



BIOTECHNOLOGY IN THE CLASSROOM
UNIVERSITY OF CALIFORNIA DAVIS

Biotechnology Laboratory

Micropipet Technique

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MICROPIPET TECHNIQUE

Dye Samples



Introduction

Micropipets are used in almost all biotechnology experiments. These precise instruments allow for the dispensing of extremely small volumes. This lab will help your students become familiar with the techniques required in later labs.

Objectives

1. Learn how to operate a micropipet.
2. Understand measuring volumes in microliters (μl).
3. Convert metric volumes.

Materials

For Each Lab Group

- 1 20 μl micropipet with tips
- 4 microtubes
- microtube rack
- 1 ml red colored water *
- 1 ml yellow colored water *
- 1 ml green colored water *
- 1 ml blue colored water *
- 50 ml beaker of colored water *
- waste container *
- waterproof pen
- wax paper *

** materials provided by instructor*

Instructor Demo Materials

- 1 100 μl micropipet with tips
- 1 1000 μl micropipet with tips

Advance Preparation

1. Aliquot 1 ml of red, yellow, green, and blue colored water for each lab group.
2. Fill a 50 ml beaker with colored water for each lab group.

Teacher Notes

USE OF MICROPIPETS:

These extremely precise and expensive instruments need to be handled gently. **For accuracy to be greatest, be sure to choose the micropipet volume that most closely matches your needs.** For example, to measure 15 μl , use a 20 μl micropipet - not a 100 μl or 1000 μl micropipet.

In this lab, students will only be using the 20 μl micropipeters. Please review setting the volume of the 100 μl and 1000 μl micropipets with your students using the demonstration instruments provided.

20 μl micropipeters:

The volume indicator consists of three number dials and is read from top to bottom. Black digits indicate tens of microliters and microliters; red digits indicate tenths of microliters.

0
7
3

7.3 μl

2
0
0

20.0 μl

⊗ DO NOT DIAL PAST 20 μl .



100 μ l micropipetors:

The volume indicator consists of three number dials and is read from top to bottom. Black digits indicate hundreds and tens of microliters; red digits indicate microliters.

0		1	
7	73 μ l	0	100 μ l
3		0	

⊗ **DO NOT DIAL PAST 100 μ L.**

1000 μ l micropipetors:

The volume indicator consists of three number dials and is read from top to bottom. Black digits indicate thousands and hundreds of microliters; red digits indicate tens of microliters.

0		1	
7	730 μ l	0	1000 μ l
3		0	

⊗ **DO NOT DIAL PAST 1000 μ L.**

To use micropipet:

- Hold micropipet in one hand. With the other hand turn the volume adjustment knob 1/3 of a revolution above the desired setting then slowly down until the required volume shows on the digital indicator. *This prevents mechanical backlash from affecting accuracy.*
- Press disposable tips firmly onto the shaft to ensure an airtight seal.
- Depress top plunger to the **first stop**. Holding the micropipet vertically, immerse the tip approximately two mm into the sample liquid. **Allow the pushbutton to return slowly to the up position!**

- Withdraw the tip from the liquid. Touch the tip end against the side wall of the receiving vessel or the inside of an agarose gel well and depress the plunger slowly to the first stop.
- Wait one second then press the plunger to the second stop, expelling any residual liquid in the tip.
- With the plunger fully depressed, withdraw micropipet and allow the plunger to return to the up position.
- Discard the tip by depressing the lower ejector button. **Use a fresh tip for the next sample to avoid contamination.**

Answers to Student Activity

1. a) 1,000,000 μ l
b) 1500 μ l
c) 0.05 ml
d) 200 μ l
2. Do not overwind the micropipet.
Make sure micropipet is held vertically when there is liquid in the tip.
Always make sure there is a tip on the micropipet when withdrawing a sample.
Do not let the plunger snap up after dispensing volume.
Use a new tip for each sample to avoid contamination.
3. 19.5 μ l 3.8 μ l
4. 100 μ l 42 μ l
5. 870 μ l 1000 μ l

MICROPIPET TECHNIQUE

Dye Samples



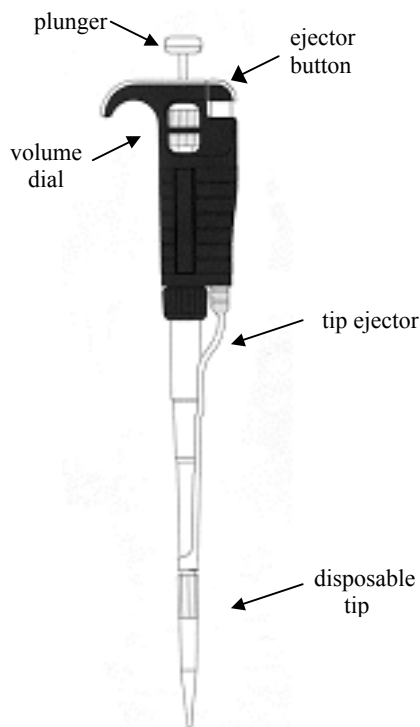
Background

Molecular biologists and biotechnologists work with such small quantities that special tools had to be developed to ensure accurate measurements. One of these tools is the micropipet. For biotechnology, it is as common a tool as a graduated cylinder.

This brief laboratory will familiarize you with equipment and volume sampling techniques necessary in later laboratories.

Objectives

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Materials

For Each Lab Group

- 1 20 μl micropipet with tips
- 4 microtubes
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- 1 ml green colored water *
- 1 ml blue colored water *
- 50 ml beaker of colored water *
- waste container *
- waterproof pen
- wax paper *

* materials provided by instructor

Instructor Demo Materials

- 1 100 μl micropipet with tips
- 1 1000 μl micropipet with tips

Precautions

- ⊗ **DO NOT** use the micropipet without a disposable tip in place. Moisture can damage the piston and reduce accuracy.
- ⊗ **DO NOT** lay a liquid loaded micropipet down. Moisture can run back inside causing damage to the micropipet.
- ⊗ **DO NOT** allow the button to snap back after pushing the plunger. Allow it to return gradually.



Procedure

PART 1: 20 μL Micropipet

1. Select a 20 μL micropipet. Rotate the dial until you get a reading of 10 μL . Your micropipet volume window should look like this:

1	Tens of microliters
0	Microliters
0	Tenths of a microliter

⊗ **When selecting a volume, turn the adjustment knob 1/3 of a revolution above the desired setting then slowly go back down until the required volume shows in the window.**

⊗ **Do not dial the micropipet above the volume written on top of the pipette.**

2. Press the micropipet into one of the tips in the boxes. Press firmly enough to ensure an airtight seal but do not use unnecessary force.
3. Depress top plunger to the **first stop**. Holding the micropipet vertically, immerse the tip approximately two mm into the colored liquid in your beaker. Release pressure on the plunger and let it slowly rise and draw the liquid up into the tip.

⊗ **Never hold the micropipet sideways when liquid is inside the tip!**
4. Withdraw the tip from the liquid. Touch the tip end against the side wall of an empty microtube and depress the plunger slowly to the **first stop**.
5. Wait one second then press the plunger to the **second stop**, forcing out any liquid left in the tip.
6. **With the plunger fully depressed to the second step**, withdraw the micropipet and allow the plunger to return to the up position.
7. Discard the tip into the waste container by depressing the lower ejector button.

PART 2: Micropipet Practice

The following section was adapted from a protocol from Gene Connection™, the San Mateo County (California) Biotechnology Education Partnership

Practicing with a P-20

1. Label three empty microtubes A, B, and C with a permanent ink marker.
2. Add the amounts of the red, yellow, blue, and green solution to tubes A, B, and C as shown in the table below.

Tube	Red Solution	Yellow Solution	Blue Solution	Green Solution	Total Volume
A	4 μL	5 μL	2 μL	0 μL	
B	6.5 μL	2.5 μL	0 μL	10 μL	
C	3 μL	0 μL	12 μL	2.5 μL	



3. Tap tube on table to pool the solutions.
4. Add up the total volume of liquid in Tube A. As a check of your technique, set the micropipet to that volume and withdraw all of the liquid in tube A. The contents should *just* fill the tip -- no air space at the bottom of the tip; no leftover liquid in the tube. Discard liquid and tip into waste beaker.
5. Add up the total volume of liquid in Tube B. Check your technique by setting the pipetor to the correct volume and withdraw all the solution in tube B.
6. Add up the total volume of liquid in Tube C. Check your technique by setting the pipetor to the correct volume and withdraw all the solution in tube C.

Assessment

1. One at a time, each student in a group of 3 should withdraw 9 μL of water from the beaker of colored water and expel the 9 μL as one drop on a piece of waxed paper. When each student has had a turn, compare the four drops. Are the drops the same size? If yes, great! If not, why not?

MICROPIPET TECHNIQUE

Dye Samples



Name: _____

1. Given the following information, complete the questions below:

$$1 \text{ ml} = 1/1,000 \text{ liter}$$

$$1 \mu\text{l} = 1/1,000 \text{ ml}$$

- a) How many microliters are in 1 liter? _____
- b) How many microliters will fill a microtube (1.5 ml)? _____
- c) Convert 50 μl to milliliters. _____
- d) You have 10 mls of water. To create a 2% dye solution out of your water sample how many microliters of dye need to be added? _____
2. List two precautions to take while using the micropipet.

3. Below are readings from a 20 μl micropipet. What volumes do they represent?

1
9
5

0
3
8

4. Below are readings from a 100 μl micropipet. What volumes do they represent?

1
0
0

0
4
2

5. Below are readings from a 1000 μl micropipet. What volumes do they represent?

0
8
7

1
0
0
