

Lab A1

Manipulating Small Volumes

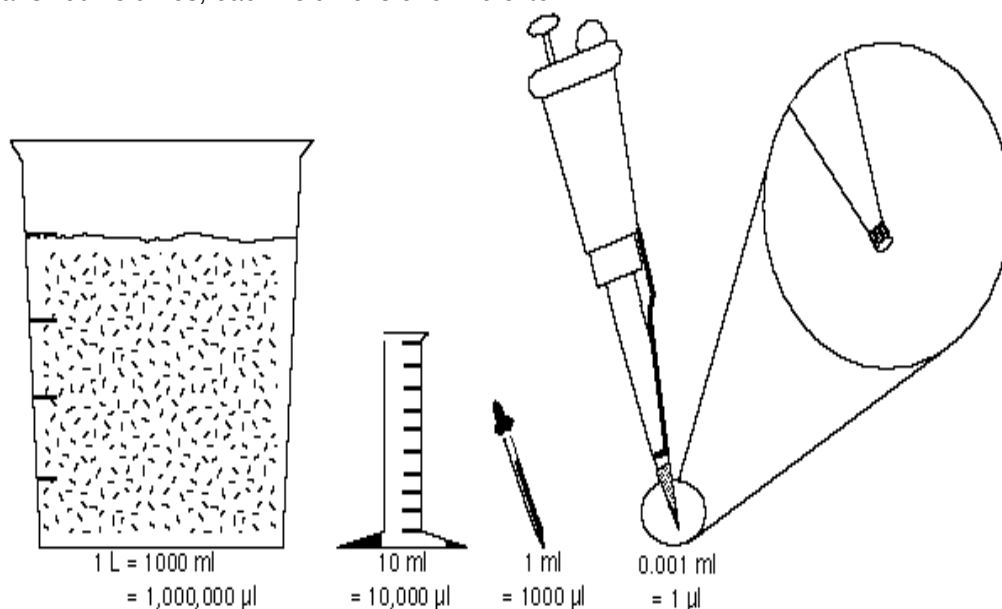
Practice using the p20 and p200 micropipettes, the microcentrifuge, and learn efficient techniques to obtain accurate results.



Background

A forensic scientist removes a microscopic amount of DNA from a dime-sized drop of blood left at the scene of a crime. A cystic fibrosis patient inhales a fine mist containing "good" copies of a gene he did not inherit. When the criminologist and the genetic engineer perform laboratory procedures involving *tiny amounts* of DNA and other chemical solutions, each uses an instrument known as a micropipet.

A micropipet is a kind of fancy eyedropper -- one that comes in many different models and volume ranges. But while an eyedropper dispenses *drops*, micropipets transfer *microliters* of fluid. In the metric system, the basic unit of volume is the liter ("L" or "l"). If you put the prefix "milli-" which means "one-thousandth", in front of liter, you are referring to one thousandth of a liter, or one milliliter ("mL" or "ml"). "Micro-" is a prefix in the metric system, which means "one-millionth" of the base unit. Therefore, one microliter (" μ L" or " μ l") is one-millionth of a liter. It may be easier for you to picture one milliliter (mL or ml) of water. If you mentally divide that milliliter of water into 1000 tiny equal-sized volumes, each volume is one microliter.



Purpose

This laboratory activity introduces micropipetting technique. As with all fine motor skills, learning how to use a micropipet takes practice and determination. You will be rewarded with excellent DNA laboratory results in this and future labs.

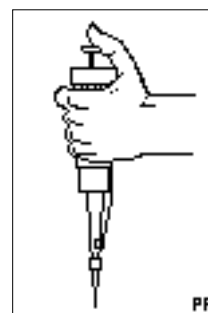
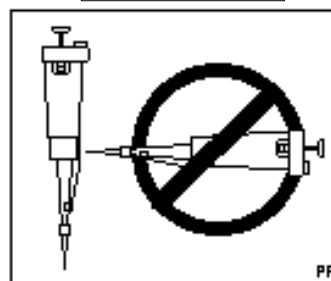
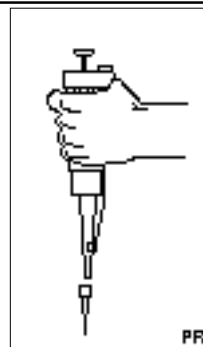
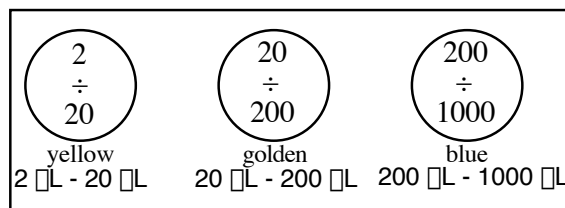
Materials per team

- | | |
|--|---|
| Solution I, colored | supply of 0.5-mL reaction tubes |
| Solution II, colored | rack for reaction tubes |
| Solution III, colored | container for waste (tips) |
| Solution IV, colored | container for liquid waste |
| fine-tip permanent marking pen | paper towels or Kimwipes to dry out tubes |
| P-20 micropipet and yellow tips | P-200 micropipet and yellow tips |
| Microcentrifuge (also called microfuge) w/0.5 mL tube adapters | |

Directions for Using Micropipets

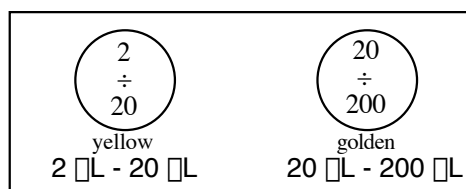
CAUTIONS

- **Set pipet volume only within the range specified for that micropipet.** Do not attempt to set a volume beyond the pipet's minimum or maximum values. The pipet is only accurate in this range and you could also possibly break it if you go too far
- **When using a micropipet, first apply a tip.** Forgetting to do this would cause liquid to be sucked into the nose cone. Since a micropipet works by air displacement (it pushes out air, then sucks up the same amount of liquid), its internal mechanism must remain dry, or it won't be accurate. This also prevents one solution from contaminating later samples.
- **Always keep a micropipet in a vertical position when there is fluid in the tip.** Do not allow liquid to accidentally run back into the nose cone or it will no longer be accurate.
- **Use your thumb to control the speed at which the plunger rises after taking up or ejecting fluid.** Releasing the plunger too abruptly will cause liquid to pop up into the nose cone and damage the piston.

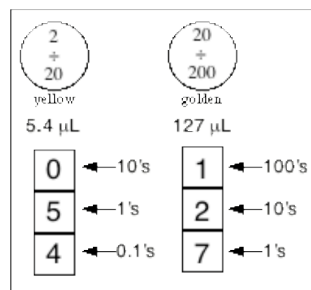


Setting and Preparing the Micropipet

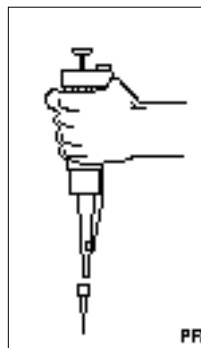
1. Check that you have the right micropipet. There may be two sizes in the lab -- a "P-20" with a yellow plunger (for 2 to 20 μ L) and a "P-200" with a golden plunger (for 20-200 μ L). In some labs, you could also wind up using a "P-1000" (for 200-1000 μ L) that has a blue plunger.



- Dial the desired volume. Each pipet has a window with 3 digits. For the P-20, the top digit is the 10's, the middle digit is the 1's, and the bottom digit (which is separated by a red line) is the 0.1's, so to set it for 5.4 μL , you would put **0 5 4**. For the P-200, the top digit is the 100's, the middle digit is the 10's, the bottom digit is the 1's, so to set it for 127 μL , you would put **1 2 7**.

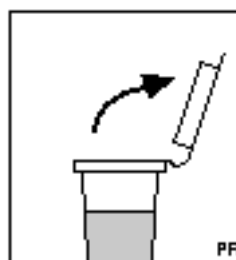


- Push the end of the pipet into the proper-size tip. The yellow tips are for P-20's and P-200's; if you ever use a P-1000, you will use larger blue tips



How to Take Up Sample with a Micropipette

- Before picking up the micropipette, open the cap or lid of the tube from which you are taking fluid (or, have your lab partner do this and hand the tube to you).

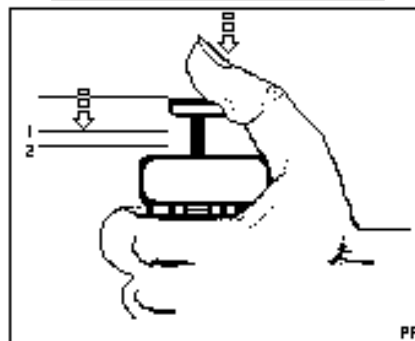


- Hold the micropipette in one hand, almost vertical; hold the tube in your other hand. Both should be at almost eye-level.

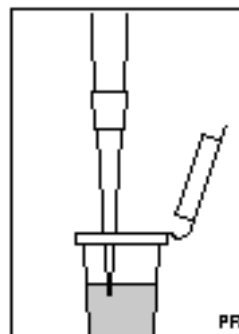
NOTE: the person holding the micropipette is also the person who should be holding the tube!



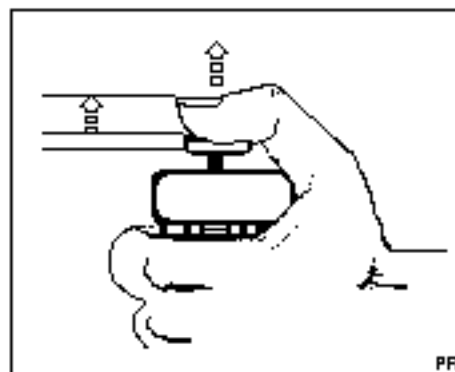
- Before lowering the tip into the liquid**, push down the plunger to the **first** stop and **hold** in this position. (DO NOT GO PAST THE FIRST STOP OR YOU WILL GET AN INCORRECT VOLUME), **then...**



7. ... dip the tip into the solution to be pipetted.

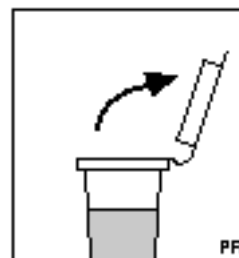


8. Suck fluid into the tip by slowly releasing the plunger. (Cap and put down the tube with the liquid or hand it to your partner.)

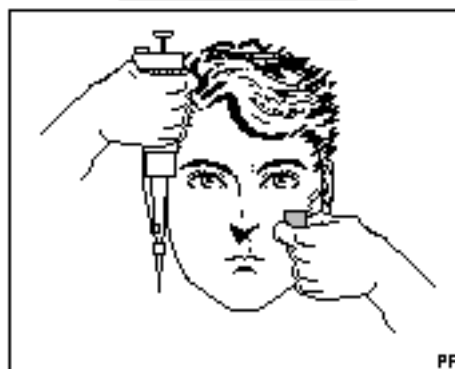


How to Release a Sample From the Micropipette

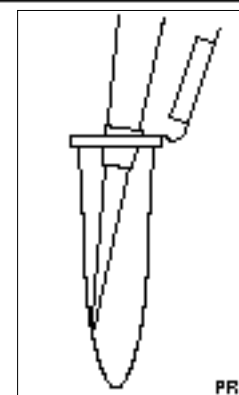
9. With your other hand (or, have your lab partner do this), open the cap or lid of the tube into which you are ejecting the fluid.



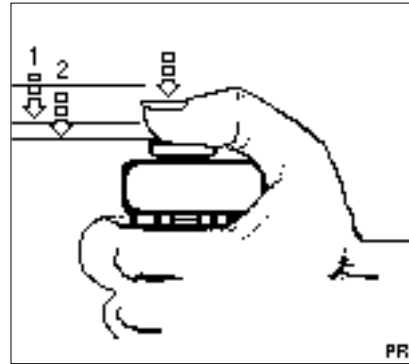
10. Hold the micropipette in one hand, almost vertical and hold the tube in your other hand. Both should be at about eye-level.



11. Touch the micropipette tip to the inside wall of the reaction tube into which you want to squirt out the sample. This creates a tiny surface-tension effect, which helps get fluid out of the tip.



12. **Push** down the plunger of the micropipet to the first stop. Then, **continue to the second stop** to squirt out the last bit of fluid, **and hold the plunger in this position.**



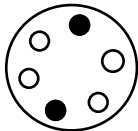
13. Slowly remove the pipet out of the tube, **keeping the plunger pushed down** to avoid sucking any liquid back into the tip.
14. Always change tips for each new reagent you need to pipet. To eject a tip, push down the large blue button on the top of the micropipet.

> **CONVENIENT STOP POINT.** Your teacher may assign additional micropipet training.

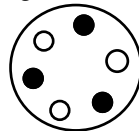
Centrifuge Instructions

- When you are adding several reagents to one tube, release each drop of reagent on the inside wall of the tube near the bottom.
- Tightly close the caps on all the tubes to be placed in the microcentrifuge (also called microfuge).
- The microfuge rotor must always be balanced - you cannot, for example, insert just one tube into a microfuge. Spinning in an unbalanced arrangement like this would damage the motor of the instrument.
- The amount of liquid in the tubes should be similar; otherwise the rotor will spin unevenly (like wet towels spinning out of balance in a washing machine). You can always prepare a "blank" tube with an equal volume of liquid (usually water) with which to balance a single tube.

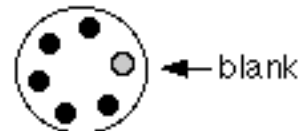
Samples of balanced rotor configurations:



2 tubes

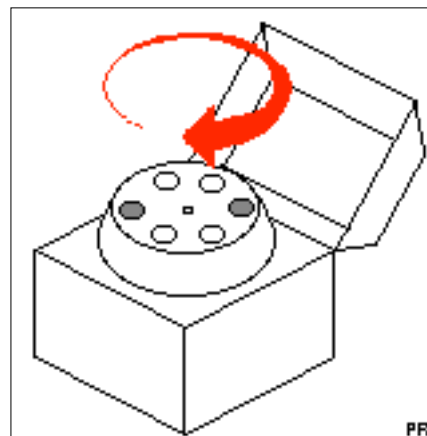


3 tubes



5 tubes (with an extra blank tube)

5. After you closed the lid of the microfuge, give the tubes a 1-2 second pulse. This will mix and pool all the reagents into a droplet in the bottom of each tube.



***NOTE:** REVIEW SECTIONS OF INSTRUCTIONS AS NEEDED AS YOU PROCEED TO PRACTICE MICROPIPETTING*

Activity: Practice with the Micropipets

Check with your instructor to see which parts you should do.

I. Practicing with a P-20

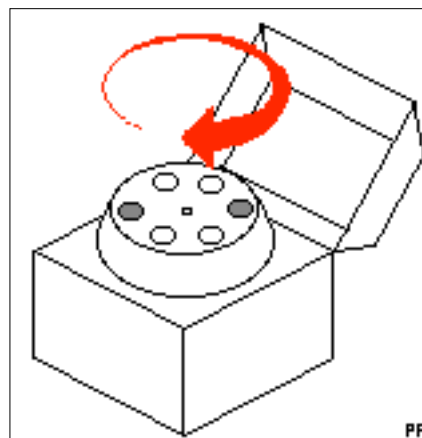
1. Label two empty reaction tubes A and B with a permanent ink marker if not already labeled.
2. Add the amounts of solutions I, II, III and IV to tubes A and B as shown in the table below. **Record** the setting for the pipet for each solution in your lab sheet

Reaction tube	Solution Volume in Each Tube							
	Solution I		Solution II		Solution III		Solution IV	Total in Tube
A	4 μL	+	5 μL	+	2 μL		-	= _____
B	6.5 μL	+	2.5 μL		-	+	2 μL	= _____

3. Spin tubes A and B in the microcentrifuge for 1-2 sec to pool the solutions.

> REFER TO PREVIOUS CENTRIFUGE INSTRUCTIONS

4. Add up the total volume of liquid in Tube A and **record** this in the table on your lab sheet. Do the same thing for tube B.
5. AS A CHECK OF YOUR TECHNIQUE, set the micropipet to the total volume and suck up all of the liquid in tube A. The contents should just fill the tip -- no air space at the bottom of the tip, no leftover liquid in the tube. **Record** your results on your lab sheet according to the following instructions, then do the same thing to tube B.
 - a. If just fills the tip, put a \checkmark .
 - b. If there is air (you didn't have enough), put a - (minus sign).
 - c. If there is left over (too much), put a + (plus sign).Discard liquid and tip into waste containers.



II. Practicing with a P-200

1. Label an empty reaction tube C if not already labeled.
2. Add the amounts of solutions I, II and IV to tube C as shown in the table below. **Record** the setting for the pipet for each solution in your lab sheet

Reaction tube	Solution Volume in Each Tube							
	Solution I		Solution II		Solution III		Solution IV	Total in Tube
C	20 μL	+	40 μL		-	+	100 μL	= _____

3. Spin tube C for 1-2 sec. (Be sure to balance your tube with either a blank or someone else's tube C).
4. Add up the total volume and **record** that on your lab sheet. Check the accuracy of your micropipetting technique with the P-200 and record your results (\checkmark , -, or +).

III. Practicing with all micropipets

1. Label an empty reaction tube D if not already labeled.
2. Write on your lab sheet the appropriate micropipet to use for each sample and what the number setting should be.
3. Add the volumes indicated below using the appropriate micropipet. When done, be sure to spin your tube D for 1-2 sec. (Be sure to balance your tube with either a blank or someone else's tube D).

Reaction tube	Solution Volume in Each Tube				Total in Tube
	Solution I	Solution II	Solution III	Solution IV	
D	15 μ L	+ 105 μ L	+ 12 μ L	+ 38 μ L	= _____

4. Check the accuracy of your micropipetting technique with the P-_____. Set the pipet to _____ μ L and withdraw the contents of tube D.

Assessment X

1. Obtain a reaction tube with 40 μ L of water.
2. Each student in a group of 4 should withdraw 9 μ L of water and put in on a piece of waxed paper as separate drops. Compare drop sizes.
3. The teacher then will withdraw the last 4 μ L.

Assessment Z

1. Obtain a tube with 10 μ L of glycerol.
2. Each student in a group of 4 should withdraw 2 μ L from the tube and place it on a piece of waxed paper as separate drops. Compare drop sizes
3. When everyone in the group is finished, call the teacher over to check your results by withdrawing the last 2 μ L.

Upon completion of this lab

- Remove liquid from all reaction tubes A-D and the two assessment tubes. DRY OUT TUBES and leave for next class. If your teacher has beakers with solutions I-IV available, refill your solution tubes.
- Dispose of designated materials as outlined by your instructor.
- Leave equipment as you found it.
- Check that your workstation is in order.
- Wash your hands.

Lab A1 Activity Sheet

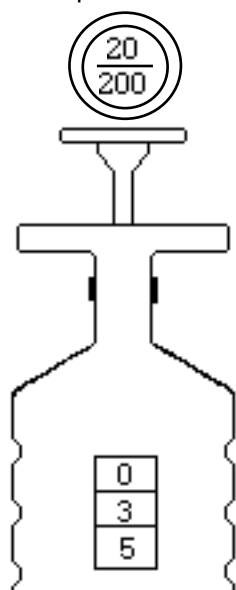
Manipulating Small Volumes



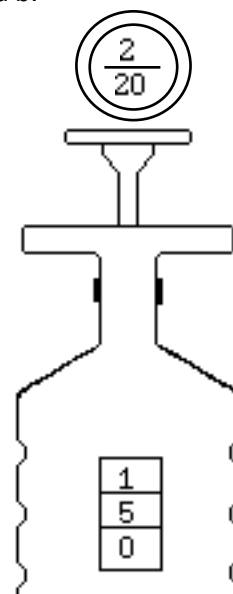
Name: _____
Period: _____ Date: _____

PRELAB:

- Complete the following conversions:
 - $1 \mu\text{L} = \text{_____ mL}$
 - $100 \mu\text{L} = \text{_____ mL}$
 - $250 \mu\text{L} = \text{_____ mL}$
 - $\text{_____ } \mu\text{L} = 1.5 \text{ mL}$
 - $\text{_____ } \mu\text{L} = 0.06 \text{ mL}$
 - $\text{_____ } \mu\text{L} = 0.003 \text{ mL}$
- Put the following volumes in order from largest to smallest.
 - 2.5 mL, 250 μL , 0.025 mL, 2.5 μL : _____, _____, _____, _____.
 - 100 μL , 0.01 mL, 250 μL , 0.015 mL: _____, _____, _____, _____.
- Explain the reason for each of the following rules:
 - Always set the micropipet within its designated range.
 - Always use a micropipet with a tip.
 - Always hold a loaded micropipet in a vertical position.
 - Always release the micropipet plunger slowly.
- Observe the volume of liquid that is measured by micropipets a and b.



a. 35 μL



b. 15 μL

Which micropipet (a or b) is the P-20? _____ What is its range? _____

Which micropipet (a or b) is the P-200? _____ What is its range? _____

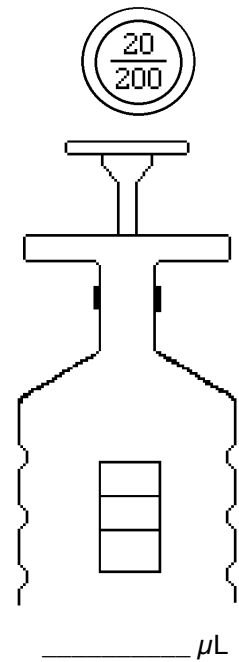
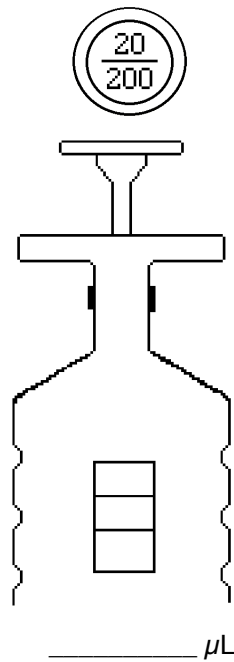
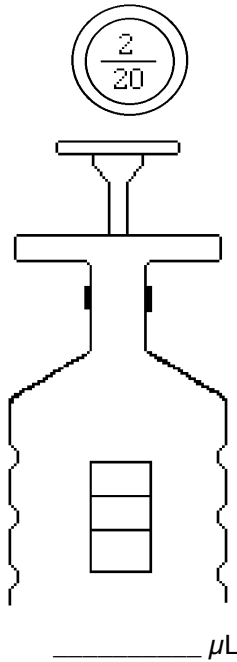
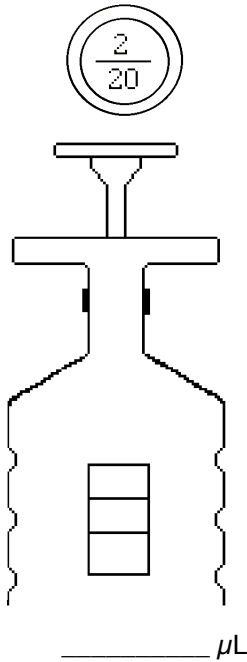
5. Select the appropriate micropipet and show what the dial should read to measure each of the following amounts of liquid. Write the amount on the line beneath each drawing.

a. 150 μL

b. 2.5 μL

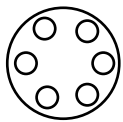
c. 84 μL

d. 7 μL

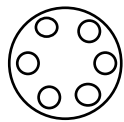


6. Why is it important to balance a centrifuge before turning it on?

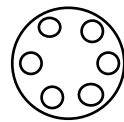
7. Show how you would arrange the given number of tubes in each centrifuge to balance the load. If you decide that you must add or remove tubes, explain.



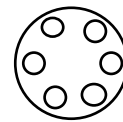
a. 1 tubes



b. 3 tubes



c. 4 tubes



d. 5 tubes

Lab A1 Lab Sheet

Manipulating Small Volumes



Name: _____
 Period: _____ Date: _____

Record your results from tubes A through D below

Tube	Pipet type(s)	Pipet Settings for Solution I	Pipet Settings for Solution II	Pipet Settings for Solution III	Pipet Settings for Solution IV	Total Volume and Settings	Accuracy (- +)
A					none		
B				none			
C				none			
D							

Lab C-1

Casting and Loading an Agarose Gel

Experience with the use of agarose gel, including casting a gel, loading samples, and separating molecules based on charge, size, etc.



Background:

In Techniques Lab B, you investigated the components of electrophoresis. You learned that electrophoresis (a term which literally means "to carry with electricity") is a technique for separating and analyzing mixtures of charged molecules. Clearly this separation wouldn't work very well if the molecules were just sprinkled on the surface of the gel box fluid! Instead, the mixture to be separated is "loaded" into slots or "wells" of a slab of jelly-like material called **agarose**. Agarose is a very pure form of **agar**, which is actually made from a kind of seaweed.

To prepare or "cast" an agarose gel, agarose powder is mixed with buffer, heated, and poured into a **casting or gel tray** containing a **comb**. When the gel has cooled and solidified, the entire casting tray is lowered into the gel box and covered with buffer that allows the electricity to flow and prevents changes in pH. The comb is removed, creating empty **wells**. Then, a micropipet (review Techniques Lab A) is used to place a small amount -- usually just a few microliters -- of the mixture to be separated into each well.

In order to track where the "invisible" DNA runs on a gel, we add two dyes to the DNA sample. One dye runs slightly faster and farther than DNA; the second dye runs slower and not as far as the DNA.

Purpose:

To practice the steps required in the casting and loading of an agarose gel. Observe the migration of four dyes commonly used to track DNA in agarose gels.

Materials per team:

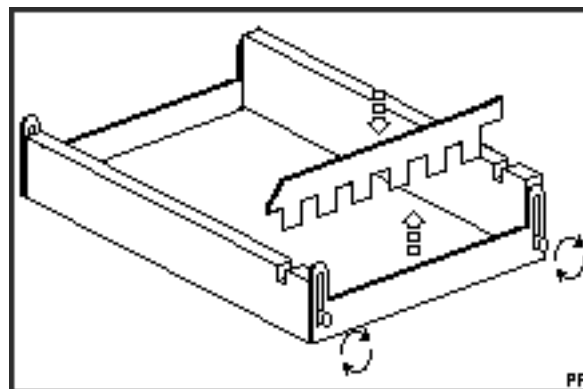
power supply	buffer [1X TAE or TBE]	glass beaker, 50 mL, for agarose
gel box with gel tray	Loading dye	beaker, 500 mL, for buffer
P-20 micropipet & tips	container for waste	liquid soap
paper towels/Kimwipes	microfuge	
agarose, [0.8%], melted and kept hot in a 65°C bath or incubator		

CAUTION:

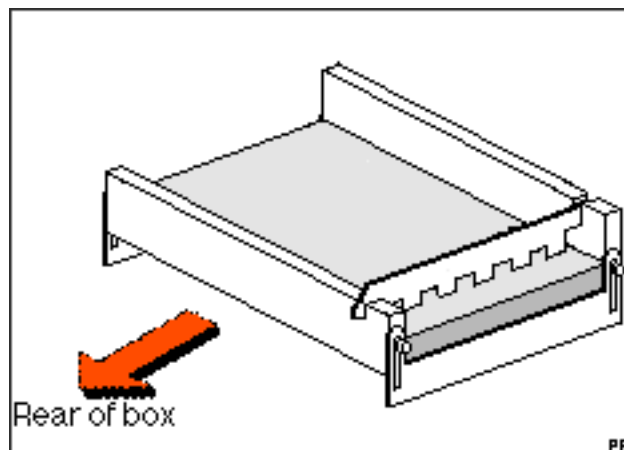
- If two teams are connecting their gel boxes to one power supply, be sure to communicate with each other whenever the power supply is turned ON or OFF. The power supply must be OFF every time anyone needs to touch or open a gel box.

Procedure: Casting a Gel

1. Loosen the screws at the ends of a casting tray, if necessary, to raise the "gates" at each end; then, tighten the screws (not too tight) until there is enough tension to hold the gates in place. Insert a comb in the end slots of the empty tray.
2. Place the prepared casting tray on a paper towel.
3. Obtain a beaker with 25-30 mL of liquid agarose, which has been kept at 65-70°C in a water bath. Pour the agarose evenly into the casting tray. **DO NOT POUR THE GEL IF AGAROSE IS ABOVE 70°C**. Clean and dry the beakers, making sure that none of the agarose is left.
4. **DO NOT JAR or MOVE** the casting tray as the gel solidifies. This ensures a smooth, even gel. As the agarose polymerizes (about 10 minutes), it changes from clear to slightly opaque.

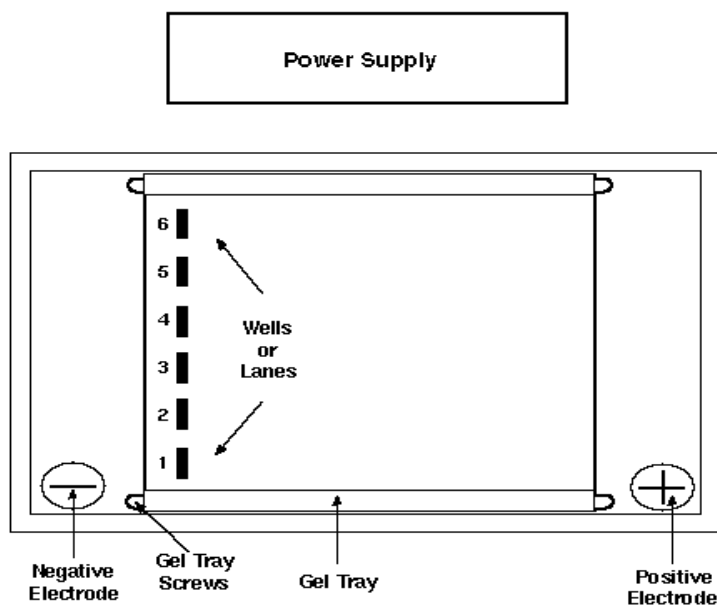


5. While you are waiting, fill the electrophoresis box with about 300 mL of 1X TAE electrophoresis buffer. (A previous class may have done this.) ORIENT THE BOX SO THAT THE WIRES ARE FACING YOU.
6. When the gel has solidified, lower and secure the "gates" at both ends of the casting tray. USING THE HIGHER SIDE OF THE CASTING TRAY AS A HANDLE, TIP THE TRAY SLIGHTLY AND INSERT THE LOWER SIDE OF THE TRAY TO THE REAR OF THE BOX. Submerge the tray onto its platform in the gel box. The comb should be located at the cathode end (black lead; (-) end). The level of the buffer should be only a few mm above the surface of the gel.
7. Carefully remove the comb from the gel (pull it straight out). You'll notice that this left behind six little empty "slots" or wells in the gel. To check to see if there is enough buffer, look to see that there is no "dimpling" of the buffer above the wells. Add more buffer if needed.



Loading the Gel

8. By convention, DNA gels are read from left to right, with the wells located at the top of the gel. With your gel lined up in its box with the wells to your left, the contents of Tube "1" should be loaded in the well closest to you. Thus, when the gel is turned so that the wells are at the top, "1" will be in the left-hand corner.

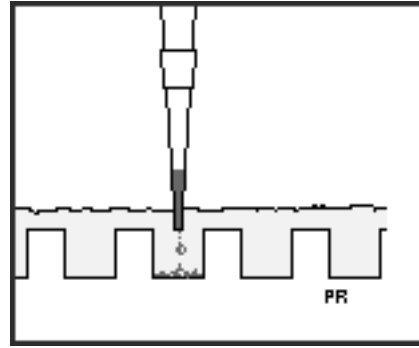


Orientation of gel for loading

Use a P-20 micropipet to practice the technique of loading a well (there are several wells in one gel, so every member of your team can practice this):

- Draw 2 μL of loading dye into the micropipet. (Remember: depress the plunger to the FIRST STOP before lowering the tip into the dye sample.)
- Steady the pipet over the well, using your second hand to support your pipetting hand or arm.

- Lower the tip of the pipet under the surface of the buffer directly over the well -- but do not lower the tip into the well itself, or you risk puncturing the bottom of the gel.
- Gently depress the pipet plunger to slowly expel the loading dye into the well. If the tip of the micropipet is centered over the well, the dye will sink to the bottom of the well.
- REMEMBER - keep the pipet plunger depressed to the **SECOND STOP** until the pipet tip is out of the gel box or you'll draw your sample back into the tip!



Electrophoresis of the Dye

9. Apply ONE drop of liquid soap to a piece of paper towel and spread evenly around the inside of the gel box LID. If previous periods already did this, do not add more soap. **DO NOT GET THE SOAP IN THE BUFFER.** Close the top of the gel box and connect electrical leads anode to anode (red to red) and cathode to cathode (black to black). Both electrodes should be connected to one power supply channel.
10. Set the power supply to approximately 100 V, and turn it ON. To double-check this, switch the display to look at the current: if one gel is running, it should read about 40 milliamps; two gels should read about 80 milliamps. (As a check to see that electricity is flowing, look for bubbles at the wire at either end of the gel box.)
11. Shortly after the voltage is turned on, you should see the dyes slowly moving through the gel toward the positive side of the gel box.
12. Electrophoreses for about 10 minutes. **Record** the location of the dyes on your Activity Sheet, p. 2.
13. Turn off the power and disconnect the leads.
14. Save the buffer for reuse by the next class. Return gels to teacher for reuse after melting.
15. **Record** your observations on your Activity Sheet.

Upon completion of this lab

- Wash empty 50 mL beakers with warm water and towel dry to make sure all the agarose is gone.
- Dispose of designated materials in the appropriate places.
- Leave equipment as you found it.
- Check that your workstation is in order.
- Wash your hands.

Lab C1 Activity Sheet

Casting and Loading an Agarose Gel

Name: _____

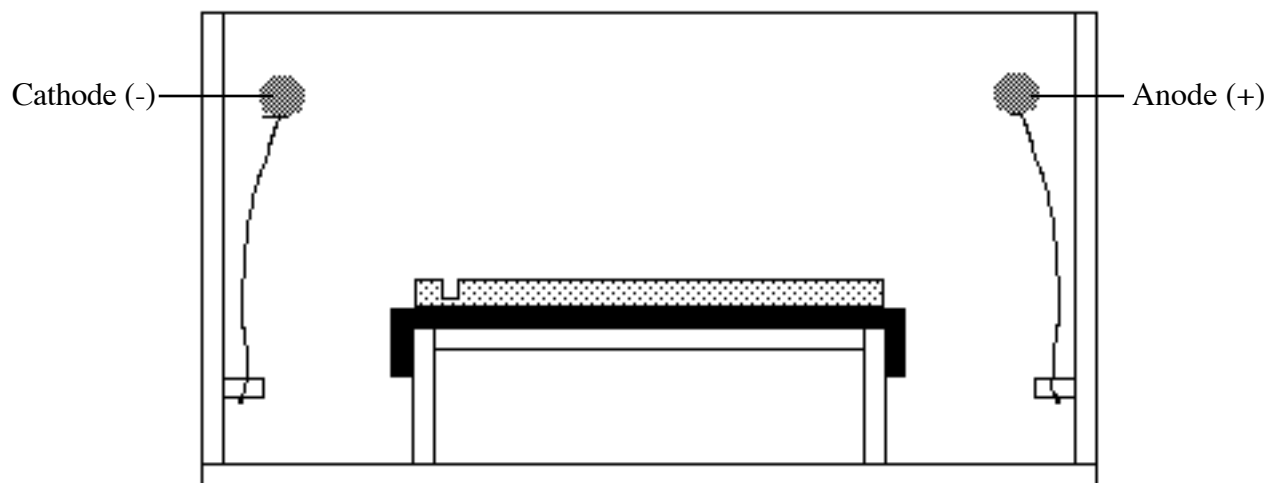
Period: _____ Date: _____



PRELAB:

1. **Record** your answers to questions a-c on the drawing of the electrophoresis gel box below.
 - a. Label the gel, gel tray, and gel tray gates.
 - b. Draw a line to show the desired buffer level. Label the line "buffer level".
 - c. Use a large dot to show the loading dye placement. Label the dot "loading dye".

ELECTROPHORESIS GEL BOX



Front View

2. Why should the agarose gel be left undisturbed while it is solidifying?
3. Why do the gates need to be lowered before you put the gel into the gel box?
4. What is the purpose of adding buffer before removing the comb?
5. When must you consult with others who are using the same power supply? Why is this consultation important?

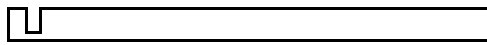
OBSERVATIONS:

6. Record the location of the loading dye(s)* on the diagrams below (BOTH the top and side views)

Fig. A. Gel: immediately after loading

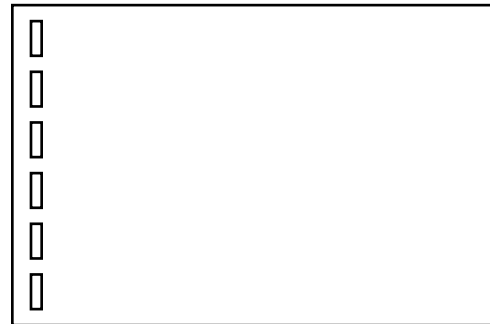


Top view of gel



Side view of gel

Fig. B. Gel: after it has been run.



Top view of gel



Side view of gel

7. Label the positive and negative ends of the gel in Figures A and B.
8. Explain why the dyes move toward the positive (anode) end of the gel.
9. Why would you want the end of the gel with the wells to be closer to the negative (cathode) end of the gel box?

POST LAB:

**Note: Two dyes were combined to form "loading dye". One of these dyes moves faster than the small pieces of DNA, the other moves more slowly than the largest pieces of DNA.*

10. In this activity, DNA was not mixed with this loading dye. However, it will be when we need to separate fragments of DNA. Mark the "Fig. B Gel: After Electrophoresis" drawing (above) to show where you would expect to find DNA.

STRAWBERRY DNA EXTRACTION

Materials (per student)

1 zip lock bag
1 strawberry
10 ml DNA extraction buffer
Filtering apparatus (cheesecloth, funnel, small beaker)
ICE COLD isopropanol
Test tubes
Bamboo skewer/glass rod/etc.

1. Place on strawberry in a Ziploc back and zip it closed.
2. Smash up the strawberry for 2 minutes\
3. Add 10mL extraction buffer to the bag
3. Smash again for 1 minute
4. Pour the extract onto the cheese cloth in the funnel and let it drip into the beaker.
5. Add filtered strawberry mush to a test tube so that it is $\frac{1}{4}$ full
6. Slowly squirt ICE COLD isopropanol down the side of the test tube until it is half full.
7. Dip a bamboo skewer (with bent tip) into the tube right where the alcohol and strawberry extract layers meet and spool the DNA

DNA extraction buffer:

2g NaCl
10 mL detergent (Dawn, tide, make sure has sodium laurel sulfate)
100 mL water

Dissolve Salt in water before adding detergent. Mix gently after adding detergent to keep sudsing to a minimum. Works best if kept in the refrigerator.