

**Class Time**

1 class periods(of 50 minutes)

**Prior Knowledge Needed**

- Basic understanding of the metric system (i.e.  $\log_{10}$ ).

**INTRODUCTION**

Much of molecular biology involves working with very small volumes. Typically, samples volumes and reagents are measured in **microliters**, or 1/1000 of a milliliter, which is abbreviated  $\mu\text{l}$ . These small volumes are measured with a tool called a **micropipettor**; examples of micropipettes are shown to the right and below.

**LEARNING OBJECTIVES**

At the end of this lesson, students will know that:

- Micropipettes are used to measure and dispense small volumes of liquids (usually less than 1 milliliter).
- Different sizes of micropipettes are used depending upon the volume of the liquid measured.
- The most common micropipettors are the P20 (which measure volumes from 2.0-20.0  $\mu\text{l}$ ), the P200 (which measure volumes from 20.0-200.0  $\mu\text{l}$ ), and the P1000 (which measure volumes from 200.0-1000.0  $\mu\text{l}$ ).
- Micropipettes should **never** be used to measure volumes above the upper limit of their capacity (for example, never measure greater than 20.0  $\mu\text{l}$  with a P20).



At the end of this lesson, students will be able to:

- Select the appropriate micropipette for the volume that they are measuring.
- Use micropipettes to measure and dispense liquids ranging from 10.0-1000  $\mu\text{l}$ .
- Confirm the accuracy of their pipetting.

**MATERIALS:**

Equipment	Quantity
P20 Pipetmen and tips	1 each per group
P200 Pipetmen and tips	1 each per group
P1000 Pipetmen and tips	1 each per group
Parafilm	1 each per student
Waste container	1 each per group
Tube of red food coloring diluted 1:10 in water, 1.5 ml per tube	1 each per group
Tube of green food coloring diluted 1:10 in water, 1.5 ml per tube	1 each per group
Tube of yellow food coloring diluted 1:10 in	1 each per group

water, 1.5 ml per tube	
Tube of blue food coloring diluted 1:10 in water, 1.5 ml per tube	1 each per group

## PROCEDURE

1. Explain to students the **aims of this lesson**. Some teachers may find it useful to write the aims on the board.

**Lesson Aim:** Learn how to use micropipettors to measure small volumes of liquids.

Teachers may also wish to discuss the *Learning Objectives* of the lesson, which are listed at the beginning of the lesson plan.

2. Tell students that micropipettes are commonly used in many types of biology labs today to measure very small volumes of liquids. To put the volumes measured in context, review the following with students:

$$1000 \text{ ml} = 1 \text{ liter (or 1.06 quarts)}$$

And

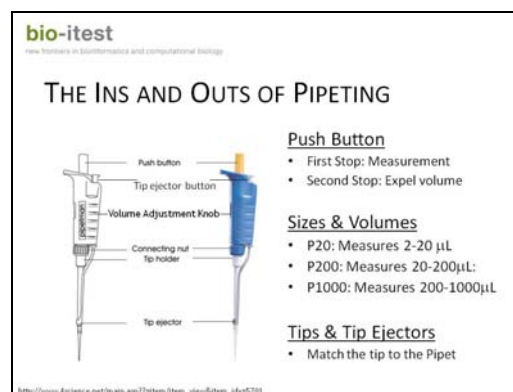
$$1000 \mu\text{l} = 1 \text{ ml}$$

3. Show **Slide #1**, “The Ins and Outs of Pipeting,” and review with students the key elements of micropipetting:

a) The Push Buttons, and the difference between the first, or “soft” stop, and the second, or “hard,” stop.

b) Sizes and volumes, in terms of which pipet should be used for which volume range.

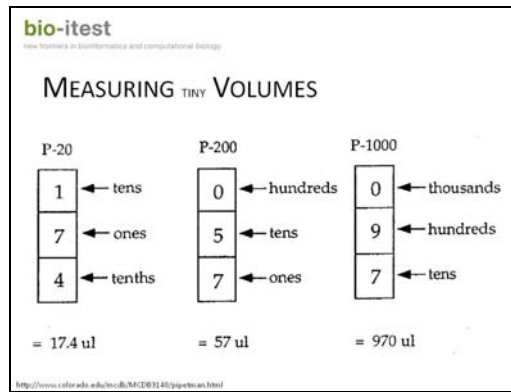
c) Tips and Tip Ejectors: Different sizes of micropipettes often require different sizes of tips. P20 and P200 micropipettes often (but not always) share the same size tip, while P1000 tips are much larger. Tips should be removed by depressing the tip ejector, not by removing the tip with your fingers.



**Micropipetting: Slide #1**

4. Show **Slide #2**, “Measuring Tiny Volumes,” and explain to students how to

read the volumes displayed on each type of micropipette: P20, P200 and P1000.



**Micropipetting: Slide #2**

5. Review these rules for using a micropipettor:

- Never use the micropipettor without a tip. This could cause damage to the instrument.
- Gently remove the disposable tips with the ejector button near your thumb. If necessary, you can remove the tip with your gloved fingers.
- Never lay a loaded pipette down or tilt it sideways because this could allow liquid to run back into the instrument.
- Never let the button snap back into place. Always allow it to rise slowly back into position.
- Never try to measure a volume that the micropipettor cannot measure. Micropipettors are only accurate in the top 75-80% of their range, and should never be used to measure volumes above their capacity. Use the table below for reference.

6. Pass out Student Handout—*Introduction to Micropipetting*, and allow them to work through the activity independently.

**Table 1: Micropipettor Reference Table**

<b><i>Micropipettor</i></b>	<b><i>Minimum Volume</i></b>	<b><i>Maximum Volume</i></b>
P2	0.2 µl	2.0 µl
P20	2.0 µl	20.0 µl
P100	10.0 µl	100.0 µl
P200	20.0 µl	200.0 µl
P1000	100.0 µl	1000.0 µl

**CREDIT**

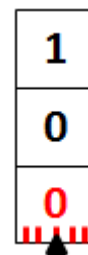
Adapted from:

<http://www.nespal.org/oziasakinslab/edout/Micropipetting.pdf>

## Introduction to Micropipetting

### Part 1: Using the 20 $\mu\text{l}$ (P20) micropipettor

1. Select the P20 micropipettor and set this to 10  $\mu\text{l}$  by rotating the knob. The volume window should look like the image to the right. (This actually indicates 10.0 microliters.)
2. Press the micropipettor into one of the small tips in the box. Press firmly enough to ensure an airtight seal but do not press so firmly as to damage the micropipettor.
3. Depress the plunger to the **first stop** position. Holding the micropipettor vertically, immerse the tip approximately 2 mm into the red colored liquid. Release the pressure allowing the plunger to move up slowly. This will draw the liquid up into the tip. (There should be no bubbles in the liquid in the tip if you have done this correctly.)
4. Withdraw the tip from the liquid and squirt the red liquid into the waste container by pushing the plunger to the **first stop** position.
5. Wait one second and then squirt out any liquid remaining in the tip by pressing the plunger to the **second stop** position or all the way down.
6. With the plunger still depressed, remove the tip from over the beaker (this beaker is your waste container) and then allow the plunger to slowly rise to the up position.
7. Discard the tip in your waste container by depressing the tip ejector button. This keeps you from ever having to touch the tip.



### Part 2: Micropipettor Challenge

8. Take a piece of Parafilm and lay it flat at your work station. This is where you will mix your dye samples.
9. Pick up the 20  $\mu\text{l}$  micropipettor and place a tip on it.
10. Measure 4  $\mu\text{l}$  of red food coloring and place this onto the Parafilm.
11. Dispose of the used tip. Get a fresh tip and measure 5  $\mu\text{l}$  of yellow food coloring. Add this to the drop of red food coloring.

12. Dispose of the used tip and get a fresh tip. Measure 2  $\mu\text{l}$  of the blue food coloring. Add this to the drop you have already created.

13. Dispose of the used tip. Get a fresh tip and measure 3  $\mu\text{l}$  of the green food coloring. Add this to the drop you have added all the other colors to.

**14. What color do you see?**

**15. What is the volume of the drop you have created on the Parafilm? How does your drop compare to the drops created by your fellow group members? (Hint: just add all the volumes you mixed to get the total.)**

16. Now set your micropipettor to the total volume of the drop and pick up the drop with the micropipette. If you did a good job, the micropipette should pick up the entire drop and there should be no air bubbles in the tip. **How did you do?**

### Part 3: 1000 $\mu\text{l}$ micropipettor challenge

17. Pick up the 1000  $\mu\text{l}$  micropipettor and place a tip on it. Now that you are experienced, we will determine just how good you are!

18. Measure the following volumes into two microcentrifuge tubes:

Tube	Red	Blue	Green	Yellow	Total	Color
#1	200 $\mu\text{l}$	300 $\mu\text{l}$	0	0		
#2	0	0	250 $\mu\text{l}$	200 $\mu\text{l}$		

19. Now calculate the total volume that should be in each tube and write it in the table above.

20. Now set your micropipettor to the total volume of the drop and pick up the drop with the micropipette. If you did a good job, the micropipette should pick up the entire drop and there should be no air bubbles in the tip. **How did you do?**

21. Continue practicing picking up, dispensing, and mixing additional volumes using the remaining food coloring until you feel confident in your micropipetting skills.