FRED HUTCHINSON CANCER RESEARCH CENTER
SCIENCE EDUCATION PARTNERSHIP

ULSTER AND RAININ VERSION

MEASURE FOR MEASURE
MICROPIPETTING AND THE METRIC SYSTEM

Concept: Work with DNA and enzymes frequently involves measuring very small volumes, often in the microliter range. A microliter (μl) is one millionth of a liter.

Liquid measurements in the metric system are made in units based on the liter where a liter is about one quart. To make these precise measurements, molecular biologists use a precision tool known as a micropipet. This tool is as basic to their lab work as a hammer is to a carpenter. Micropipets come in many models and sizes. You will be using micropipets similar to those found in the Fred Hutchinson Cancer Research Center research labs.

Objectives: In this lab, you will learn to use micropipets accurately and to measure volumes using metric units including microliters. Mastery of this technique is essential for good results in the activities to follow.

Use the following information to calculate metric volume conversions.

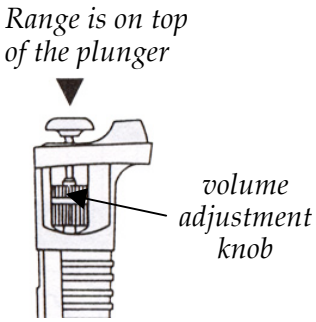
1 liter = 1000 ml (milliliters) 1 ml = 0.001 liter
1 liter = 1,000,000 microliters 1 microliter = 0.000001 liter

microliter = μl = (in lab jargon) lambda (λ)

For accurate measurements and to prevent damage to the micropipets, follow these important guidelines:

- Set the volume only within the range of your micropipet.
- Have the proper size disposable tip in place on your micropipet before immersion into any solution.
- Always keep the micropipet in a vertical position when there is liquid in the tip. In a horizontal position, fluid can leak back into the piston.
- Use your thumb to control the speed at which the plunger rises after taking up or ejecting liquid. Letting the plunger snap back damages the piston and the volume dispensed may be inaccurate.

SETTING AND PREPARING THE MICROPIPET



*There are **four** common sizes of micropipets. See the guide below for these sizes and volume ranges.*

1. Choose a micropipet and set the volume by rotating the black volume adjustment knob. Make sure to recognize the decimal point for your micropipet (indicated by the → in the table below) and to stay within the volume range.
2. Using the guide below, select the correct tip for your micropipet. Firmly push the end of the micropipet into the open end of the tip while the tip is still in the tip box. (Avoid twisting the micropipet as this can unscrew the shaft.) Lift the tip from the box, but don't touch the tip near the smaller end. Touching the tip contaminates it and will contaminate your samples.

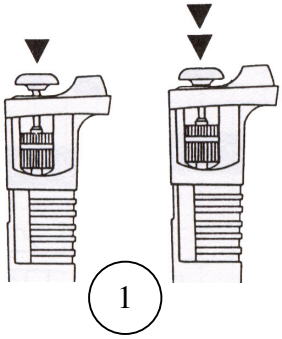
Refer to the Protocol Card for more information

Ulster and Rainen Micropipet and Tip Guide

Size	Range	Top view and Color		Example Setting	Tip size and color	Tip sample
		Ulster	Rainen			
P-10	0.5-10 µl	0.5 / 10 white	P 10 silver	→ 0 6 5 = 6.5 µl	micro white	
P-20	2-20 µl	2 / 20 yellow	P 20 yellow	→ 1 7 8 = 17.8 µl	medium white or yellow	
P-200	20-200 µl	20 / 200 gold	P 200 yellow	→ 1 5 0 = 150 µl		
P-1000	200-1000 µl	200 / 1000 blue	P 1000 blue	→ 0 6 7 = 670 µl or 0.67 ml	large white or blue	

→ decimal point

OPERATING THE MICROPIPET



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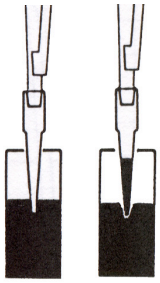
1. Find the stops

Hold the micropipet in your hand.
Use your thumb to depress the plunger.
Practice slowly depressing the plunger and feeling the two "stops."



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2. *Draw some sample fluid from your microtube*

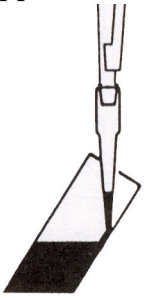
Push the plunger knob down to the first stop.
Keeping the plunger down, lower the tip into the sample fluid just below the surface (~2 to 3mm). Gradually release the plunger.
Do this slowly to avoid sucking liquid inside the shaft of the pipet.
After filling, wait 1 second then remove tip from liquid.

3. Check the tip of the pipet

See how much fluid you have. Make sure that there are no air bubbles trapped in the tip. If so, redraw the sample. Make sure that there are no drops clinging to the outside of the tip.

4. Release the fluid from the pipet

Lower the tip into the microtube and lightly touch the side or bottom of the tube.
Slowly depress the plunger to the first stop.
Wait 1 second, then push plunger to the second stop to expel the last bit of fluid so that the pipet tip has no fluid left in it. Keep the plunger pushed down as you take the pipet out of the tube, then slowly release the plunger.



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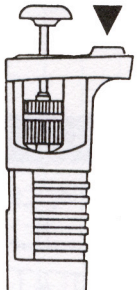

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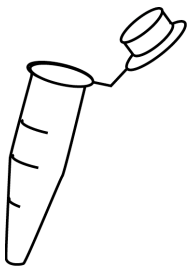
5. ***Eject the tip*** into a waste tip container by pushing the tip ejector button.

Viscous fluids: If you are pipetting a liquid that is very thick or viscous (such as sample loading buffer or restriction enzymes--both contain glycerol or ficoll), it is especially important to insert the disposable tip just into the liquid that you are measuring (say 2 mm). If you immerse the tip fully, large volumes of liquid will stick to the outside of the tip giving you a very inaccurate measurement. With viscous solutions, it is also important to move the plunger up and down slowly. *Being able to do this makes you a real pro!*



1. **Metric conversions**: complete the following

a. 1 ml = _____microliters	d. 1 μl = _____ml
b. 10 microliters = _____ml	e. 20 μl = _____ml
c. 100 microliters = _____ml	f. 2 ml = _____μl

2. 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 _____, _____, _____, _____

3. Explain the reason for each of the following rules:
   a. Always use the micropipet within its designated range:

   _____

   b. Always use a disposable tip on a micropipet:

   _____

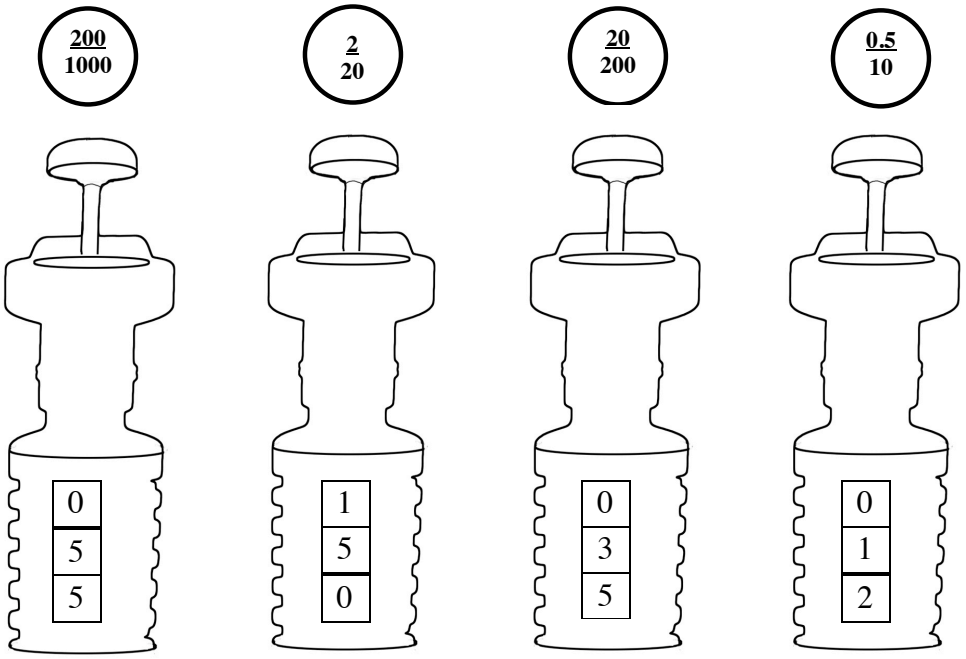
   c. Always hold a loaded micropipet in a vertical position:

   _____

   d. Always release the micropipet plunger slowly:

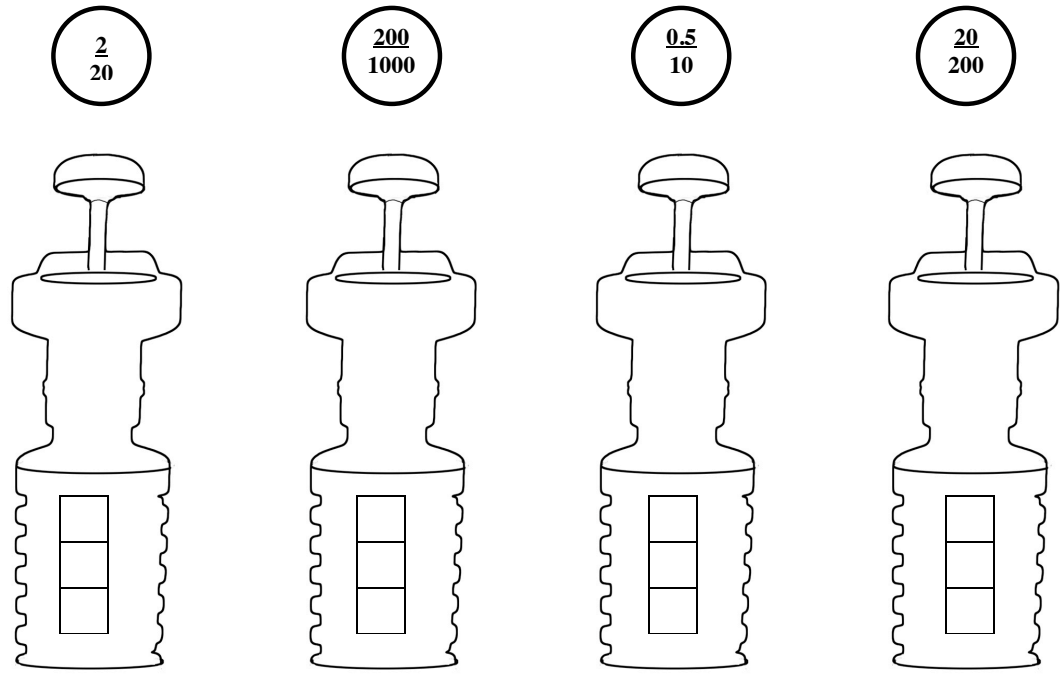
   _____

4. Under each micropipet, indicate the size of the micropipet, the set volume and the range for that micropipet (*the first one is filled out as an example*).



Size (P-?)	P-1000			
Volume:	550 μl			
Range:	200 – 1000 μl			

5. Select the appropriate micropipet and show what the dial should read to measure each of the following volumes: 150 µl, 1.5 µl, 300 µl, and 17.3 µl. Also write the amount on the line beneath each drawing.



_____ _____ _____ _____

6. **Volume comparisons**

Practice measuring 1, 5, 10, 20, 100, and 500 µl. On a piece of waxed paper or Parafilm release the drops and visually compare the sizes. Draw the actual size of each droplet on the chart below. (If you wet the lab table before setting the waxed paper down, the paper will not curl up as much.)

Work on your technique. Be smooth. Try to pick up and dispense the same drop several times

1.0 µl	5.0 µl	10.0 µl
20.0 µl	100 µl	500 µl

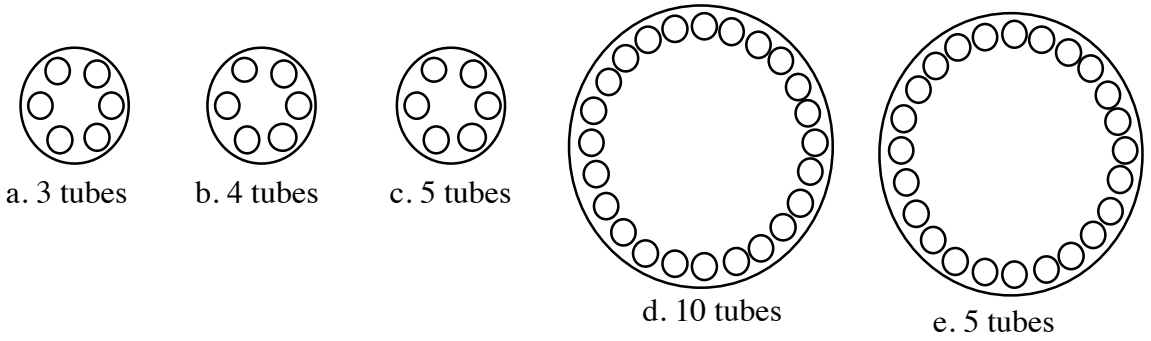
7. Put one drop from an eyedropper or plastic transfer pipet on the piece of wax paper away from your other drops. **Estimate** its size in µl. _____

8. **Microcentrifuge instructions**
   - Close the caps on the tubes.
   - The rotor must always be balanced—you cannot, for example, insert only one tube into a microcentrifuge (microfuge). Spinning in an unbalanced arrangement like this would damage the motor of the instrument. Use an extra balance tube if necessary.
   - After you have closed the lid of the centrifuge, give the tubes a 1-3 second spin. This will mix and pool all the reagents into a droplet in the bottom of each tube.
   - Wait for the rotor to stop before opening the centrifuge lid.

9. Why is it important to balance a centrifuge before turning it on?

_____

10. Show how you would arrange the given number of tubes in each centrifuge to balance the load. If you decide that you must add or remove tubes, explain.



11. Measurement Matrix

Label three empty microtubes A, B, and C, with a permanent marker pen.

Add solutions I, II, III, and IV to tubes A-C as shown in the matrix. To help you stay organized and prevent cross contamination, after pipetting each liquid place a check mark next to that space on your chart.

Always use a new tip for each new liquid being added to the tubes or when adding a new liquid to a tube already containing some.

Micropipet Matrix

Tube	Micropipet	Solution I	Solution II	Solution III	Solution IV	Total
A	P-10	1.8 μl	4.1 μl	3.7 μl	–	
B	P-20	3.4 μl	–	2.6 μl	12 μl	
C	P-200	105 μl	42 μl	–	50 μl	

Spin the tubes in the microcentrifuge (microfuge) for a few seconds to pool the solutions. **See centrifuge instructions in 8 above**.

As a check of your pipetting accuracy, do the following exercise. Set the P-10 to 9.6 μl. Slowly attempt to suck in all of the fluid in tube A. The contents should just fill the tip--no air space at the bottom of the tip, no leftover fluid in the tube. Repeat with tube B and the P-20 (total 18 μl) and with tube C and the P-200 (total 197 μl).

OBSERVATION

12. What is the approximate volume of a microcentrifuge tube? _____μl

13. If **practice agarose gels** are available:

Load 15μl from tubes B and C and all of tube A into separate wells. How much fluid do the gel wells hold?_____ Use an empty well to find out. You may flush out the wells and load them again for practice.