An Introduction to Microvolumetrics and Pipetting

INTRODUCTION

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 two 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 1900's, 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.

When you take a chemistry class, one of the things you will quickly notice is the differences 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, 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 $3,600,000,000 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, like 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

P-20 micropipette (2-20 µL)
P-200 micropipette (50-200 µL)
P-1000 micropipette (100-1000 µL)
Centrifuge
Yellow Solution
Red Solution
Blue Solution
Green Solution
Distilled H_2O (dH_2O)
Four 1.5 mL microfuge tubes
weigh boat
waste container
Disposable pipette tips
Plastic microfuge tube rack
Permanent marker

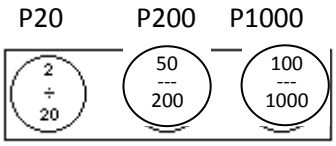
METHODS

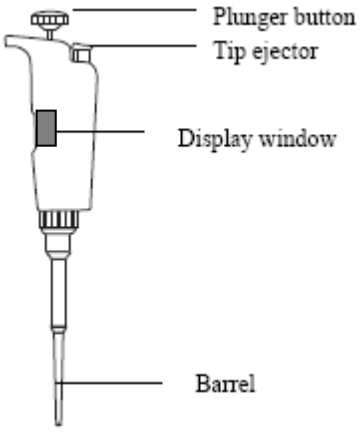
The Micropipette

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 mainly 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.

How to use the micropipette

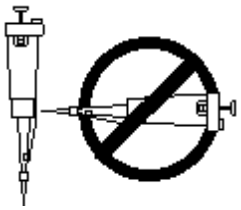
Please read and follow these precautions:





• Be careful to always select the correct micropipette and to keep each micropipette volume within its range.

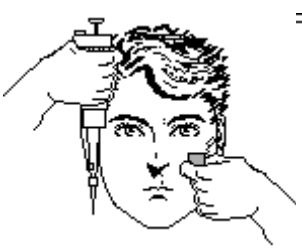
• Do not use the micropipette without the proper disposable tip or the pipette barrel will be contaminated.

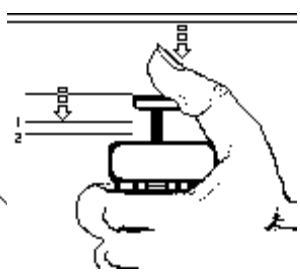


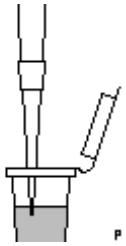
• Do not lay down a micropipette with fluid in the tip or hold it with the tip pointed upward, fluid could leak back into the barrel of the pipette.

- If the disposable tip is not firmly seated onto the barrel, the pipette will not take up or expel the full dialed volume.

• Avoid letting the plunger "snap" back when withdrawing or ejecting fluid; it will eventually destroy the piston and may pull fluid up into the pipette barrel .

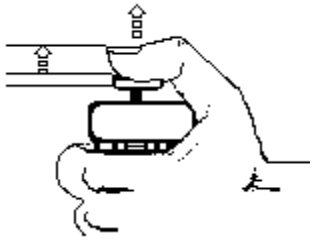




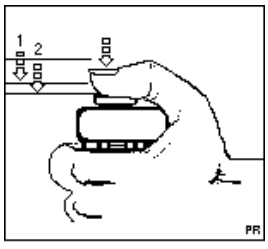


• When aspirating (drawing-up) a solution, push the plunger to the *first stop (air will be expelled to the measured volume), next* lowering the pipette tip just below the level of the solution that you are sampling. You should be holding the tube, containing the solution in your hand, at eye-level.

It's important to actually see the solution enter the pipette tip.



• As you slowly release the plunger the tip of the micropipette will take up the measured quantity of liquid. Be certain that the disposable tip is below the surface of the liquid to avoid aspirating air into the tip.

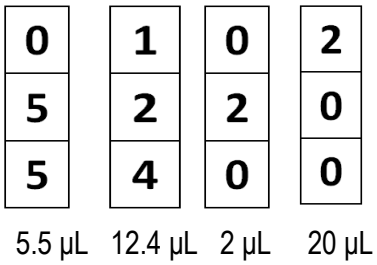


• When dispensing (pushing out) the liquid, place the pipette tip into the tube that will receive the solution. Touch the micropipette tip to the lower inside wall of the reaction tube into which you want to expel the sample. This creates a tiny surface-tension effect which helps coax fluid out of the tip. *Slowly push down the plunger of the micropipet to the first stop. Then, continue to the second stop* to expel the last bit of fluid, *holding the plunger in the same position.* Slowly remove the pipet out of the tube, *keeping the plunger pushed down* to avoid aspirating any liquid back into the tip. Do not forget to work at eye level to 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 are 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 onto 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 Basics

1. **Changing the Volume**: Find the display window on the handle of the micropipette and note its setting. Turn the black dial knob, clockwise to decrease the volume or counterclockwise to increase the volume. Turning this knob changes the distance the plunger will travel. The figures at the right represent some pipette settings for the P20 and the volumes of liquid dispensed.



2. **Disposable tip:** Place a disposable tip onto the end of the pipette barrel. Avoid touching the pointed end, as this will contaminate the tip. **Remember that you must have a tip in place when using the pipette.** If you notice that your micropipette is not properly taking up a liquid sample the disposable tip may not be firmly in place on the micropipette. If this is the case use your thumb and index finger to grab the base of the disposable tip to check that the tip if firmly seated onto the micropipette.

3. **Taking up and expelling a sample:** Place your thumb on the plunger. Push down on the plunger with your thumb and notice that it has a "stop" position. This first stop position has expelled enough air to then take in the dialed volume of liquid. While holding the microfuge tube at eye level, lower the disposable tip of the micropipette to below the liquid surface while holding the plunger at the first stip. Slowly allow the plunger to rise while watching that the disposable tip remains under the surface of the liquid sample. Deposit the sample onto a low wall of the desired microfuge tube by pressing the plunger slowly. After hitting the first stop, exert a little more pressure with your thumb, and push the button of the plunger to the second stop. The second stop pushes a small volume of air into the tip to eject the solution. Keep your thumb depressed all the way on the plunger until the micropipette tip is out of the microfuge tube.

Pipetting Exercise 1

1. With your lab partner, use a permanent marker to label four clean dry reaction tubes A+, A-, B+, and B-
2. Today and for most labs you and your lab partner will share reagents (lab chemicals) with the other members at your lab station. Often all shared reagents will be kept on ice when not in use. Set up your shared REAGENTS by first filling one Styrofoam cup labeled REAGENTS with ice. Obtain the following five microfuge tubes: dH2O, Red, yellow, green, and blue solution and set them on ice in the REAGENTS ice cup.
3. As you work with the reagents today hold reagent tubes by the rim of the microfuge tube rather than by the bottom to avoid warming the samples. Pour melted ice water down the sink periodically to avoid submerging any of your tubes.
4. Lab partners will set up one Styrofoam ice cup to keep your four labeled tubes (A+, A, B+, B-) on ice at all times. Each lab pair will have one Styrofoam cup containing their four microfuge tubes.
5. The table below summarizes the contents of each tube. Follow the directions below to set-up the samples. **Reminder: add the samples to the sides of the microfuge tube near the bottom without allowing your micropipette tip to touch the other solutions in the tube.**

Tube	dH2O	Red solution	Yellow Solution	Blue Solution	Green Solution	Total Volume
A+	3.5 µL	-	8 µL	4.5 µL	4 µL	20 µL
A-	7.5 µL	-	8 µL	4.5 µL	-	20 µL
B+	5 µL	6 µL	5 µL		4 µL	20 µL
B-	7.5 µL	7.5 µL	5 µL		-	20 µL

6. Set the P-20 micropipette to 3.5 µL and dispense dH2O into the A+ tube. Change the volume and add dH2O to the remaining tubes. Eject the tip into the plastic waste container and replace with a fresh tip.

7. Place 6 µL of *red solution* into tube B+ and7.5 µL of red solution into tube B-. Eject the tip into the plastic waste container and replace with a fresh tip.

8. Use a fresh tip and dispense 8 µL of yellow solution into tube A+ and A-, and 5 µL of *yellow solution* into tubes B and B-.

9. Use a fresh tip and dispense 4.5µL of *blue solution* into tube A+ and A-.

10. Show your instructor one of your tubes in which four distinct drops are clinging to the walls of your reaction tube.

11. Use a fresh tip and dispense 4µL of *green solution* into tube A+. Add the green solution directly into the solution at the bottom of the microfuge tube. If your solutions are still clinging to the sides of tubes A+ and B+, gently tap each microfuge tube on the table and the solutions should fall to the bottom of the tube. After addition of the green solution to tube A+, gently pump the solution in and out with the micropipette going only to the first stop to mix the reagents. Once mixed, expel all of the solution from the micropipette tip by pushing down to the first then second stop. Cap the tube and repeat for B+. Be certain to use a new tip for each tube A+ and B+ to avoid contamination.

Checking the accuracy and consistency of pipetting

12. Tubes A+, A-, B+ and B- should each contain 20 µL of solution.

13. Place your closed microfuge tubes in a balanced position in the centrifuge. Spin for 2 seconds to bring the contents of your tube to the bottom (mix).

14. Set your P-20 micropipette to 20 µL and place a fresh tip on to the barrel.

15. ***To save materials we will be using one disposable tip for all four microfuge tubes in this step*.** Aspirate (draw up) all 20 µL from tube A+. Dispense all of the contents of tube A+ (20 µL) in one drop in a weigh boat. Do the same for tubes A-, B+, and B- creating four separate drops of 20 µL in your weigh boat.

16. Show your instructor your four 20 µL drops, and your four used reaction tubes,  and the number of tips that are in your waste container.

17. Clean up.

- Wipe out your weigh boat with a small piece of paper towel.
- Rinse out tubes tubes A+, A-, B+ and B- with a small amount of distilled water. Violently shake out excess water. Return wet tubes to the WET TUBE bin on the cart at the front of the room. Leave all caps OPEN so they will dry out.
- Replace the four tubes you rinsed out with four clean dry tubes to your bin.
- Return red, yellow, green, blue, and dH2O solutions to the microfuge rack on the cart at the front of the room.
- Empty your ice cups.
- Empty your waste bin containing only disposable pipette tips into the one waste bin for tips on the cart.
- Return your two dry weigh boats, two boxes of pipette tips, two permanent markers, one empty REAGENTS cup, two empty ice cups, two waste bins, and a total of eight clean dry microfuge tubes to your bin. Place your bin on the cart.
- Return micropipettes rack to the front cart.