

MICROPIPETTING TECHNIQUE ACTIVITY

Learning Outcome: The students will develop the skills necessary to use a micropipettor and an understanding of extremely small volumes associated with molecular biology.

High School Georgia Performance Standards

SCSh4 Students use tools and instruments for observing, measuring and manipulating scientific equipment and materials.

SCSh7 Students analyze how scientific knowledge is developed.

Middle School Georgia Performance Standards

S7CS4 Students use tools and instruments for observing, measuring and manipulating equipment and materials in scientific activities.

Materials:

Provided in kit	Provided by teacher
7 Large-range micropipettors	
7 Boxes large-range micropipette tips	Yellow food coloring
7 Small-range micropipettors	Red food coloring
7 Boxes small-range micropipette tips	Green food coloring
1.5 ml Microcentrifuge tubes	Blue food coloring
Roll of Parafilm	Small beakers (or disposable plastic medicine cups)

Materials to be assembled for a class of 28 students (7 groups of 4 students each).

7 large-range micropipettors
Large-range micropipette tips
7 small-range micropipettors
Small-range micropipette tips

1 bottle red food coloring
1 bottle green food coloring
1 bottle blue food coloring
1 bottle yellow food coloring
1 roll Parafilm
7 small beakers
72 microcentrifuge tubes

Duration of activity

Prep time: Setting equipment at each work station and making colored water solutions - approximately 30 minutes.
Class time: One 55 minute period for introduction and activity.

Advanced Preparation:

- Dispense 5 μ l of dye into a microcentrifuge tube, then fill to the 1.5 ml line with tap water (do this for each of the four colors). Each group should have four tubes (one of each color) to act as their stock dye solution.

Additional tips:

- Please be sure that the students have mastered these skills before moving on to the next lab activity.
- Make certain you emphasize the four following rules for using a micropipettor.
 - Never use the micropipettor without a tip.
 - Never lay a loaded micropipettor down or tilt it as this could allow liquid to run into the pipette.
 - Never allow the button to snap back into place. Always allow the button to slowly return to the up position.
 - Never adjust the micropipettor beyond its measurable range. In other words, don't use the small range pipette (2 μ l - 20 μ l) to measure anything below 2 μ l or above 20 μ l.

MICROPIPETTE TECHNIQUE USING FOOD COLORING



Molecular biologists and biotechnologists generally work with very small volumes called microliters which can be abbreviated as μl . These small volumes are measured using a tool called a micropipettor (pictured above). This lab will allow you to practice using a micropipette to measure very small volumes. To relate these volumes to something you might understand, look at the following.

$$1000 \text{ ml} = 1 \text{ L or } 1.06 \text{ quart}$$

So to relate this to microliters:

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

As you can see, 1 microliter (μl) is an extremely small volume. In the field of molecular biology it is sometimes important to be able to measure 1 μl .

Rules for using a micropipettor:

- Gently remove the disposable tips with your fingers.
- Never use the micropipettor without a tip. This could cause damage to the instrument.
- 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. An example would be to try to measure 25 μl with a 2 μl - 20 μl micropipettor. That would damage the micropipettor.

PROCEDURE

Part 1: Using the 20 μl micropipettor

1. Select the 20 μl micropipettor and set this to 10 μl by rotating the knob. The volume window should look like this: (This actually indicates 10.0 microliters.)

1	Tens of microliters
0	Microliters
0	Tenths of a microliter

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

1. Take a piece of Parafilm and lay it flat at your work station. This is where you will mix your dye samples.
2. Pick up the 20 μl micropipettor and place a tip on it.
3. Measure 4 μl of red food coloring and place this onto the Parafilm.
4. Dispose of the used tip. Get a fresh tip and measure 5 μl of yellow food coloring. Add this to the drop of red food coloring.
5. Dispose of the used tip and get a fresh tip. Measure 2 μl of the blue food coloring. Add this to the drop you have already created.
6. Dispose of the used tip. Get a fresh tip and measure 3 μl of the green food coloring. Add this to the drop you have added all the other colors to.
7. What color do you see?
8. 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.)
9. 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 μl micropipettor challenge

1. Pick up the 1000 μl micropipettor and place a tip on it. Now that you are experienced, we will determine just how good you are!

1	Thousands of microliters
0	Hundreds microliters
0	Tens of microliters
0	Microliters

2. Measure the following volumes into two microcentrifuge tubes:

Tube	Red	Blue	Green	Yellow	Total	Color
#1	200 μl	300 μl	0	0		
#2	0	0	250 μl	200 μl		

1. Now calculate the total volume that should be in each tube.

2. Which of the data is qualitative and which is quantitative?