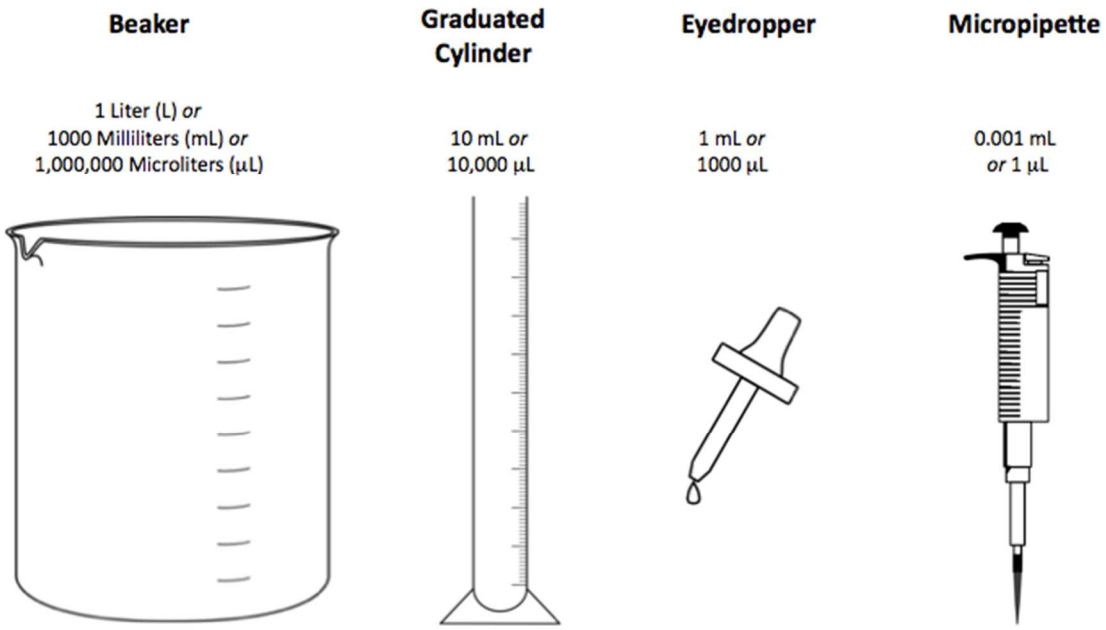
Measuring and Moving Small Volumes: An Introduction to Micropipettes

Student Guide

Introduction

A forensic scientist removes a microscopic amount of DNA from a drop of blood left at the scene of a crime. A cystic fibrosis patient inhales a fine mist containing “good” copies of a gene he did not inherit. When the criminologist and the genetic engineer perform laboratory procedures involving tiny amounts of DNA and other chemical solutions, each uses an instrument known as a micropipette.



A micropipette is a sophisticated eyedropper - one that comes in many different models and volume ranges. But while an eyedropper dispenses drops, micropipettes transfer microliters of fluid. In the metric system, the basic unit of volume is the liter (L). If you put the prefix "milli-" which means "one-thousandth", in front of liter, you are referring to one thousandth of a liter, or one milliliter (mL). "Micro-" is a prefix in the metric system, which means “one-millionth” of the base unit. Therefore, one microliter (µL) is one-millionth of a liter. It may be easier for you to picture one milliliter (mL) of water. If you mentally divide that milliliter of water into 1000 tiny equal-sized volumes, each volume is one microliter.

Laboratory science often involves working with very small volumes of liquid; frequently millionths of liters are used. One millionth of a liter is equal to one microliter, abbreviated 1 µL. 1 L = 1,000 mL = 1,000,000 µL.

It would be very difficult to measure such small volumes without a very accurate and precise instrument. The instrument most often used to measure microliters is called a micropipette. Different micropipettes are used to measure different volumes.

How to Use a Micropipette in 10 Steps

Lt. yellow Goldenrod Blue

1. SELECT the correct size micropipette for the volume you need
- 2 – 20 µL: select the micropipette with the light yellow top
- 20 – 200 µL: select the micropipette with the goldenrod top
- 200 – 1000 µL: select the micropipette with the blue top

2. SET the micropipette to the appropriate volume by adjusting the dial

Set volume only within the range specified for that micropipette. It is only accurate in this range and damage occurs when it is set beyond the minimum or maximum.

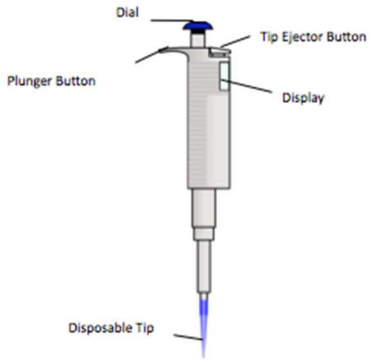
Pipettes only work in one direction. They will not turn around back to zero after turning to the highest number, the direction must be reversed to return to lower numbers.

3. PLACE a clean, disposable tip on the micropipette

Use new disposable tip every time. The small tips (yellow or white) are for the P20 and P200. The large tips (blue or white) are for the P1000.

4. PRESS the plunger down to the first stop and hold

Keep the micropipette in a vertical position when there is fluid in the tip. Failure to do this will cause liquid to enter the nose cone. Since a micropipette works by air displacement, its internal mechanism must remain dry.



5. INSERT the tip beneath the surface of the liquid

Eye level pipetting allows you to see the liquid moving and ensures accuracy.

6. TRANSFER liquid up into the micropipette tip by slowly releasing the plunger

Control the speed at which the plunger rises after taking up or ejecting fluid with your thumb. Releasing the plunger too quickly will cause leakage or bubbles that will trap air and make the measurement inaccurate.

7. DISPENSE liquid by pressing the plunger to the first stop

Touch the tip to the inside wall of the reaction tube into which you want to unload the sample. This creates a tiny surface-tension effect, which helps the drop of fluid transfer from the tip to the tube.

8. EXPEL any remaining liquid by pressing the plunger to the second stop.

The second stop is used only to push out any last remaining drops from the tip

9. REMOVE the tip from the sample drop (pull away from the tube) before releasing the plunger

Releasing the plunger too soon will suck up liquid from your sample, causing error.

10. DISCARD the tip by pressing the ejector button

Never re-use a tip. Can you think why?

How to Read Micropipette Dials

20 µL Micropipette (P20), light yellow

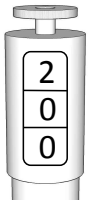
The 20 µL micropipette allows for transfer of volumes ranging from 2 – 20 µL.
It is the smallest of the three micropipettors that you will use, and often has a yellow top.
You should notice a red line between the second and third number in the setting window. This represents a decimal.

1. Select the P20 micropipette and rotate the dial until you get a reading of 20 µL. Your micropipette volume window should look like this:

20.0 µL

Microliters (1

Tenths (0.1) c *ers*

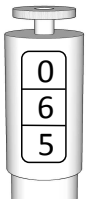


Tens (10) of Microliters

2. Next, rotate the dial to set the micropipette to a reading of 6.5 µL. Your micropipette volume window should look like this:

6.5 µL

Tenths of I



Tens of Microliters

Microliters

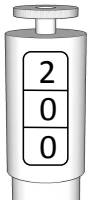
200 µL Micropipette (P200), goldenrod

The 200 µL micropipette allows for transfer of volumes ranging from 20 – 200 µL.
It is the middle size of the three micropipettors that you will use, and often has a goldenrod top.

1. Select the P200 micropipette and rotate the dial until you get a reading of 200 µL. Your micropipette volume window should look like this:

200 µL

Tens Microliters



Hundreds of Microliters

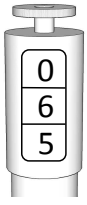
Microliters

2. Next, rotate the dial to set the micropipette to a reading of 65 µL. Your micropipette volume window should look like this:

65 µL

Tens of Microliters

Microliters



Hundreds of Microliters

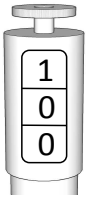
3. Where would the dial be set to measure 20 µL? Discuss with your partner then set to this level.

1000 µL Micropipette (P1000), blue

The 1000 µL micropipette allows for transfer of volumes ranging from 200 – 1000 µL.
It is the largest of the three micropipettors that you will use, and often has a blue top.

1. Select the P1000 micropipette and rotate the dial until you get a reading of 100 µL. Your micropipette volume window should look like this:

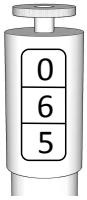
1000 µL



Thousands of Microliters
Hundreds of Microliters
Tens of Microliters

2. Next, rotate the dial to set the micropipette to a reading of 65 µL. Your micropipette volume window should look like this:

650 µL



Thousands of Microliters
Hundreds of Microliters
Tens of Microliters

3. Where would the dial be set to measure 200 µL? Discuss with your partner then set to this level.

4. Why can you not use this pipette to measure 20µL?

Key Points to Remember

- Know what size micropipette you are using before you set the dial
- The highest setting for each micropipette is:
  - For the **P20**: the **tens** place
  - For the **P200**: the **hundreds** place
  - For the **P1000**: the **thousands** place
- Remember that the dial can have the same reading, but if the size micropipette is different, the volume will be different. 6.5 µL, 65 µL, 650 µL look the same on the dial of a p20, p200 and p1000 respectively.
- By knowing the maximum volume of the micropipette (usually written on the top), you can figure out what each of the digits on the readout means.

Pre-Lab Worksheet – Setting the Dial

Name _____

Date _____ **Class Period** _____

1. Explain the reason for each of the following rules:

   a. Always set the micropipette within its designated range.

   b. Always use a micropipette with a tip.

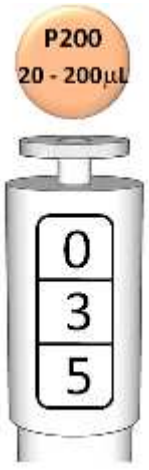
   c. Always hold a loaded micropipette in a vertical position.

   d. Always release the micropipette plunger slowly.

   d. Always operate the micropipette by holding it at eye level.

2. Observe the volume of liquid that is measured by micropipettes A and B.

A)



B)

Which micropipette (A or B) is the P-20? _____ What is its range? _____

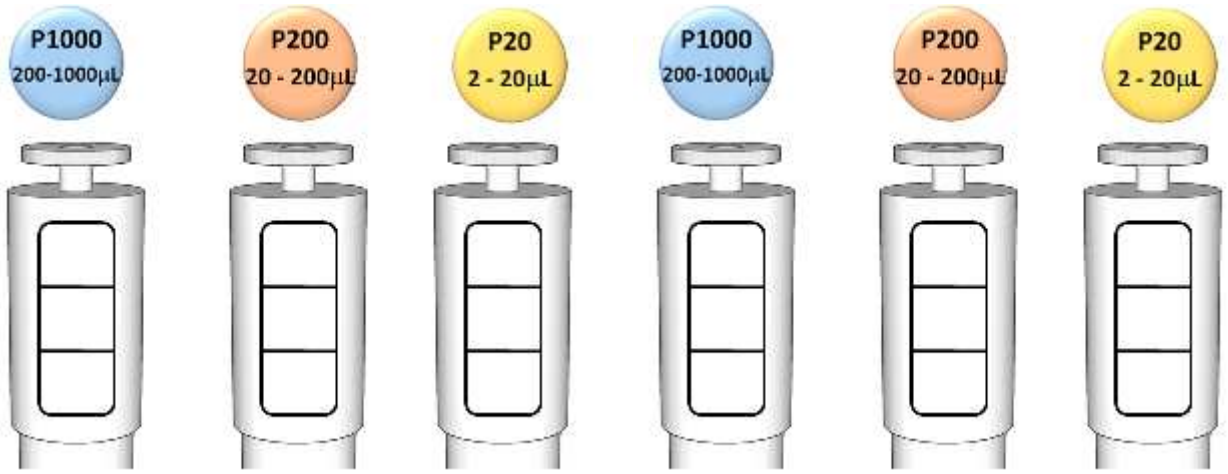
Which micropipette (A or B) is the P-200? _____ What is its range? _____

At which volume is the P200 set? _____

At which volume is the P20 set? _____

3. Select the appropriate micropipette and write the amount in the dial box that corresponds to the six volumes:

a) 175 µL
b) 220 µL
c) 11.7 µL
d) 984 µL
e) 21 µL
f) 3.1 µL



4. Take the P20 and set it to 8.3 µL
Take the P200 and set it to 83 µL
Take the P1000 and set it to 830 µL

Write in the space below what you observe about the dial settings in all three micropipettes. What is the same about them, and what is different? Why?

5. Complete the following conversions:

a. 1 µL = _____ mL

b. 100 µL = _____ mL

c. 250 µL = _____ mL

d. _____ µL = 1.5 mL

e. _____ µL = 0.06 mL

f. _____ µL = 0.003 mL

6. Put the following volumes in order from **largest** to **smallest**.

a. 2.5 mL, 250 µL, 0.025 mL, 2.5 µL: _____, _____, _____, _____.

b. 100 µL, 0.01 mL, 250 µL, 0.015 mL: _____, _____, _____, _____.

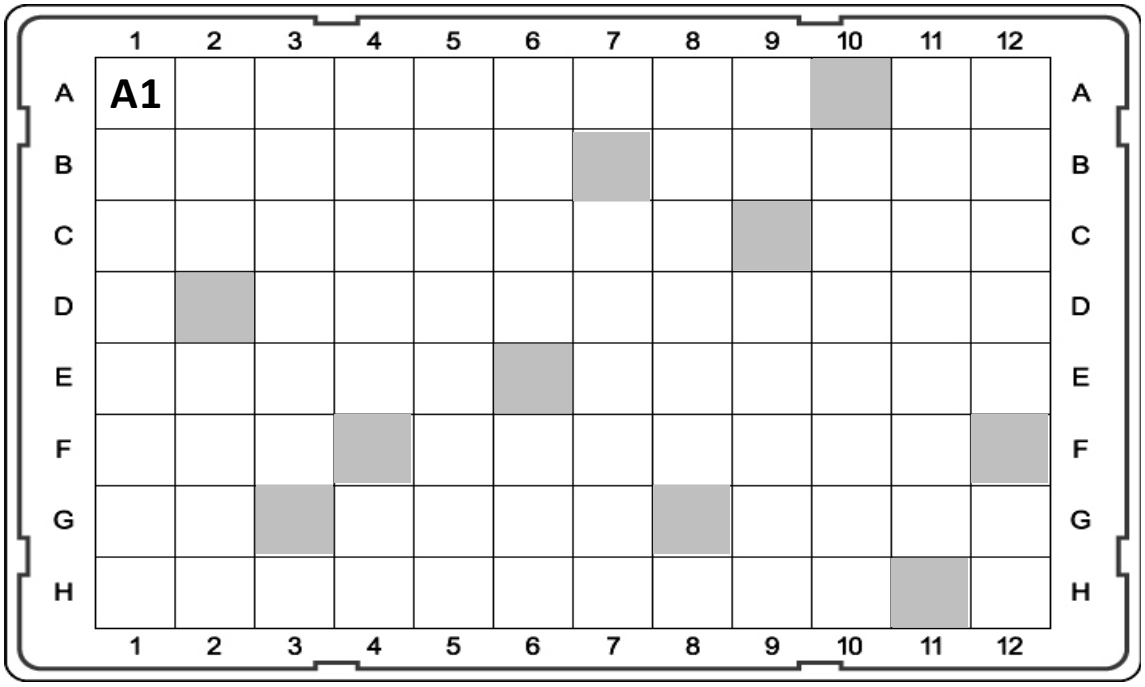
Lab Activity #1: Color by Numbers – With Micropipettes!

In this activity, you will use the tools of a science laboratory to create an art project. You will hone in on your pipetting skills by using a Cartesian coordinate system modified for a 96-well reaction plate.

A **96-well plate** is a universal tool used in biological research laboratories. It is used to culture living cells, to analyze DNA, to measure proteins, and for many other laboratory procedures. It consists of a grid of 12 columns and 8 rows. The columns are numbered 1-12, and the rows are lettered A-H, as seen in the image below.

You will use the **P200 micropipette** to precisely deliver a volume of **100 µL** of different colored dyes into the 96-well plate according to a specific coordinate key. In the plate map below, coordinate A1 is indicated. Look at the 10 grey boxes on the plate map and fill in the coordinates:

Your teacher will have grid templates to hand out, with a variety of images. You can also create your own! After creating your image, you will lock-in the colors with a "plate sealer", and you can take your science art project home.

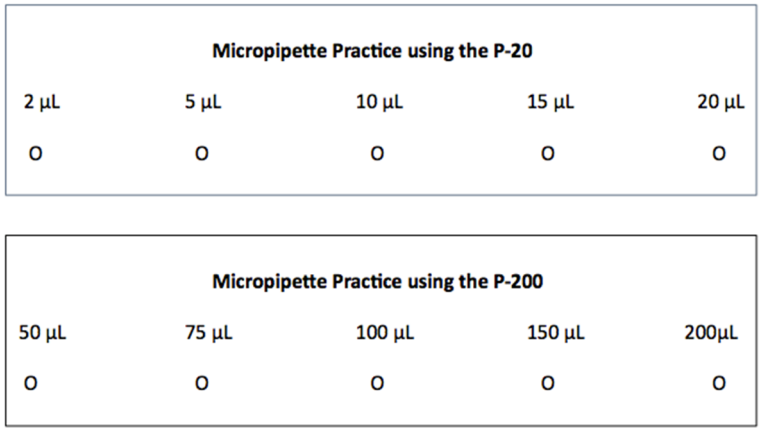


Lab Activity #2: Precision Pipetting

Biotechnology laboratory experiments often require you to move very tiny amounts of liquid between different tubes. In this lab activity, you will learn how to be very precise with delivering small volumes. This lab activity takes advantage of the surface tension of liquids to help you with visualizing small volumes.

Steps:

1. Your teacher will hand out practice cards with a water-proof surface. You will use these to micropipette the suggested volume into the circle below it. The cards will look like this:



2. You will be given a small tube that contains colored liquid. Use the liquid in this tube to withdraw the exact volume with your micropipette and transfer to the water-proof surface as indicated on the card.

3. Hold the micropipettes at eye-level when withdrawing liquid form the tube. That way, you can see the liquid entering the micropipette tip, and will begin to get comfortable with how different sized volumes look when inside a micropipette tip.

4. Watch the liquid entering and exiting the micropipette tip and observe the size of the drop.

5. Perform a repeated dispense-withdraw cycle so that you can observe the liquid entering and exiting the tip. How does the speed and pressure on the plunger affect the precision of your volumes?

6. Observe the different size drops. How do these small volumes compare to everyday measurements you might use, like a teaspoon?

7. Compare your drops to those of a table mate. What can this tell you about your pipette skill, or that of your table mates? If someone's droplet sizes don't look like they are getting increasingly larger in size, that person should wipe off their card and repeat the pipetting. Help them improve their skill so they do it right!

8. Have your teacher check your group's cards before moving on. Accurate pipetting skill is important for future labs!

Lab Activity #3
ROY, Gee & BIV's Challenge

Name _____

Date _____ **Class Period** _____

Purpose

This laboratory activity introduces micropipetting technique. As with all fine motor skills, learning how to use a micropipette takes practice and determination. You will be rewarded with excellent laboratory results in this and future experiments.

Background

ROY, Gee, and BIV are having problems with their science lab. Use the following table and the directions that follow to help them by constructing your own spectrum. It is important that you follow the directions and use the most accurate pipetting technique as possible.

Constructing the Spectrum

1. The table below contains instructions about how to construct the spectrum.
   - Label six empty tubes #1-#6 with a permanent marker.
   - Complete the table by filling in all white boxes with the correct value for each of the 6 tubes, along with the dial setting and the micropipette size to use. Follow the examples that are given.
   - You must fill in the table before starting the experiment!

		Tube #1	Tube #2	Tube #3	Tube #4	Tube #5	Tube #6	Dial	Which Pipette?
Step 1	Add 38 µL of red water into tube #1	+ 38 µL Red						0 3 8	P-200
Step 2	Add 44 µL of yellow water into tube #3			+ 44 µL Yellow					
Step 3	Add 50 µL of blue water into tube #5					+ 50 µL Blue			
Step 4	Transfer 8 µL from tube #1 into tube #2	- 8 µL	+ 8 µL						
Step 5	Transfer 8 µL from tube #1 into tube #6								
Step 6	Transfer 8 µL from tube #3 into tube #4								
Step 7	Transfer 14 µL from tube #3 into tube #2								
Step 8	Transfer 14 µL from tube #5 into tube #4								
Step 9	Transfer 14 µL from tube #5 into tube #6								
Final volume									

2. Place your tubes in order and observe them.

3. Check your pipetting accuracy:

    1. Hold all six tubes up to eye level. Do the volumes look the same? _____
    2. Set your micropipette to measure the expected volume that you calculated for each tube.
    3. Remove that volume from tube #1.
    4. Use the symbols below to record your level of accuracy.

        a. **-** if there is air at the bottom of the tip. You added too little to the tube.

        b. **+** if there is liquid left in the tube. You added too much to the tube.

        c. ★ If the tip is full, with nothing left in the tube. Great Job!

Tube #	Starting Volume (µL)	Final Expected volume (µL)	Accuracy (-, +, ★
1	38 µL		
2	0 µL		
3	44 µL		
4	0 µL		
5	50 µL		
6	0 µL		

4. Micropipette the contents of each tube onto a piece of filter paper or paper towel and observe the different colored dots. Alternatively, you can use a plastic surface like a petri dish or the back of the precision micropipette card from Activity #2.

Compare the colored spot sizes. Are they the same size? _____

5. Explain your lab results:

What errors did you make in accuracy?

How can you prevent these errors in the future?

What other possible errors were you able to avoid this time, but need to remember in the future?

6. Follow your teacher's instructions for labeling your filter paper with team number, names, and period number.

7. CLEAN UP: Please follow your teacher's instructions for clean-up.