Name: _____ MOLE BIO/BIOCHEMISTRY

MOLEBIO LAB #1: Microquantity Measurement

Introduction:

This lab introduces micropipetting and sterile pipetting techniques used throughout this course. Mastery of these techniques is important for good results in pretty much all experiments that follow. Most of the lab exercises are based on *microchemical* protocols that use very small volumes of DNA and other reagents. These require use of an adjustable micropipette that measures as little as on microliter (μL) – a millionth of a liter.

Many experiments require growing *Escherichia coli* in a culture medium that provides an ideal environment for other microorganisms as well. Therefore, it is important to maintain sterile conditions to minimize the chance of contaminating an experiment with foreign bacteria or fungi. *Sterile conditions* must be maintained whenever living bacterial cells are to be used in further cultures. Use sterilized materials for everything that comes in contact with a bacterial culture: nutrient media, solutions, pipettes, micropipette tips, inoculating and spreading materials, flasks, culture tubes, plates, or anything else that can be contaminated.

SAFETY

THE SOLUTIONS FOR THIS PART OF THE LAB ARE COLORED WATER. THE SAFETY PRECAUTIONS LISTED BELOW ARE TO PREVENT DAMAGE TO THE MICROPIPETTE.

- NEVER SET THE MICROPIPETTE TO A VOLUME BEYOND ITS RANGE.
- NEVER ATTEMPT TO USE THE PIPETTE WITHOUT A TIP IN PLACE.
- NEVER LAY DOWN A PIPETTE THAT HAS A FILLED TIP.
- NEVER LET THE PLUNGER SNAP BACK AFTER WITHDRAWING OR EJECTING FLUID.
- NEVER FLAME THE MICROPIPETTOR TIP.

You should already be familiar with metric units of measurement and their conversions. We concentrate on liquid measurements based on the liter, but the same prefixes also apply to dry measurements based on the gram. The two most useful units of liquid measurement in molecular biology are the milliliter (mL) and the microliter (μL).

1 mL = 0.001 liter	1000 mL = 1 liter
1 μL = 0.000001 liter	1000000 μL = 1 liter

Procedure:

USING A PIPETTE WITH A PIPETTE PUMP

1. Take a 10 mL glass (or nalgene) pipette. Carefully place non-tapered end of the pipette into the white end of the pump. Make sure the plunger is fully depressed. Place the tip of the pipette below the solution's surface in the beaker. Using your thumb, slowly spin the wheel of the pump down. This will draw fluid into the pipette. For our pipettes, you should fill past the "0" line and then dispense any extra so that the bottom of the (small) meniscus is now at the "0" line.

2. Touch the tip of the pipette to the inside of the beaker to remove the drop hanging from the tip. If this drop is not eliminated, the volume transferred will be slightly higher than the volume desired.
3. To transfer the solution into the desired vessel, use the trigger to dispense. Carefully watch the volume so as not to dispense too much. Touch the tip of the pipette to the wall of the receiving vessel to remove any liquid from the outside of the tip.
4. Practice pipetting by withdrawing the following volumes from a stock beaker (filled with water) and dispensing them into the above test tubes the following volumes: a) 1.0 mL b) 2.5 mL c) 4.1 mL d) 6.7 mL e) 8.6 mL f) 10.0 mL
5. Now, determine the mass of the volume of water you dispensed. (If pure water, then density = 1.0 g/mL) To do this, use an electronic balance and a 250 mL (or other acceptable volume) beaker. Place the empty beaker on the platform and then tare the balance. Remove the beaker and place it on the lab counter. Dispense the correct amount of water for each trial into the beaker. Record the mass of the water – and then tare the balance. Repeat for b) through f).
6. Calculate the percent error for each of the six trials using the formula below:

$$Percent\ Error = \frac{Experimental\ Value - Theoretical\ Value}{Theoretical\ Value}\ X\ 100$$

7. Show your work in question 1…

USING A MICROPIPETTE

8. Rotate the volume adjustor to the desired volume. Notice the slight change in the plunger length as the volume is changed.
9. Push the pipette end firmly in the proper size tip.
10. When withdrawing or expelling fluid, always hold the tube firmly between thumb and forefinger. Hold the tube at nearly eye level to observe the change in fluid level in the pipette tip. It is important that you watch while you pipette. Do not pipette with the tube in the test tube rack or have another person hold the tube while pipetting.
11. Each tube must be held in the hand during each manipulation. Grasping the tube body, rather than the lid, provides more control and avoids contamination from the hands.
12. Hold the pipette in a vertical position when filling.
13. Most digital micropipettes have a two-position plunger with friction "stops". Depressing to the first stop measures the desired volume. Depressing to the second stop introduces an additional volume of air to blow out any solution remaining in the tip. **Pay attention to these friction stops, which can be felt with the thumb.**
14. To draw fluid, depress the button to the **first stop**, and hold in this position. Then, dip the tip into the solution to be pipetted, and draw the fluid into the tip by *gradually* releasing the plunger.
15. Slide the tip out along the inside wall of the reagent tube to dislodge any excess fluid adhering to the outside of the tip.
16. To expel the sample, touch the pipette tip to the inside wall of the reaction tube into which you wish to empty the sample. This creates a capillary effect which helps draw fluid out of the tip.
17. Slowly depress the button to the **first stop**. **Pause**. Then press on to the **second stop** to blow out the last bit of fluid. **Hold** the button down in the second position for a second or two.
18. Slide the pipette out of the reagent tube with the button depressed to the second stop to avoid sucking any liquid back into the tip.

19. To eject the tip, depress the separate thumb button to 'launch' tip into a waste jar.
20. To prevent contamination of your reagents:
    - Always add appropriate amounts of a single reagent sequentially to all reaction tubes.
    - Release each reagent drop onto a new location on the inside wall of the reaction tube. In this way you can use the same tip to pipette reagent into each reaction tube.
    - Use a fresh tip for each new reagent you pipette.
21. Obtain the large-range (100 μL — 1000 μL) micropipette.
22. Label two (2) 1.5 mL tubes and label them A and B.
23. Using the matrix designed for your micropipette, fill each tube to the desire volume.

Tube	Solution I	Solution II	Solution III	Solution IV
A	100 μL	200 μL	150 μL	550 μL
B	150 μL	250 μL	350 μL	250 μL

24. Close the tops, and place the reaction tubes in a balanced configuration in the microfuge rotor. Spinning tubes in an unbalanced position will damage the microfuge rotor. See below for balanced rotor positions. If you have an odd number of tubes, you can put in blanks that will balance out the arrangement.

Balanced Rotor Configurations

25. Spin tubes for a 1-2 second pulse in the microfuge. This will mix and pool reactants into a droplet in the bottom of each tube.
26. You added a total of 1000 μL (1 mL) of reactants into each test tube. Now, set your pipette to 1000 μL (which equals 1 mL), and very carefully withdraw the solution from each tube. Discard into the waste jar.
    - Are you able to just fill the tip? *You should be able to…*
    - Did you find that a small volume of fluid is left behind? *There shouldn't be…*
    - Did you find that after extracting all the fluid you are left with a small air space in the tip? *There shouldn't be…*

27. Obtain the mid-range (10 μL — 100 μL) micropipette.
28. Label two (2) additional 1.5 mL tubes and label them C and D.
29. Using the matrix designed for your micropipette, fill each tube to the desire volume.

Tube	Solution I	Solution II	Solution III	Solution IV
C	15 μL	25 μL	32 μL	28 μL
D	11 μL	44 μL	18 μL	27 μL

30. Close the tops, and place the reaction tubes in a balanced configuration in the microfuge rotor. **Spinning tubes in an unbalanced position will damage the microfuge rotor.**
31. Spin tubes for a 1-2 second pulse in the microfuge. This will mix and pool reactants into a droplet in the bottom of each tube.
32. You added a total of 100 μL of reactants into each test tube. Now, set your pipette to 100 μL, and very carefully withdraw the solution from each tube. Discard into the waste jar.
    - Are you able to just fill the tip? *You should be able to…*
    - Did you find that a small volume of fluid is left behind? *There shouldn't be…*
    - Did you find that after extracting all the fluid you are left with a small air space in the tip? *There shouldn't be…*
33. Obtain a 0.5 — 10 μL micropipette.
34. Label three (3) additional 1.5 mL reaction tubes and label them E, F, and G.
35. Use the matrix below to add each solution sequentially to each of the three (3) tubes. Be sure to use a fresh pipette tip for each change in solution.

Tube	Solution I	Solution II	Solution III	Solution IV
E	4 μL	5 μL	1 μL	----
F	4 μL	5 μL	----	1 μL
G	4 μL	4 μL	1 μL	1 μL

36. Close the tops, and place the reaction tubes in a balanced configuration in the microfuge rotor. Spinning tubes in an unbalanced position will damage the microfuge rotor.
37. Spin tubes for a 1-2 second pulse in the microfuge. This will mix and pool reactants into a droplet in the bottom of each tube.
38. You added a total of 10 μL of reactants into each test tube. **Now, set your pipette to 10 μL, and very carefully withdraw the solution from each tube.** Discard into the waste jar.
    - Are you able to just fill the tip? *You should be able to…*
    - Did you find that a small volume of fluid is left behind? *There shouldn't be…*
    - Did you find that after extracting all the fluid you are left with a small air space in the tip? *There shouldn't be…*

Micklos, David A. Freyer, Greg A. *DNA Science*. 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2003. 321-330. Print.

1. Show your work for the percent error for all six dispensed samples with the 10 mL glass pipette and then record in Table 1.

   a)

   b)

   c)

   d)

   e)

   f)

volume dispensed (mL)	expected mass of volume (g)	actual mass of volume (g)	difference (g)	Percent Error
1.0				
2.5				
4.1				
6.7				
8.6				
10.0				

2. Using the diagram below, draw **balanced** rotor configurations for 5, 7, 8, 9, and 10 tubes.
   HINT: You must use at least that number of tubes…

3. Which of the following shows an unbalanced rotor? LOOK CAREFULLY!
   (put an **X** through each configuration that is unbalanced)

4. The small range digital micropipette measures volumes between 0.5 μL and 10.0 μL. If you wish to dispense seven and five-tenths microliters of a fluid with the instrument, what sequence of numerals would you see on the digital dial?
   a) 75/00
   b) 75/10
   c) 00/75
   d) 07/50

5. A student presses the button on the micropipette to the first position, places it in a liquid and slowly releases the button. What will most likely occur?
   a) ejection of the tip
   b) fluid will be drawn up into the tip
   c) the last drop of fluid will be pushed ouT of the tip
   d) most, but not all fluid will be expelled from the tip

6. A student with a full micropipette tip depresses the button on the micropipette to the second position. What will most likely occur?
   a) ejection of the tip
   b) most of the fluid will be removed from the tip
   c) fluid will be drawn up into tip
   d) fluid will be ejected and then redrawn into the tip

7. On a large-range Eppendorf digital micropipette, what volume of liquid is indicated by these numbers **0 5 0 0**
    a) 5 μL
    b) 50 μL
    c) 500 μL
    d) 5000 μL

8. Complete the following conversions:
    a) 0.167 mL to μL _____
    b) 0.05 mL to μL _____
    c) 42 μL to mL _____
    d) 182 μL to mL _____
    e) 0.9 μL to mL _____

9. Identify four (4) important precautions in micropipette use:

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