Laboratory #1 – Pipette and Micropipette

Reagents/Supplies:

Micropipettes (P10, P20, P200, P1000)
Pipettes (1ml, 2ml, 5ml)
Micropipette tips
Micro centrifuge tubes
Sharpe pen
250ml Erlenmeyer flask
Water
Fine balance
Balance

Overview:

The proper use and technique of using a pipette and a micropipette is essential in molecular biology. For this lab we will practice using a pipette by moving water from a flask to a culture tube. We will practice using a micropipette by moving water from a flask to a micro centrifuge tube.

Liquid Units:

Become familiar with the metric units of measurement and their conversions. The following examples are based on the Liter but the same prefixes also apply to dry measurements based on the gram.

1 ml = .001 Liter ($1x10^{-3}$)
1,000 ml ($1x10^{3}$) = 1 Liter
1 µl = .001 ml ($1x10^{-3}$)
1,000 µl = ($1x10^{3}$) = 1 ml
1 µl = .000001 Liter ($1x10^{-6}$)
1,000,000 µl ($1x10^{6}$) = 1 Liter

Metric System Units

The table below describes the prefixes for metric units commonly used in molecular biology.

Prefix	Meaning	Exponential Notation
mega	one million	10^{6}
kilo	one thousand	10^{3}
milli	one-thousandth	10^{-3}
micro	one-millionth	10^{-6}
nano	one-billionth	10^{-9}
pico	one-trillionth	10^{-12}

Below are tables of commonly used molecular biology metric units for volume, mass and number of molecules.

Volume	Symbol	Equivalent
liter	l	
milliliter	ml	10^{-3} l
microliter	µl	10^{-6} l

Mass	Symbol	Equivalent
gram	g	
milligram	mg	10^{-3} g
microgram	µg	10^{-6} g

Molecules	Symbol	Equivalent
mole	mol	
millimol	mmol	10^{-3} mol
micromol	µmol	10^{-6} mol
nanomol	nmol	10^{-9} mol
picomole	pmol	10^{-12} mol

Pipette:

A pipette is used to transfer a specific volume of liquid. We will use several different pipettes throughout this course (1ml, 2ml, 5ml). The pipettes are wrapped individually and are sterile until opened. It is important to remember several things when using pipettes:

- Do not open up the wrapper until you are ready to use the pipette.
- Try not to make contact with any part of the pipette, especially the tip. If the tip touches you or anything else consider it contaminated and use a new pipette.
- Do not cross contaminate solutions by using the same pipette. Always use a sterile pipette when transferring liquid to different solutions.

A pipette bulb is used to withdraw liquid from a solution by creating a vacuum in the pipette as the dial on the bulb is rotated. A vacuum release button allows the solution to be released.

Micropipettes:

A micropipette is used to measure and transfer small volumes of liquid. The volume of air space in the barrel is adjusted by screwing the plunger farther in or out of the piston, and the volume is displayed on a digital readout. Depressing the plunger displaces the specified volume of air from the piston. Releasing the plunger creates a vacuum, which draws an equal volume of fluid into the tip. The withdrawn fluid is then expelled by depressing the plunger again.

The volume range for a micropipette can vary. Some micropipettes are set to a fixed volume. The micropipettes we will use are adjustable within a specified range. A small volume pipette such as the P10 has a range of 0.5-10µl. Another small volume pipette we will use is the P20 which has a range of 1-20µl. A mid-range micropipette such as the P200 has a volume range of 20-200µl. A large range micropipette such as the P1000 has a range of 100-1000µl. The precision of these pipettes vary depending on the quality of the pipette and the manufacturer.

Take the following precautions when using a micropipette:

- NEVER rotate the volume adjuster beyond the upper or lower range of the pipette.
- NEVER invert or lay the micropipettor down with a filled tip; fluid can run back into the piston.
- NEVER let the plunger snap back after withdrawing or expelling fluid; this could damage the piston.
- NEVER immerse the barrel of the micropipettor in fluid.
- NEVER flame the tip of the micropipettor.
- NEVER reuse a tip that has been used to measure a different reagent.

Pipette tips come in individually packed, sterilized containers. It is essential that the correct tip is used for the selected micropipette. The tips are usually color coded to the correct micropipette.

How to use a micropipette:

1. Rotate the volume adjuster to the desired setting.

2. Firmly seat a proper-sized tip on the end of the micropipettor.

3. When withdrawing or expelling fluid, always hold the tube firmly between your thumb and forefinger. Hold the microcentrifuge tube nearly at eye level to observe the change in the fluid level in the pipette tip. If possible do not pipette with the tube in the test tube rack.

4. For best control, grasp the micropipettor in your palm and wrap your fingers around the barrel. Work the piston with your thumb. Hold the micropipettor almost vertical when filling it.

5. Most micropipettors 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. Notice these friction stops. They can be felt with the thumb.

6. To withdraw the sample from a micro centrifuge tube.

    a. Depress the plunger to the first stop and hold it in this position. Dip the tip into the solution to be pipetted, and draw fluid in to the tip by gradually releasing the plunger. Be sure that the tip remains in the solution while you are releasing the plunger.

    b. Slide the pipette tip out along the inside wall of the reagent tube to dislodge any excess droplets adhering to the outside of the tip.

    c. Check that there is no air space at the very end of the tip. To avoid future pipetting errors, learn to recognize the approximate levels to which particular volumes fill the pipette tip.

    d. If you notice air space at the end of the tip or air bubbles within the sample in the tip, carefully expel the sample back into its micro centrifuge tube.

7. To expel the sample into a reaction tube.

    a. Touch the tip of the pipette to the inside of the reaction tube into which the sample will be emptied. This creates a capillary effect that helps draw fluid out of the tip.

    b. Slowly depress the plunger to the first stop to expel the sample. Depress to the second stop to blow out the last bit of fluid. Hold the plunger in the depressed position.

c. Slide the pipette out of the reagent tube with the plunger depressed to avoid sucking any liquid back into the tip.

d. Manually remove or eject the tip into a beaker kept on the lab bench for this purpose. The tip is ejects by depressing a separate tip ejection button.

9. To prevent cross contamination of reagents

a. Always add appropriate amounts of a single reagent sequentially to all reaction tubes.

b. Release each reagent drop onto a new location on the inside wall, near the bottom of the reaction tube. In this way, the same tip can be used to pipette the reagent into each reaction tube.

c. Use a fresh tip for each new reagent to be pipetted.

d. If the tip becomes contaminated, switch to a new one.

Procedure:

A. Pipette

1. Obtain a 250ml Erlenmeyer flask and fill it with tap water. Also obtain a 10 ml culture tube. Remove the cap from the culture tube.

2. Place the culture tube on the balance and zero the balance.

3. Obtain a 5ml pipette and open the package so that the end is exposed. Do not remove the entire pipette.

4. Place the pipette bulb on the end of the pipette. Be sure that the pipette is firmly secure in the pipette bulb.

5. Remove the pipette from the packaging. Be sure not to touch the tip to anything.

6. Holding the flask with one hand and the pipette with the other, insert the pipette into the flask and withdraw 2.5ml of water by turning the dial. Try to keep the pipette at eye level and be sure to measure the correct amount of fluid based on the level of the meniscus.

7. Withdraw the pipette from the flask and place the flask on the table.

8. Pick up the culture tube and place the pipette into the culture tube. Press the pressure release valve on the pipette bulb in order to release the fluid.

9. Obtain the mass of the water by weighing the culture tube again. Record the mass of the water in table 1 under the actual weight.

10. Empty the culture tube.

11. Repeat steps 6 through 10 two more times and obtain an average mass for your three tries.

12. Repeat the procedure for the 1ml pipette. For the 1ml pipette measure .4ml.

13. Repeat the procedure for the 2ml pipette. For the 2ml pipette measure 1.5ml.

14. Complete data table 1 by finding the %error for each sample and the average error.

B. Micropipette

1. Obtain eight micro centrifuge tubes. Label each one according Data table 2.

2. Weigh a micro centrifuge tube on the fine balance and zero the balance.

3. Following the instructions on “How to use a micropipette”, add the specified amount of water to the micro centrifuge tube. Refer to data table 2 for the amounts to be added to each tube.

4. Place the micro centrifuge tube on the fine balance to determine the weight of the water. Record the weight in Data table 2.

5. Empty the centrifuge tube.

6. Measure and record the weight for each volume three times.

7. Complete the percent error, average error and standard deviation in data table 2.

Data:

% Error = [| actual value – expected value | ÷ expected value] x 100

Average error = (Σ error) ÷ (# trials)

Standard deviation = $(\Sigma(x-u)^2 / (N-1))^{1/2}$

Σ = sum
x = % error
u = average error
N = total number of errors calculated (in our case 3)
½ = Square root of the total sum

Standard deviation helps determine whether the variation in your data is significant. Two experiments could have the same mean and have very different standard deviations. For example, if you measured, in one experiment, weights of 4.0, 4.5, and 3.5 and in another experiment you measured weights of 1.0, 7.0, and 4.0, the first experiment would have a mean (average) of 4.0 and a standard deviation of 0.5 while the second experiment would have a mean of 4.0 and a standard deviation of 3.0. A large standard deviation indicates a significant variation in the data. Therefore, the data collected may not provide much information. A standard deviation closer to zero indicated little variation in the data. Therefore, the data collected may be more informative.

Table 1

Pipette	**Experimental Weight**	**Actual Weight**	**% Error**	**Average Error**
2.5ml	2.5g	1.	1.	
		2.	2.	
		3.	3.	→
.4ml	.4g	1.	1.	
		2.	2.	
		3.	3.	→
1.5ml	1.5g	1.	1.	
		2.	2.	
		3.	3.	→

Expt. 1A) Testing the Accuracy of Your Pipetting:

Learning how to pipet small quantities of liquid is critical for the success of your experiments on this research project. What may seem as relatively small errors in pipeting may have a large effect on the outcome of your experiments and whether you will get publishable results. It is therefore extremely important that you learn to use the proper techniques when pipeting. Below is an exercise to measure your accuracy in pipeting small amounts of liquid.

Note: This exercise is required for all students

1. **Set a P-20 pipetor to 2 µl.**

2. **Your instructor will pipet 2 µl of Blue Dye onto a piece of filter paper for you to use as a standard. (Note: This is <u>NOT</u> gel loading dye. Please discard after this experiment.)**

3. **Pipet 2 µl of the Blue Dye onto filter paper adjacent to the spot done by the instructor.**

4. **Compare the size of the spots. Are they the same size?**
   a) If your spot is bigger than the instructor's control spot, then that suggests that you have either set the pipet incorrectly or that you have gone down to the second stop on the plunger to draw up the liquid.

   b) If your spot is smaller than the instructor's control spot then that suggests you did not press the plunger all the way down to the first stop, your tip was not in the liquid when you drew up the sample, or that your tip was not firmly seated on the pipetor (allowing air to leak in instead of pulling up liquid).

   Call an instructor to review your pipeting technique if your spot is significantly larger or smaller than the instructor's control spot so that they can review your pipeting technique.

5. **Repeat the spotting of 2 µl 4 more times. Check to see if all the spots are the same relative size. If they are not, then that indicates that your pipeting is not reproducible.**

6. **Repeat the spotting of 2 µl 5 more times, but for one of the spots go down to the second stop to draw up the liquid. Does that spot look significantly different than the others?**

7. **Have your lab partner try to guess which spot was incorrectly pipeted.**

8. **Change the P20 to pipetor 3 µl and spot 3 µl of the blue dye 5 times. On one spot use the second stop. Compare the sizes of the spots to make sure they are the same except for the incorrect one. See if your partner can identify the incorrect spot.**

9. **After the experiment is complete, discard the tube with dye (to prevent it being confused with 10X Gel Loading Dye). Write you school number and your name on the filter paper. Your instructor will collect these and send these to the WSSP staff.**

Table 2

Micropipette	**Volume**	**Expected Weight**	**Actual Weight**	**% error**	**Average Error**	**Standard Deviation**
P10 (low volume)	2µl	0.002g				
	2µl	0.002g				
	2µl	0.002g			→	→
P10 (high volume)	8µl	0.008g				
	8µl	0.008g				
	8µl	0.008g			→	→
P20 (low volume)	2µl	0.002g				
	2µl	0.002g				
	2µl	0.002g			→	→
P20 (high volume)	18µl	0.018g				
	18µl	0.018g				
	18µl	0.018g			→	→
P200 (low volume)	30µl	0.03g				
	30µl	0.03g				
	30µl	0.03g			→	→
P200 (high volume)	185µ1	0.185g				
	185µ1	0.185g				
	185µ1	0.185g			→	→
P1000 (low volume)	250µ1	.250g				
	250µ1	.250g				
	250µ1	.250g			→	→
P1000 (high volume)	850µ1	.850g				
	850µ1	.850g				
	850µ1	.850g			→	→

Results:

A. Pipette

1. How does the percent error vary from trial to trial? Does your technique improve resulting in a smaller percent error with each trial? Explain why or why not.

2. Did the pipette volume affect your %error? Which pipette volume did you find the most difficult to use? What might be the reason for this?

B. Micropipette

1. This experiment should indicate the accuracy of your pipette. Analyze your data for the high and low volume for each pipette. Does the standard deviation indicate a significant variation in the data? If yes, do you believe that this variation is due to the accuracy of the micropipette or due to poor technique? Explain your answer by using the data to support your conclusion.