Record the mass (to nearest 0.01 g) of your empty tube here.**

6. In the following steps you will be pipetting 600 µL of distilled water from a small beaker into your microcentrifuge tube. Within your group, look at the micropipettes available at your lab bench and choose the correct size micropipette for transferring 600 µL. The person that has this micropipette should set it to this volume.

IMPORTANT:DO NOT try to force the volume-setting dial above or below its stated range. Doing so can break the micropipette.

7. Locate the box that contains the tips for transferring 600 µL of solution. Open the box but do not touch the tips with your hands. Lower the tapered end of the micropipette onto one of the tips then push firmly. Lift the micropipette and attached tip from the box and **close the box**. Always keep the box closed when tips are not being obtained.

8. Using one hand, hold the micropipette and press down on the plunger with your thumb or index finger (whichever feels more comfortable). Note that there are two places the plunger stops. **The first stop is for filling the tip and the second is for dispensing the solution**. Practice a few times until you can easily feel the difference between the two stops.

9. Press down to the first stop. Submerge the end of the tip just under the surface of the distilled water that you poured in a small beaker. **If you submerge more than just the end of the tip, liquid will collect on the sides of the tip** and drip into the collection when you deliver it. This will result in a larger volume of liquid than was desired.

10. While the end of the tip is still submerged, slowly release the plunger. If you release the plunger too quickly, the liquid may splash upwards into the micropipette resulting in less volume that can be dispensed. If you are pipetting viscous (thick) liquids and you release too quickly the liquid won't enter the tip fast enough and your measurement will be inaccurate. Thus, **you should always pipette slowly**. Be careful not to remove the tip from the liquid before it is filled with the desired volume or you will get an air bubble in the tip and less liquid than was desired. If you released the plunger slowly and kept the tip in the liquid but you still got a bubble, you probably pushed the plunger down to the second stop instead of the first. If that was the case, practice the stops again.

11. **While the end of the tip is still submerged, slowly release the plunger.** Carefully lift the micropipette with tip containing 600 µL of distilled water. Slowly and carefully dispense the solution into the 1.5 mL microcentrifuge tube. Look to see if the solution is near the 0.6 mL mark on the tube. This is just a quick check to make sure you used the correct micropipette and set it correctly. Keep your tube so that you can compare with the other members of your group in step 14.

12. Pass the micropipette to the next student in the group who will repeat this procedure starting at step 8. While you are waiting for the other students in your group to complete the procedure, practice opening and closing microcentrifuge tubes with one hand and setting another micropipette with the other hand. Instructions on how to do so can be found above in step 7.

13. At this point, all students in the group should have transferred 600 µL of distilled water into their respective microcentrifuge tubes. Compare the volume of tube contents with your lab partners. Be sure that everyone in the group has transferred the correct volume of solution. Any volume discrepancies among members in your group should be brought to your instructor's attention. It is crucial that each student knows how to properly use a micropipette before proceeding. **Were there any volume discrepancies in your group? If so, to what did you attribute these differences?**

14. A better check for accuracy is by weighing. The density of distilled water is 1 g/mL. **If there are 1000 µL in 1 mL, how many grams should 600 µL of distilled water weigh?**

15. Measure and record the mass (to the nearest 0.01 g) of your tube plus its contents.

16. **Calculate the mass of the contents by subtracting the initial tube weight (see #5) from the mass of the tube plus solution (see #16). Show your work below.**

17. **Compare the expected mass of the contents (see #15) to their actual mass (see #17). Was there a discrepancy? If so, to what do you attribute this difference?**

18. Each person should discard their micropipette tip into the designated "waste" beaker by pressing the eject button. **Do not dispose of your tube**. Pour out the contents of the microcentrifuge tube into the same waste beaker, rinse and dry your tube. You will use your microcentrifuge tube for Procedure 4

Procedure 4. Using a microcentrifuge

A microcentrifuge is a common piece of lab equipment. It has many uses in molecular biology including mixing substances and collecting small droplets in the bottom of the tube. It works by spinning small (≤ 2 mL) liquid samples at high speeds. In this procedure you will prepare several dye mixtures with the aid of a microcentrifuge.

1. First, you are going to measure different amounts of colored water into the 4 microcentrifuge tubes that were previously used by your group in Procedure 3. Obtain the 4 empty tubes, close them, and label the lids with the numbers 1 – 4. Always label on the top so that it can be read without removing the tube from the rack. Only use a permanent marker, such as a Sharpie, that will not erase or bleed if it gets wet.

2. Using one hand (as described in Proc. 3 step 7), open the lids of all the microcentrifuge tubes so they are ready to receive the solutions. Before you begin measuring, think about what will be the most efficient way of dispensing the amount.

IMPORTANT: Always use a new tip for each different liquid that is being transferred. A new tip should also be used if it touches a liquid other than the one that is being transferred.

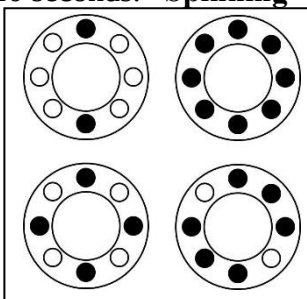
You may want to first fill all of the empty tubes that have the same measure of liquid so you do not have to change the setting too often. Sometimes it matters which ingredient is added first, as is the case when diluting acids and bases. In this procedure, the order does not matter. **Outline the procedure that you plan to use for filling the tubes according to the amounts and contents listed in the table below.**

Tube #	Contents	Total volume (µL)
1	2.5 µL blue & 7.5 µL red	
2	4.0 µL blue & 6.0 µL red	
3	7.5 µL blue & 2.5 µL red	
4	6.0 µL blue & 4.0 µL red	

3. Using the information presented in the table above, measure the amounts of each colored water solution into the indicated tubes. **Mix the contents by pipetting up and down several times in a slow and controlled manner.** DO NOT pipet so vigorously that you make bubbles. This can degrade some sensitive solutions such as enzymes, and can also contaminate the micropipette. You may want to close the tubes as they are filled to avoid accidentally filling the same tube twice.

4. Place the tubes in the microcentrifuge, opposite one another for balance as shown in Figure 1. Close the lid of the microcentrifuge and spin it for 10 seconds. **"Spinning down"** the samples in the microcentrifuge collects all liquid at the bottom of the tube where they are also mixed.

5. Remove your tubes from the microcentrifuge and check the accuracy of your measurements by setting a micropipette to the total volume in the tube (refer to Table 1 above) and slowly withdrawing all of the solution. Your pipetting was accurate if you leave no solution behind and have no air bubble in your micropipette tip. Your instructor may check your tubes before discarding them, as he or she may wish to watch you draw up the amount to check accuracy.



F*igure 1*. *Examples of proper ways to load a microcentrifuge that can hold up to 8 tubes. Note that odd numbers of tubes should never be run in the microcentrifuge.*

Procedure 5. Loading a gel and running gel electrophoresis

You should recall from Procedure 2 that gel electrophoresis is used to separate substances based on their size and electrical charge. In this procedure, you will use your hardened gel from Procedure 2 to run gel electrophoresis on several colored samples that are known to have different molecular sizes and electrical charges.

1. Your agarose gel should now be set. Carefully remove the rubber end caps and comb from the gel casting tray, taking care not to rip the wells. The gel should remain on the casting tray.

2. Place the casting tray with gel on the platform of your group's electrophoresis chamber. Be sure that the negative side of the tray aligns with the negative end of the chamber indicated by the black lead.

3. Fill the chamber with electrophoresis buffer to a level that fills both troughs and just covers the surface of the gel (~ 2 mm). **Make sure that the sample wells left by the comb are completely submerged.** There may be some slight "dimples" left around the wells.

> *NOTE: If your chamber already contains buffer, it is necessary to remix the ions by gently rocking the chamber back and forth. This will redistribute the ions that have accumulated at either end of the chamber.*

4. Each group should obtain the micropipettes that can be used to transfer up to and including 10 µL of solution as well as their appropriate tips. **Which micropipettes (give the volume range) can be used to perform this task?**

5. In this step, your group will add 5-10 µL of six different colored samples into the wells in the gel. Before obtaining and loading the samples you will need to make a drawing of your gel. The gel drawing should show the location of the wells and indicate which sample will be placed in each of the wells along with the initials of the person who will load each sample. Each student should load one or two wells until all six samples are in the gel. **Make your drawing in Figure 2 below, where you will also record the results.**

   After you have made your drawing, you may load the gel with the samples. Remember to use a new tip for each sample. Also be careful not to puncture the bottom of the well.

6. Thoroughly clean up any liquids that may have spilled on your lab bench. Also be sure that the power supply to the electrophoresis chamber is unplugged and switched off before proceeding.

7. Place the lid on the electrophoresis chamber and connect the black and red leads to their corresponding black and red terminals.

8. Plug in the power supply for the electrophoresis chamber, set it to 100 volts (V) and run the machine (runner symbol). The run light will illuminate, signifying that power is running to the cell.

9. Observe the tiny bubbles that form in the troughs along the platinum electrodes.

10. Let the gel run undisturbed for approximately 20 minutes. During this time you should check often to ensure that the dyes do not run off the end of the gel. While gel is running you should also work on answering the review questions below. You may discuss the answers with your group members, but your written responses must be in your own words. This is your homework to be turned in next lab for a grade.

11. After 20 mins, turn off the power supply and unplug the unit.

12. Remove the gel tray with gel from the chamber and place it on several layers of paper towels on your lab bench. Record your observations of your gel results by completing Figure 2 below, where you should have already labeled the wells with samples and student initials. Use colored pencils to indicate the results of your group's gel. Please be neat, as this will be turned in as part of your homework grade.

Name: **Class Day & Time:**

INTRODUCTION TO MOLECULAR BIOLOGY LAB HOMEWORK

Figure 2. Diagram of agarose gel showing locations of loading wells and samples after running electrophoresis.

1. What is meant by aseptic technique? Describe the aseptic technique you will be expected to use in future labs when working with DNA samples.

2. How many µL is the micropipette shown at the right set to measure?

How many mL is this?

3. Why should you avoid touching the micropipette tips?

4. Why should you avoid submerging the micropipette tip too deep in the liquid?

5. What happens if you push the plunger to the second stop before drawing up the liquid?

6. What does the phrase “pipetting up and down” mean and why is this technique used?

7. On what part of a microcentrifuge tube should you write a label?

8. Describe the order in which you filled the tubes in step 2 of Procedure 4. Did this order result in maximum efficiency? If not, what order would be most efficient?

9. What does the phrase “spinning down” mean and why is this technique used?

10. Why was the comb placed in the middle rather than at one end of the gel for this electrophoresis experiment?

11. Which colored sample likely contained the smallest molecule? How did you arise at this conclusion?

12. List the colored samples that have a positive charge. How did you arise at this conclusion?

13. If you were pouring a gel to run DNA through, where would you place the comb? Explain your answer.