

# Molecular Biology Lab

## Lab #1 Tools of the Trade

Name \_\_\_\_\_

### Metric System

The metric system is a system of measurements based on the power of 10. The base units are; the meter (m) for length, the liter (L) for volume and the gram (g) for weight. These were derived from each other and relate to natural substances such as water. For example, 1 milliliter of water weighs 1 gram and measures 1 cubic centimeter. Other units were also derived from these; in the Celsius temperature scale water freezes at 0°C and boils at 100°C, also a calorie is the energy required to raise 1 ml of water 1 degree Celsius.

Multiples of metric units are related by powers of ten. This is consistent with the decimal system of mathematics and thus simplifies conversion from one unit to another (1 gram = 1000 milligrams = 0.001 kilogram). Below is a table of metric units and their prefixes

Prefix	abbreviation	Power of 10	Mass unit	Length unit	Volume unit
*mega-	M	10 <sup>6</sup> or 1,000,000	megagram, Mg	megameter, Mm	megaliter, ML
kilo-	k	10 <sup>3</sup> or 1000	kilogram, kg	kilometer, km	kiloliter, kL
hecto-	h	10 <sup>2</sup> or 100	hectogram, hg	hectometer, hm	hectoliter, hL
deka-	da	10 <sup>1</sup> or 10	dekagram, dag	dekameter, dam	dekaliter, daL
<b>base unit</b>	-----	10 <sup>0</sup> or 1	<b>gram</b>	<b>meter</b>	<b>liter</b>
deci-	d	10 <sup>-1</sup> or 0.1	decigram, dg	decimeter, dm	deciliter, dL
centi-	c	10 <sup>-2</sup> or 0.01	centigram, cg	centimeter, cm	centiliter, cL
milli-	m	10 <sup>-3</sup> or 0.001	milligram, mg	millimeter, mm	milliliter, mL
*micro-	μ	10 <sup>-6</sup> or 0.000001	microgram, μg	micrometer, μm	microliter, μL

\*Note that these units are more than a power of 10 difference from the previous units.

Use the metric table above to complete the following conversions

#### Volume

1 L = \_\_\_\_\_ ml

0.1 L = \_\_\_\_\_ ml

1 L = \_\_\_\_\_ μl

1 ml = \_\_\_\_\_ μl

0.3 ml = \_\_\_\_\_ μl

#### Weight

1 g = \_\_\_\_\_ mg

0.01 g = \_\_\_\_\_ mg

1 g = \_\_\_\_\_ μg

1 mg = \_\_\_\_\_ μg

0.45 mg = \_\_\_\_\_ μg

#### Length

1 m = \_\_\_\_\_ km

0.01 m = \_\_\_\_\_ mm

0.1 m = \_\_\_\_\_ μm

1 mm = \_\_\_\_\_ μm

45 cm = \_\_\_\_\_ m

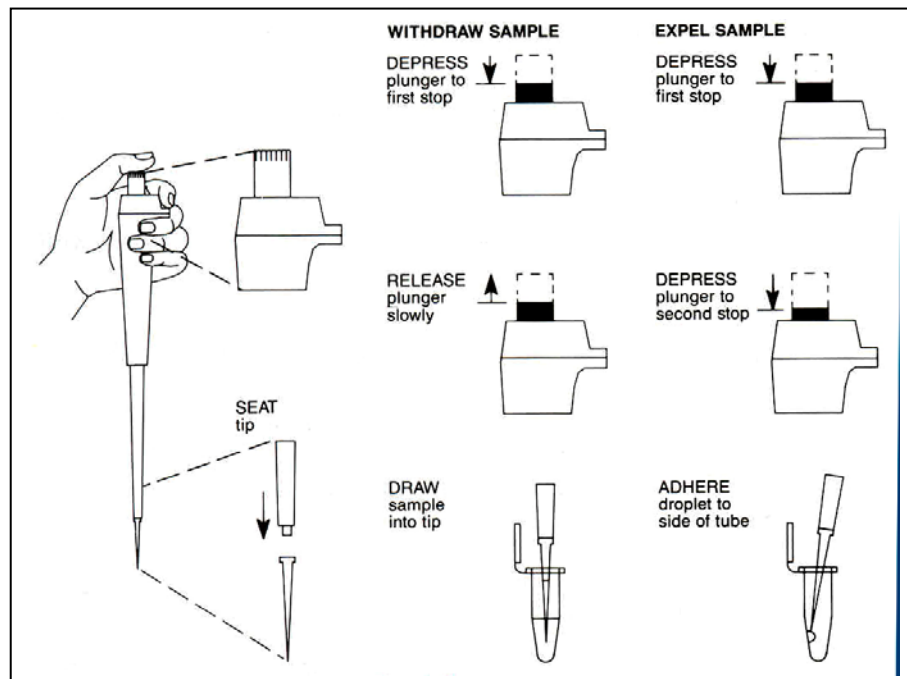
## Use of Micropipettors

Micropipettors are designed to measure and deliver small volumes of fluid. To achieve the highest accuracy, you must use the appropriate size micropipettor. The optimal range of a micropipettor is 10%-100% of its maximal volume. The optimal ranges of micropipettors used in the lab are listed in the table to the right.

Micropipettor	Optimal Range
P10	1-10 $\mu\text{l}$
P20	2-20 $\mu\text{l}$
P100	10-100 $\mu\text{l}$
P200	20-200 $\mu\text{l}$
P1000	100-1000 $\mu\text{l}$

## Pipetting Directions

1. Set the volume by rotating the black volume adjustment wheel. Always set the volume from the same direction. Go 1/3 turn above the desired volume then adjust down to the desired volume.
2. Place a disposable tip on the end of the micropipettor.
3. When withdrawing or expelling liquid always hold the tube in your hand at eye level so you can see what's happening. Don't pipet from a tube in the rack or have another person hold the tube while you pipet.
4. When withdrawing liquid the micropipettor should be almost vertical.
5. Micropipettors have a two-position plunger with friction stops. Pushing the plunger to the first stop measures the correct volume.
6. To withdraw a fluid sample from a tube
  - a. Push the plunger down to the first stop.
  - b. Place the tip into the fluid and gradually release the plunger.
  - c. Check to see that there is no air at the end of the tip.
7. To expel fluid into the reaction tube
  - a. Touch the tip to the inside wall of the reaction tube. This provides a capillary effect that will help to draw the fluid out.
  - b. Slowly depress the plunger to the first stop. Depress the plunger to the second stop to blow all the sample out of the tip.
  - c. Keep the plunger depressed as you remove the tip from the sample to avoid sucking fluid back into the tip.
  - d. Eject the disposable tip into the waste container



8. When pipeting viscous solutions
  - a. Withdraw fluids more slowly and leave the tip in the sample 2 seconds after releasing the plunger to allow the viscous solution to slowly fill the tip.
  - b. Expel fluids slowly, giving them time to leave the tip
  - c. When expelling a viscous solution into a non-viscous buffer, it may be useful to pipet up and down, allowing the buffer to “wash” the viscous solution out of the tip.

### Pipetting Small Volumes

1. Label six microcentrifuge tubes as 1-6
2. Using the table below as your guide, add the indicated volumes of colored solutions to each tube. Colored solutions are to be found on a tray at your bench.
3. Be certain to use the most accurate micropipettor for each volume.
4. Calculate the final volume for each tube and enter that value in the table below.
5. Balance the tubes in the microcentrifuge and spin them for several seconds.

Tube	Green solution	Red solution	Blue solution	Yellow solution	Total volume
1	10 $\mu\text{l}$	650 $\mu\text{l}$	150 $\mu\text{l}$	40 $\mu\text{l}$	
2	55	400	380	15	
3	150	20	60	270	
4	400	80	15	5	
5	4	3	2	1	
6	3	1	2	4	

### Pipeting Viscous Solutions

1. Add 50  $\mu\text{l}$  of tap water to a microcentrifuge tube.
2. Obtain a tube of blue loading dye (this is a viscous solution).
3. Add 10  $\mu\text{l}$  of loading dye to the tube containing the water, but first look back at the procedure for pipeting viscous solutions. Observe the reaction tube carefully as you expel the viscous solution into the buffer.

Did the viscous solution spontaneously mix with the buffer or did it stratify at the bottom?

Did you pipet up and down? Did that help to expel the viscous solution from the tip?

4. Hold the reaction tube at the top between your thumb and forefinger then flick the bottom of the tube to mix the viscous solution with the buffer. Continue until the solution is homogeneous
5. Obtain a second microcentrifuge tube.
6. Add 10  $\mu\text{l}$  of water to this tube.
7. Add 1  $\mu\text{l}$  of loading dye to this same tube. Remember to barely immerse the tip in the viscous solution before withdrawing fluid. If you immerse the tip deep into the viscous solution there will be more solution on the outside of the tip than the inside and you will deliver more than you intended to the reaction vessel.
8. Pipet up and down in the reaction tube to expel all of the viscous solution, but avoid introducing bubbles into the mix. Bubbles create shear forces than can damage enzymes and other proteins in your reactions. If you have introduced bubbles, you can remove them by centrifugation at high speed for a several seconds.

## Gel Electrophoresis

DNA fragments of different size can be separated one from another by gel electrophoresis. DNA is a negatively charged molecule owing to the phosphate groups in the DNA backbone. When placed in an electric field, the negatively charged DNA moves toward the positive pole (cathode). The gel is a matrix of fibers surrounded by water. DNA is loaded into wells in the gel and an electric field is applied. Smaller DNA fragments move more quickly toward the cathode, while larger DNA fragments move more slowly because each fragment must wind its way through the matrix of fibers.

There are 2 common types of gels used in molecular biology; agarose gels and polyacrylamide gels. Agarose gels have a porous matrix and are used to separate relatively large DNA fragments (200 to 10,000 basepairs). The concentration of agarose can be varied depending on DNA fragment size. A higher concentration of agarose produces a denser matrix and thus facilitates the separation of smaller DNA fragments. A lower concentration of agarose improves the resolution of larger DNA fragments. Polyacrylamide produces a gel matrix that is much denser than agarose. Polyacrylamide gels are used to separate small DNA fragments (<400 basepairs) and proteins.

Prior to electrophoresis, DNA samples are mixed with 1/10 volume of loading dye. The loading dye contains 2 negatively charged blue dyes that move toward the positive pole in the electric field. The dyes don't bind to DNA, but co-migrate alongside DNA and function as a visual indicator of the rate of electrophoresis. The smaller of the 2 dyes (bromphenol blue) co-migrates with DNA fragments of about 300 basepairs. The loading dye also contains glycerol, a dense syrupy liquid. Glycerol increases the density of the DNA sample and thus keeps the sample in the well of the gel even when the wells are covered with a buffer solution.

DNA is invisible as it migrates through a gel. DNA must be stained with a dye before we can see it. There are several dyes that will bind to DNA; the most commonly used are Methylene Blue and Ethidium Bromide (EtBr). Methylene Blue is less dangerous to use, but is less sensitive (more DNA is required). EtBr is a fluorescent dye that is much more sensitive, but is carcinogenic and thus requires more safety precautions. EtBr is a flat molecule that intercalates between the bases of DNA. EtBr is relatively colorless under visible light, but fluoresces bright orange when exposed to ultraviolet (UV) light. We will use agarose that already contains EtBr. As DNA migrates through the gel it binds large quantities of EtBr, making the DNA visible under UV light.

### Pouring, Loading and Running an Agarose Gel

1. Place the casting tray into the clamp as demonstrated by the instructor. Be certain that the tray is level.
2. Place a comb in the tray.
3. Put on a pair of vinyl gloves. Obtain molten agarose from the 55<sup>0</sup>C incubator. Work quickly and then return it to the incubator as soon as you are done so it doesn't harden before others can use it. The agarose contains ethidium bromide (EtBr), which is toxic, so avoid contact with your skin.

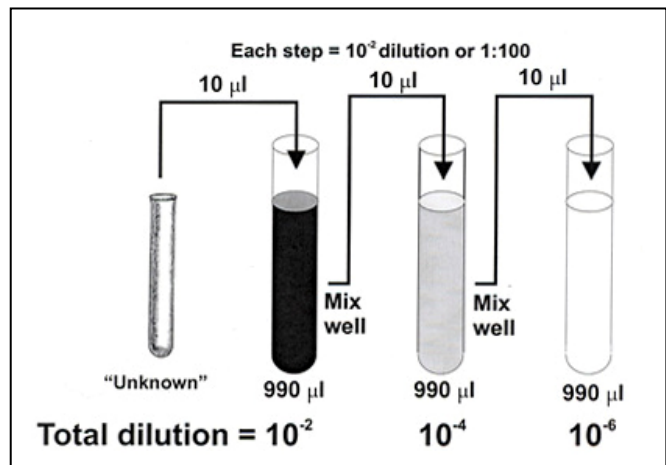
4. Pour enough agarose into the tray such that the height of the gel is about 5mm. The gel should cover only 1/3 the height of the comb. Use a pipet tip to move any bubbles to the side of the casting tray. The gel will become cloudy as it solidifies (about 10-15 min).
5. After the agarose has solidified, remove the comb and then open the clamp and place the tray in the electrophoresis chamber. The tray must slip into its locked position, at which time it will be level in the chamber.
6. Fill the electrophoresis chamber with enough 1X TBE (found at teachers bench) to just cover the gel. The gel should be completely submerged, but just barely. TBE is a salt buffer that conducts electricity.
7. Prepare your DNA samples. Add 1  $\mu$ l of loading dye to DNA tubes 1-4 (found on your tray) and mix well. Spin the DNA in a microcentrifuge at top speed for several seconds to concentrate the solution at the bottom of the tube. Each tube contains 10  $\mu$ l of restriction enzyme-digested Lambda phage DNA.
8. The instructor will demonstrate how to load a gel. Don't proceed until you have observed this demonstration.
9. Load your DNA samples 1-4 from left to right. Load the middle 6 lanes, avoiding the outside lanes (DNA doesn't run even in outside lanes, but rather slants up toward the outside).
10. Put the plastic lid on the electrophoresis chamber, being careful not to disturb the DNA samples.
11. Plug the electric leads into the power supply (red on red, black on black) and turn it on. Set the power supply to 100 volts (measure in volts, not amps). Observe the chamber. There should be bubbles arising near the negative pole (black anode). Within minutes you should be able to see the dye migrating toward the positive pole (red cathode). If these indicators are opposite, then you have set it up backwards and it must be quickly switched.
12. Run the gel until the dark blue dye (bromphenol blue) has migrated approximately  $\frac{3}{4}$  the length of the gel. Turn the electricity off.
13. Put on a pair of vinyl gloves to protect yourself from the EtBr in the gel and from the EtBr that has diffused into the buffer. Remove the casting tray from the electrophoresis chamber then slide the gel into a plastic weigh boat.
14. While still wearing gloves, carefully transfer the gel to the UV light box. Turn on the power to the box. You should now be able to see the orange bands of DNA. Each band represents thousands of DNA fragments, all of the same size. The width of the band is determined by the width of the wells in the gel.
15. The instructor will take a picture of the gel and print a copy for you to staple in your lab report.
16. While still wearing gloves, dispose of the electrophoresis buffer by filtering it through charcoal, as demonstrated by the instructor.
17. Rinse the electrophoresis chamber and lid with tap water, and leave it upside down at your bench to dry.

## Serial Dilution

A serial dilution is a stepwise dilution of a substance in a solvent. Typically the dilution factor remains constant at each step. The most common dilutions factors are 10, 100 and 1000. The concentration of the original solution and the desired final concentrations will determine how great the dilutions need to be. Serial dilutions are used to accurately create highly diluted solutions of solutes, or to accurately measure the concentration of virus cultures or cell cultures.

This technique involves the transfer of a small amount of an original solution to another container which is then brought up to the original volume

using the required solvent. In the example to the right, transfer of 10  $\mu\text{l}$  of the original solution into a 2<sup>nd</sup> tube containing 990  $\mu\text{l}$  of solvent is a 1:100 dilution. Transfer of 10  $\mu\text{l}$  from the 2<sup>nd</sup> tube into a 3<sup>rd</sup> tube containing 990  $\mu\text{l}$  of solvent is another 1:100 dilution. Transfer of 10  $\mu\text{l}$  from the 3<sup>rd</sup> tube into a 4<sup>th</sup> tube containing 990  $\mu\text{l}$  of solvent is another 1:100 dilution. The total dilution at this point is 1 million-fold or  $10^6$ . One could continue this dilution pattern until the final desired concentration is achieved. If the original solution contained  $5 \times 10^8$  cells/ml, then the final tube has a concentration of  $5 \times 10^2$  cells/ml because we have simply divided our original concentration by 100 at each step.



## Serial Dilution Procedure

1. Obtain the microcentrifuge tube labeled MB (methylene blue). You will find this in the rack at your bench.
2. Obtain 3 glass culture tubes and place them in a rack. Label them 1-3.
3. Using a 5 ml pipet, deliver 2.7 ml of tap water into each of the 3 culture tubes.
4. Using your P1000 micropipetor, add 0.3 ml of MB into tube #1 and mix. Hold the top of the tube between your thumb and index finger then flick the bottom of the tube with your other index finger to create a swirling vortex that will effectively mix the solution.
5. Pipet 0.3 ml of tube 1 mixture into tube 2 and mix.
6. Pipet 0.3 ml of tube 2 mixture into tube 3 and mix
7. Save these tubes and their contents for the Spectrophotometer Exercise

## Questions

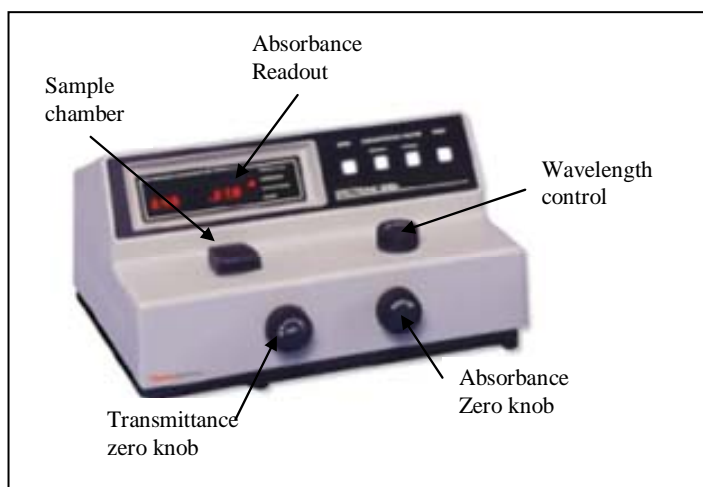
What is the dilution factor at each step? \_\_\_\_\_

If the original MB solution has a concentration of 1 Molar (1 M), what is the concentration of MB in each of the dilutions?

Tube 1 \_\_\_\_\_ Tube 2 \_\_\_\_\_ Tube 3 \_\_\_\_\_

## Spectrophotometry

A spectrophotometer can measure the relative concentration of a solute in a solution or the concentration of cells in a cell suspension by measuring the amount of light that the sample can absorb. If a sample is more concentrated then it will absorb more light. Different substances preferentially absorb different wavelengths of light. For example, methylene blue preferentially absorbs red light (670nm), while chlorophyll preferentially absorbs blue light (450 nm). A spectrophotometer can be set to emit any wavelength of light within the visible spectrum. Typically a test tube containing a liquid sample is placed in the sample chamber, a particular wavelength of light is emitted from a light bulb and directed toward the sample, and the sample absorbs some of the light, while the remainder is transmitted through the sample. A light detector senses the transmitted light, and then the machine subtracts the transmitted light from the emitted light to find the amount of light absorbed. Absorbance values are read from the Absorbance Scale on the machine.



Typically a test tube containing a liquid sample is placed in the sample chamber, a particular wavelength of light is emitted from a light bulb and directed toward the sample, and the sample absorbs some of the light, while the remainder is transmitted through the sample. A light detector senses the transmitted light, and then the machine subtracts the transmitted light from the emitted light to find the amount of light absorbed. Absorbance values are read from the Absorbance Scale on the machine.

### Procedure:

1. Set the wavelength control knob to 670 nm. This is the peak absorbance wavelength for methylene blue.
2. Set the filter to the 600-950 nm range (lever on bottom left, not shown in the drawing above)
3. With the sample chamber empty and the lid closed, adjust the 0% T knob (left knob) until the scale reads zero Transmittance (far left zero line).
4. Set the display mode to absorbance by pressing the mode control key.
5. Pipet 3 ml of water into a small glass test tube; this is your blank. Place the blank into the sample chamber and close the lid. Adjust the 100% T/OA knob (right knob) until the scale reads zero absorbance (far right zero line). The spectrophotometer is now calibrated. It will ignore the contribution of water to the absorbance readings of the methylene blue solution.
6. In the previous lab procedure you made serial dilutions of a methylene blue solution. You will now measure the absorbance readings of those samples.
7. Place tube #1 into the sample chamber. Note the absorbance value and enter it in the space below. The absorbance scale is a logarithmic scale so be very certain you understand how to read the correct values from the scale.
8. Measure the absorbance values for samples 2 and 3. You need not recalibrate between samples.

Sample 1 \_\_\_\_\_

Sample 2 \_\_\_\_\_

Sample 3 \_\_\_\_\_

# Questions

1. Which gel matrix is better for separating molecules in the following situations?
  - a. Separating DNA fragments smaller than 200 basepairs in length \_\_\_\_\_
  - b. Separating Proteins \_\_\_\_\_
  - c. Separating DNA fragments greater than 200 basepairs in length \_\_\_\_\_
2. Why does DNA move toward a positive pole (cathode) when placed in an electric field?
3. What are 2 functions of the loading dye used in electrophoresis of DNA?
4. How does ethidium bromide (EtBr) illuminate DNA?
5. You are given a test tube containing a cell culture with  $1 \times 10^7$  cells/mL. You are to produce a diluted culture that contains less than 100 cells/mL. What dilutions must you perform in order to arrive at the desired result?
6. How does a spectrophotometer function to measure the relative concentration of dye molecules in a solution?

Notes:

