An Introduction to Microvolumetrics and Pipetting

The purpose of this laboratory is to provide you with a hands-on experience using some of the important tools and techniques commonly used in molecular biology and introduce you to some of the volumetric measurements that are most often used in this field of science. The laboratory will provide you with an opportunity to practice some of the skills you will need to build a recombinant DNA molecule. The instruments and supplies that you will be using over the next few weeks are identical to the ones that are used in research laboratories.

While the theoretical foundations upon which biotechnology and DNA sciences have been built extend back to the early 1900s, most of the laboratory techniques utilized are relatively recent. And though the techniques you will be learning over the next few weeks have become routine in modern research laboratories, few high school and college students have an opportunity to do such sophisticated molecular biology.

If and when you take a chemistry class, one of the things you will quickly notice is the differences in the quantities of reagents and chemicals that you use. In a typical chemistry lab, volumes are measured in large graduated cylinders. Solutions are often measured in 50, 100 or 200 milliliters (mL) volumes. Weights of solids are generally expressed in grams (g). In the molecular biology lab, volumes are frequently measured in microliters (µL); 1 µL is equal to 0.001 mL. Weights are often expressed in terms of micrograms (µg) or nanograms (ng); 1 µg is equal to 0.000001 gram and 1 ng is equal to 0.000000001 gram.

You might be wondering why molecular biologists use such small volumes and amounts of materials. The reason is related to the cost of these materials and the difficulty involved with obtaining them. For example, you will be given some specially engineered plasmids (DNA) in the next laboratory. If this DNA were sold "by the pound," it would cost around $360,000,000 per pound. So don't be surprised if we only give you a tiny amount of these DNA molecules. The reason why these chemicals are so expensive is related to the difficulty in preparing them in pure form. Many of these chemicals are produced within living organisms, such as bacteria, and have to be purified and separated from all of the other thousands of substances in the cell. Molecular biology, however, really requires this level of purity and precision. As you do this lab work, keep in mind that you are doing real-world molecular biology.

Materials

REAGENT

Solution 1
Solution 2
Solution 3
Distilled H_2O (dH_2O)
0.8% Agarose gel (pre-made)
1 x SB (or 0.5x TBE)

EQUIPMENT & SUPPLIES

1.5 mL microfuge tubes
P-20 micropipettor (2-20 µL)
Disposable pipette tips
Permanent marker
Electrophoresis equipment
Power supply
Plastic microfuge tube rack

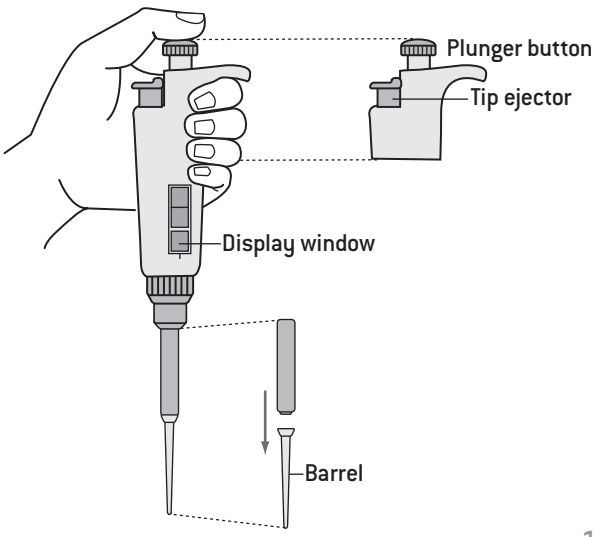
Methods

The Digital Micropipette

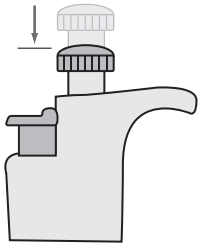
Molecular biology protocols require the use of adjustable micropipettes. Micropipettes are used to dispense different volumes of liquids. While researchers will have several kinds of micropipettes at their lab bench, these laboratories have been designed to utilize a P-20. The P-20 is engineered to dispense liquid volumes between 2 and 20 µL. This is a high-quality, precision instrument, and it is essential that you learn to use it properly.

Please read and follow these precautions:

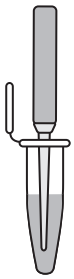
- Do not set the adjustment below 2 µL or above 20 µL unless instructed to do so by your teacher.
- Do not use the micropipette without the proper disposable tip firmly attached to the barrel. Failure to use a pipette tip will contaminate the pipette barrel.
- Do not lay down a micropipette with fluid in the tip or hold it with the tip pointed upward. If the disposable tip is not firmly seated onto the barrel, fluid could leak back into the pipette.
- Avoid letting the plunger "snap" back when withdrawing or ejecting fluid; it will eventually destroy the piston.



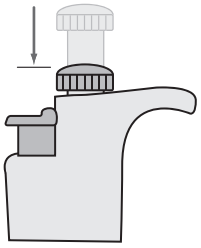
- When aspirating (drawing up) a solution, push the plunger to the *first stop* and lower the pipette tip below the level of the solution that you are sampling. You should be holding the tube containing the solution in your hand about eye level. It's important to actually see the solution enter the pipette tip.



- *Slowly* release the plunger and allow the liquid to move into the pipette tip. Be certain that you're not aspirating air into the tip.



- When dispensing (pushing out) the liquid, place the pipette tip into the tube that will receive the solution. Position the tip so that it touches the side and near the bottom of the tube. Slowly push down on the plunger to the *first stop and then to the second stop*. Keep your thumb on the plunger and remove the tip from the tube into which you're dispensing the liquid. This will avoid reaspirating the liquid into the pipette tip. Be certain that you see the solution leaving the tip.

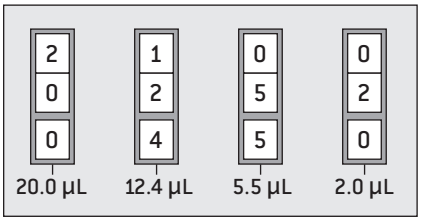


- Remove the tip by ejecting it into a waste container; there is an eject button on the pipette. If you're dispensing the same reagent into separate tubes and there is no danger of cross contamination, you can use the same tip several times. To avoid contamination, it is good practice to deposit each reagent on to the sidewall near the bottom of the microfuge tube without touching any of the other reagents. This technique allows you to use the same tip to dispense a reagent into several tubes that contain a different reagent.

- When dispensing a new reagent, always *use a fresh tip* to avoid contamination.

Pipetting Exercise 1

1 Find the display window on the handle of the micropipette and note its setting. Turn the knurled knob in the handle clockwise to decrease the volume or counterclockwise to increase the volume. Turning this knob changes the distance the plunger will travel. The figures below represent some pipette settings and the volumes of liquid dispensed.



2 Place a disposable tip onto the end of the pipette barrel. Using your thumb and index finger in a twisting motion, check to see that the tip is firmly seated onto the barrel. Avoid touching the pointed end, as this may contaminate the tip.
Remember that you must have a tip in place when using the pipette.

3 Place your thumb on the button that activates the plunger. Push down on this button with your thumb and notice that it has a "stop" position. If you exert a little more pressure with your thumb, you can push the button of the plunger to a second stop. The second stop pushes a small volume of air into the tip to eject the solution.

Pipetting Exercise 2

1. Use a permanent marker to label three reaction tubes A, B and C.

2. The table on page 1.4 summarizes the contents of each tube, but follow the directions that begin with step 3 to set up the samples.

Tube	dH_20	Solution 1	Solution 2	Solution 3	Total volume
A	2 µL	4 µL	–	4 µL	10 µL
B	2 µL	–	8 µL	–	10 µL
C	2 µL	–	–	8 µL	10 µL

3. Set the P-20 micropipette to 2 μL and dispense dH_20 into tubes A, B and C.

4. Eject the tip into the plastic waste container and replace with a fresh tip.

5. Place 4 μL of solution 1 into tube A.

6. Eject the tip into the plastic waste container and replace with a fresh tip.

7. Use a fresh tip and dispense 4 μL of solution 3 into tube A.

8. Use a fresh tip and dispense 8 μL of solution 2 into tube B.

9. Use a fresh tip and dispense 8 μL of solution 3 into tube C.

10. Save all three tubes for the next part of the lab.

Checking the Accuracy and Consistency of Pipetting

1. Tubes A, B and C should each contain 10 μL of solution.

2. Set your P-20 micropipette to 10 μL and place a fresh tip onto the barrel.

3. Carefully check the volume of each of microfuge tube. There should be 10 μL in each of these tubes.

4. Save tubes A, B and C for the next part of the lab.

Using Gel Electrophoresis to Separate Molecules

Gel electrophoresis is a method that uses an electrical current and a gel matrix (meshwork) to separate molecules such as DNA and proteins. The molecules that are being separated are either negatively charged or are made to be negatively charged. Using an electrical current, the charged molecules are then forced through a meshwork of material that will sort out the molecules according to their sizes, although molecular shape and degree of electro-negativity will influence movement through the gel. Because the molecules are negatively charged, they will migrate through the gel toward the positive (red) electrode. The more negatively charged, the faster the molecule will migrate.

In this laboratory, your teacher has made a gel composed of agarose, a polysaccharide (complex sugar). The agarose is mixed with an electrolytic solution called Sodium Borate (SB). This solution contains ions, which are electrically charged atoms. These ions help conduct the electrical current through the gel. As the molecules are drawn toward the positive electrode, the smaller molecules are able to move in and around this agarose network much more quickly than the larger molecules. Thus, over the length of the gel, the molecules become separated by size.

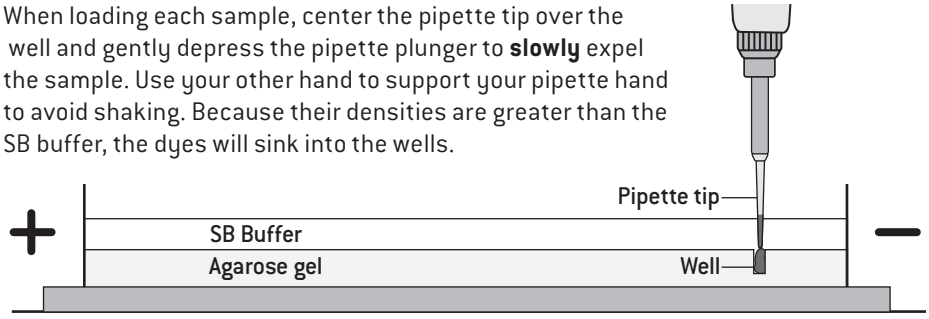
1. Your teacher has already prepared an agarose gel for you, but you will need to cover the agarose gel with the appropriate amount of SB buffer to run the gel properly. Two groups will share each gel. Take the box to the power supply you will use to run the gel.

2. Check to make certain that the gel is positioned in the gel box so that the "wells" of the gel are located toward the negative (black) electrode. The dyes are negatively charged and they will move toward the positive (red) electrode.

3. Fill the box with 1x SB buffer (there are several plastic containers containing this buffer in the lab) to a level that just covers the entire surface of the gel to a depth of 1–2 mm. Check to see that the gel is covered with buffer and that no "dimples" appear over the wells; add more buffer if needed.

4. Set the micropipette to 10 μL and load each sample into a separate well as indicated by your teacher. Use a **fresh tip** for each sample. Remember that your group will be sharing this gel. One group will load their samples in three wells on the left while the other group will use the three wells on the right. You may wish to record which solution you place in each well.

5. When loading each sample, center the pipette tip over the well and gently depress the pipette plunger to **slowly** expel the sample. Use your other hand to support your pipette hand to avoid shaking. Because their densities are greater than the SB buffer, the dyes will sink into the wells.



6. Close the cover tightly over the electrophoresis chamber. Connect the electrical leads to the power supply. Be certain that both leads are connected to the same channel with the cathode (–) to cathode (black to black) and anode (+) to anode (red to red).

7. Turn on the power supply and set the voltage to 130–135 v.

8. After two or three minutes, look at the dyes to make certain they are moving toward the positive (red) electrode. You should begin to see the purple dye (called Bromophenol blue) beginning to separate from the blue dye (Xylene cyanole).

9. In approximately 10 minutes, or when you can distinguish all three dyes, turn **off** the power switch and unplug the electrodes **from the power supply**. Do this by grasping the plug at the power supply—not by yanking on the cord. Carefully remove the cover from the gel box so that you can better see the dyes in the gel.

10. On a piece of notebook paper, record the banding or color pattern in each of the lanes containing your samples. Use this information to answer the questions in the "Conclusions."

11. Leave the gels in the gel box.

Conclusions

1a The dyes that you separated using gel electrophoresis were:
Orange G (yellow), Bromophenol blue (purple) and Xylene cyanole (blue).
What electrical charge did these dyes carry?

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1b What evidence allowed you to arrive at this conclusion?

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2a Molecular size can play a role in separation with small molecules moving through the gel matrix more rapidly than larger molecules. The formula (or molecular) weights for these dyes are Orange G (452.38), Bromophenol blue (669.98) and Xylene cyanole (538.62). From your results, did it appear that these molecules were separated clearly on the basis of size?

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2b What other factors may have played a role in the separation of these dyes?

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2c Which *tube* contained a single dye? A, B or C?

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2d Name this dye.

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3 When aspirating a solution, why is it important to actually *see* the solution enter the pipette tip?

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4a After loading your gel, did any solution remain in tubes A, B or C?

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4b What could account for solution remaining in these tubes?

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