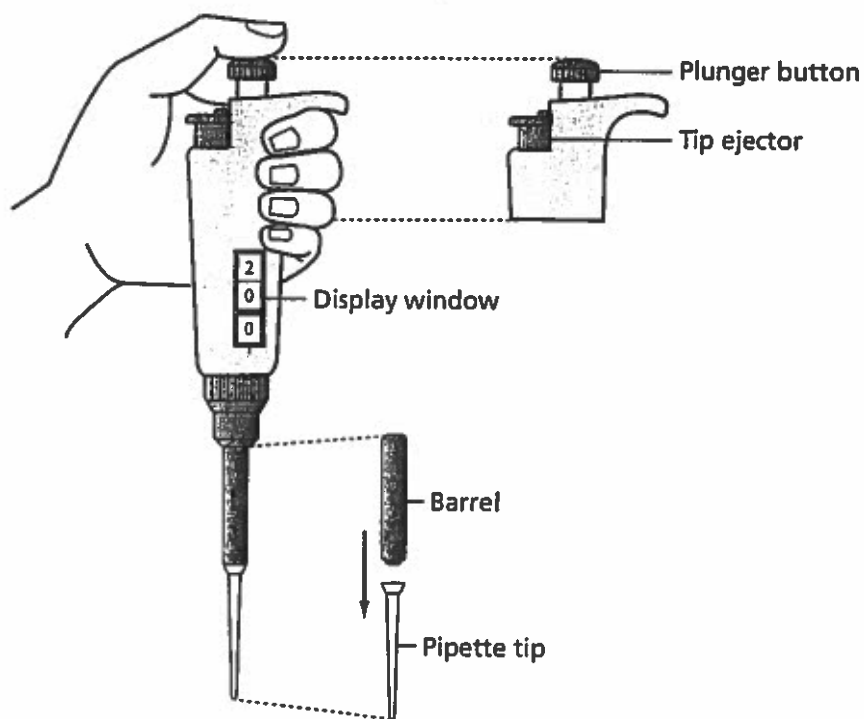


LABORATORY 1.1: HOW TO USE A MICROPIPETTE

The purpose of this laboratory is to introduce you to an important tool used in genetic engineering: the micropipette, shown in Figure 1.1. A micropipette is used to transfer very small and exact volumes of liquids in either milliliters (mL, thousandths of a liter) or microliters (μL , millionths of a liter), which are the measurements of volume most often used in genetic engineering. This laboratory will give you the chance to learn how to use the micropipette and to see the relative size of different amounts of solution measured by this very precise tool and how precise the amounts that you can measure with it are.

Figure 1.1: A P-20 micropipette



BEFORE THE LAB

Respond to the following questions with your group and be prepared to share your answers with the class.

1. Why do you think it is necessary to use very small and exact volumes of reagents in biotechnology?
2. Read through the Methods section on pages A-19 through A-21 and briefly outline the steps, using words and a flowchart.

MATERIALS

Reagents

- A plastic microfuge tube rack with a microfuge tube of red dye solution (RD)

Equipment and Supplies

- P-20 micropipette (measures 2.0–20.0 μL)
- Tip box of disposable pipette tips
- Laminated micropipette practice sheet
- Waste container for used tips and microfuge tubes (will be shared among groups)

SAFETY:

- All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles. Please refer to your teacher's instructions.
- Wash your hands well with soap after completing the lab.



METHODS

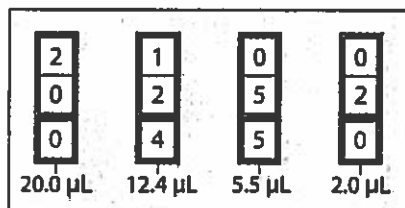
1. Check your rack to make sure that you have the reagent listed.
2. Review the parts of the micropipette shown in (see Figure 1.1 on page A-18).
3. Find the display window on the handle of the micropipette.
4. Turn the plunger button on the top of the micropipette clockwise—to the right—to decrease the volume, or counterclockwise—to the left—to increase the volume.



5. Figure 1.2 shows four micropipette volumes. Practice setting the micropipette to these volumes.

LAB TECHNIQUE: Never set the P-20 micropipette lower than 2.0 μL or higher than 20.0 μL or you could damage the equipment.

Figure 1.2: Four micropipette volumes



The display window of a micropipette shows how much fluid it will load and dispense. Four examples of displays and the corresponding amounts are shown.

6. Review the laminated micropipette practice sheet. Each group member will pipette five drops of different volumes onto the sheet. Pipetting consists of two parts: loading the liquid into the micropipette, and dispensing the liquid from the micropipette.



7. Load the micropipette with 20.0 μL of RD by doing the following:

- a. Set the micropipette to 20.0 μL .
- b. Open the tip box. Lower the micropipette onto a tip and press down firmly (do not touch the tip with your fingers). Close the box when done.
- c. Bring the micropipette and the RD tube to eye level.
- d. Use your thumb to press the plunger to the first stop position, which is your first point of resistance.

LAB TECHNIQUE: When loading the micropipette, only press the plunger to the first stop or you will draw too much solution into the pipette tip.

- e. Put your pipette tip into the RD and slowly release the plunger to draw up the solution.

LAB TECHNIQUE: 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.

8. Dispense RD onto the laminated sheet by doing the following:

- a. Place the pipette tip over the 20.0 μL circle.



- b. Use your thumb to press the plunger to the first stop position and then press down to the second stop.

LAB TECHNIQUE: When dispensing liquid from the micropipette, press the plunger to the first stop to dispense most of the liquid and then press the plunger to the second stop in order to dispense the last little bit.



- c. With the plunger still depressed, pull the pipette out of the tube—this prevents you from accidentally pulling the liquid back into the tip.
9. Without setting down the micropipette, twist the plunger button to set it to 15.0 μL and repeat steps 7b–8c, dispensing over the 15.0 μL circle.
10. Without setting down the micropipette, twist the plunger button to set it to 10.0 μL and repeat steps 7b–8c, dispensing it over the 10.0 μL circle when dispensing the liquid.
11. Without setting down the micropipette, twist the plunger button to set it to 5.0 μL and repeat steps 7b–9, dispensing it over the 5.0 μL circle.
12. Without setting down the micropipette, twist the plunger button to set it to 2.0 μL and repeat steps 7b–9, dispensing it over the 2.0 μL circle.
13. Use the tip ejector to place your pipette tip into the waste container.

STOP AND THINK:

- When loading or dispensing a solution, why is it important to actually see the solution enter or leave the pipette tip?
- You were instructed to avoid contact with the pipette tips—for example, you were asked to put the pipette tip on without using your hands, to avoid setting down the micropipette, to use the ejector button to remove the tip, and to keep the tip box closed. If you were working with plasmids and bacterial cells, why would these precautions be important?



14. Using the micropipette practice sheet, each person in your group should have a chance to load and dispense the five drops of different volumes, with each person using a new pipette tip.
15. When everyone in your group has had a chance to dispense RD onto the micropipette practice sheet, draw the approximate sizes of each drop in your notebook (or take a photograph and tape it into your notebook) and label them with the amounts.