7.02
Training Lab

In the course of the training laboratory today, you will complete five "stations" that are intended to prepare you for work in the 7.02 teaching laboratory this semester. For each station, read the background material that is provided, then complete the practice problems or laboratory exercise(s) that follow. The stations you will complete today are:

Station I: Laboratory Mathematics

Station II: Introduction to Pipetting

Station III: Using the Spectrophotometer

Station IV: Working with Bacteria

Station V: Loading Agarose Gels

Although during the semester 7.02 students work in teams of two, today each student will work independently. You should work through the exercises at your own pace, and be sure to ask the teaching staff any questions you might have.

Station I: Laboratory Mathematics

Note: The information and practice problems below are meant to refresh your memory of these concepts. If they are completely new to you, or if you are having trouble with the practice problems, please ask for extra help.

Background Information:

Part A: The Metric System, Scientific Notation, and Unit Conversions

The Metric System

Reagents in the molecular biology laboratory are measured in the units of grams, liters, and moles. These units will be appended with prefixes to modify the unit by a power of ten, as noted in the table below:

10^3	= 1000	= 1000/1	= 10^3/1	kilo (k-)
10^0	= 1	= 1/1	= 10^0/1	unit (-g, -l, -mole…)
10^{-3}	= 0.001	= 1/1000	= 1/10^3	milli (m-)
10^{-6}	= 0.000001	= 1/1000000	= 1/10^6	micro (μ- or u-)
10^{-9}	= 0.000000001	= 1/1000000000	= 1/10^9	nano (n-)

Scientific Notation

Scientific notation expresses numbers so there is one digit to the left of the decimal point and that number is multiplied by a power of ten. When expressed in scientific notation, 2334 becomes 2.334 x 10^3 and 0.0041 becomes 4.1 x 10^{-3}. Expressing your data in scientific notation makes computation much easier!

Moles, molarity and molecular weight

molarity. (M) The concentration of a solution measured as the number of moles of solute per liter of solution. For example, a 6 M HCl solution contains 6 moles of HCl per liter of solution.

mole. (mol)
1. The amount of a substance that contains as many atoms, molecules, ions, or other elementary units as the number of atoms in 12 grams of carbon 12. The number is 6.0225 X 10^{23}, or Avogadro's number. Also called gram molecule.
2. The mass in grams of this amount of a substance, numerically equal to the molecular weight of the substance. Also called gram-molecular weight. 1 mole of molecules has a mass equal to the molecular weight in grams.

gram-molecular weight or molecular weight. (MW)
The mass in grams of one mole of a substance. For example: sodium chloride, NaCl, has a MW of 58.44grams/mole.

Unit Conversions

The metric system and scientific notation go hand in hand, making unit conversions straightforward. For example 100 μl can be converted to ml by writing the starting volume in scientific notation (1.00×10^2 μl) and multiplying by the power of ten that separates the units (1 ml = 1×10^3 μl). It is a good practice to write out these conversions so that the units will cancel properly when you multiply through, as in the example below:

$$1 \times 10^2\ \mu l \times \frac{1\ ml}{1 \times 10^3\ \mu l} = 1 \times 10^{-1}\ ml$$

Part B: Making Dilutions

Many solutions used in the molecular biology laboratory are stored as concentrated "stock solutions." The researcher will dilute these stocks to "working strength" for his/her experiments. Dilution factors of 1:2, 1:5, 1:10 and 1:100 are common. To make a 1:10 dilution, one "part" of the original (concentrated) solution is mixed with 9 "parts" of a diluent such as water. Thus, there is 1 "part" of the concentrated solution in a **total** of 10 "parts." The second number in the ratio is known as the dilution factor (DF). To calculate the concentration of the dilute solution, divide the concentration of the original solution by the DF.

For example, imagine you have a 5M stock solution of sodium chloride (NaCl). You want to make 100 μl of a 0.5M NaCl solution. To do this, you would make a 1:10 dilution of the stock by mixing 10 μl of the 5M stock solution with 90 μl of water.

A Useful Formula for Dilutions

When the dilution factor is less obvious, the formula $\mathbf{c_1V_1 = c_2V_2}$ can be used, where:

- $\mathbf{c_1}$ is the starting concentration of the stock solution;
- $\mathbf{c_2}$ is the desired concentration;
- $\mathbf{V_1}$ is the volume of stock solution you'll need (usually this is your unknown); and
- $\mathbf{V_2}$ is the final volume of your diluted solution.

For example, imagine that you want to make 1 ml of 0.2M Tris, and you have a stock solution of 1.5M Tris. Applying the formula above:

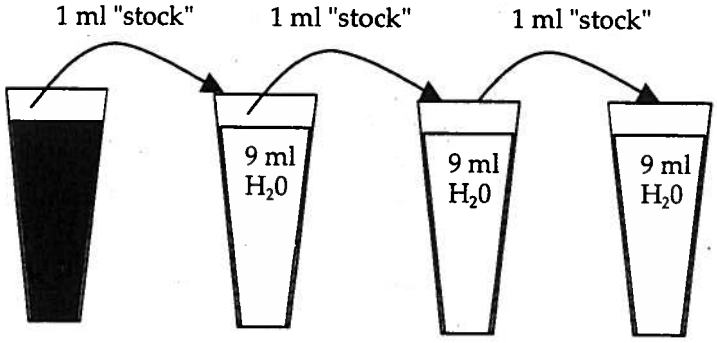
$$1.5M\ (\mathbf{V_1}) = 0.2M\ (1\ ml)$$
$$(\mathbf{V_1}) = 0.133\ ml$$

To determine how much water to add you would subtract $\mathbf{V_2 - V_1}$ (in this case 1 ml – 0.133 ml = 0.867 ml).

Serial Dilutions

When solutions must be diluted several orders of magnitude, then **serial dilutions** are made. You will be doing this frequently in both the Genetics and Protein Biochemistry modules of 7.02. In a serial dilution, the concentrated stock is progressively diluted, for example using a 1:100 dilution as the new "stock" for another 1:100 dilution. These steps can be repeated as necessary. One benefit to serial dilutions is that small volumes of each dilution can be made accurately. On the other hand, any pipetting or calculation error is propagated through every dilution.

Example: Making 1:10 serial dilutions of a 100 mg/ml saline solution



Concentration: 100 mg/ml 10 mg/ml 1.0 mg/ml 0.1 mg/ml

Station I: Practice Problems:

Express the following in scientific notation:

1. 3000 Answer: _____
2. 2 Answer: _____
3. 0.0045 Answer: _____
4. 0.000000876 Answer: _____

Perform the following unit conversions. Show your calculations, and express your answer in scientific notation.

1. The amount of agarose you want to weigh is 1.9 g. How many μg is that?

2. The volume you want to measure is 100 ml. How many liters is that?

3. Your reaction generates 0.1 μmoles of product. How many nmoles is that?

4. How many ng is .023 g?

Calculate the mass needed to make each solution:

1. You want to make a 100 ml of a stock solution of 5 M NaCl. MW of NaCl = 58.44

2. You want to make a 500 ml of a solution that is 5 mM $MgCl_2$. MW $MgCl_2$ = 203.30

Answer the following questions about dilutions:

3. You have a 5X stock solution of DNA loading dye. What volume of stock and what volume of water will you use to make 20 μl of DNA loading dye with a final concentration of 1X?

4. Describe how you would make a 100 ml solution of 3 mM EDTA, starting with a stock solution of 0.1 M EDTA.

5. A concentrated culture of bacteria has approximately 1×10^8 cells/ml. What is the concentration of bacteria after it has been diluted 1:100? What is the concentration of bacteria if a 1:2 dilution was made of the 1:100 dilution?

Station II: Introduction to Pipetting

Background Information:

Part A: Pipetman Use and Care

Pipetmen (or micropipettors) are some of the most important tools in a molecular biology laboratory. Each station has 3 Pipetmen labeled with a station letter to be shared by the T/R and W/F laboratory sections. These precision instruments are designed to deliver exact volumes of liquid within their specified ranges. They are fairly delicate and cost $200 each, so it is important that you know how to use them properly. Below is a table showing some of their specifications.

Pipetman	µl Range	Max. dial reading	Tip size	Tip color
P-20	1-20	20.0	small	clear or yellow
P-200	10-200	200	small	clear or yellow
P-1000	100-1000	1.00 (ml)	large	blue

How to use a Pipetman:

1. Select the Pipetman that will deliver the volume you need most accurately. (Hint: they are more accurate in their higher range than their lower range. Which one would you choose to deliver 15 µl?)

2. Using the black knurled knob, dial to the correct volume setting. Using the maximum dial reading from the table above, determine the correct reading for your volume (Example: 600 µl should read "060" on the P1000). **DO NOT DIAL PAST THE MAXIMUM DIAL READING.** This will cause the instrument to go out of calibration and have to be sent out for repair. You will also be without a Pipetman for the rest of the term, along with your counterparts on the other lab day. Sample volumes for each pipetmen are shown below:

	P20	**P200**	**P1000**
Dial Setting:	**1**	**1**	**1**
	0	0	0
	0	0	0
Volume Delivered	10 µl or 0.010 mL	100 µL or 0.100 mL	1000 µL or 1.00 mL

Note that due to different volume ranges, the same dial setting on the P20, P200, and P1000 will yield three different volumes!

3. Grasp the Pipetman in your hand so the blue "lip" at the top of the barrel is over the knuckle of your index finger. Holding it vertically, firmly push the end of the barrel into the top of the appropriate size tip. The tip should fit snugly to deliver an accurate volume.

4. Using your thumb, push the plunger down to the **FIRST** stop. Submerge the end of the tip in the liquid to be transferred. Do not submerge the whole tip. Gently let the plunger up to suck up the liquid. Don't just let go, as the liquid might jump up and go inside the Pipetman. When the plunger is all the way up, remove the end of the tip from the liquid.

5. Place the end of the tip onto the side of the container into which you are pipetting the liquid. Push the plunger down. When you get to the first stop, keep pushing to the **SECOND** stop to eject all the liquid.

6. To remove a used tip, place the tip into the tip disposal container and depress the white button opposite the blue lip. This will cause the ejection of the tip. All used tips are disposed of in the grey "sharps" container at the end of the benches.

Tips for using Pipetmen

- Keep tip boxes containing sterile tips closed as much as possible to prevent airborne contamination. Bear in mind that the Pipetman itself is not sterile.
- Do not do anything that might cause the plunger to become bent, i.e. vortexing it (yes, it's been done!). This ruins the instrument.
- When using nonsterile tips, handle only the top of the tips to avoid contaminating the end with substances on your skin.

Part B: Pipetting Larger Volumes

A pipette is used to accurately measure and transfer volumes of liquid ranging from 1 to 10 milliliters (ml). They come in many different sizes. In 7.02, you may be using 1, 5, and 10 ml pipettes. You should choose the smallest pipette that allows you to measure the desired amount of liquid. Also, be sure to use care when inserting the glass pipette into the pipette pump. If forced, the glass may break—possibly injuring your hand.

The side of the pipette is marked to indicate the volume of liquid. When you look closely at the liquid in a pipette, you will notice that there is not a flat surface. Rather, you will see a concave curve known as a *meniscus*. Always measure volumes from the bottom of the *meniscus*.

Tips for serial dilutions

- Label all tubes carefully before beginning the dilutions
- Change pipettes (or Pipetman tips) every time you make a new dilution. (If the tips aren't changed, any concentrated solution that remains on the tip from a previous dilution will contaminate the other samples, and the concentrations will be incorrect.)
- Mix each sample well (by pipetting up and down several times) before transferring liquid from one dilution to the next.
- Keep track of the tubes you have finished by moving each tube to the row behind in the rack as you finish the dilution.

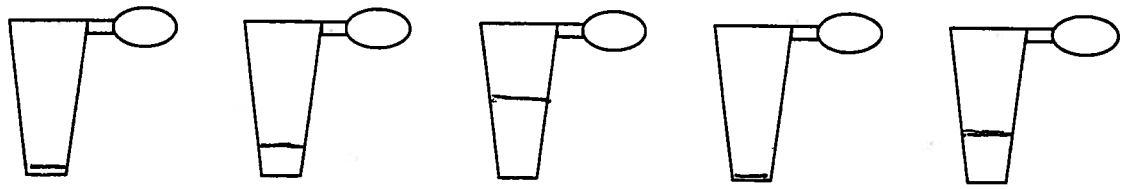
Station II Exercises:

Exercise 1: Pipetting basics

1. Write down which Pipetman (P20, P100, P1000) you would use for each of the following volumes:

17 µl _____
0.130 ml _____
730 µl _____
0.007 ml _____
0.225 ml _____

2. Label five 1.5 ml Eppendorf tubes with the above volumes, and pipette those volumes of water CORRECTLY into the tubes. Draw the results in row of tubes below:



17 µl 0.130 ml 730 µl 0.007 ml 0.225 ml

3. Label another 1.5 ml Eppendorf tube "0.130 ml/wrong." Dial the Pipetman correctly, but this time pipette the water INCORRECTLY (that is, by sucking up by pushing down to the SECOND stop) and dispense it into the Eppendorf tube. Compare the 0.130 ml tubes ("correct" and "wrong").

Is there a visual difference between the tubes that were pipetted CORRECTLY and INCORRECTLY? YES or NO

What volume was actually pipetted into the "0.130 ml/wrong" tube? _____

Take home message:
Remember what you saw, and how volumes varied when pipetting correctly and incorrectly. Always suck up liquid using the Pipetman's FIRST stop, and release by pushing through to the SECOND stop!

Exercise 2: Pipetting Volatile vs. Non-volatile Substances

During 7.02, you will be pipetting two volatile substances: ethanol and isopropanol. These substances behave differently than water. In order to prepare yourself to pipette these substances carefully, practice with isopropanol by performing the following exercise:

1. Pipette up 750 μl of isopropanol, and hold it in the tip over an empty Eppendorf tube. How many drips come out during a 30 second period? _____

2. Repeat the experiment above with water. How many drips come out during a 30 second period? _____

3. Suggest two ways to prevent volatile substances from "dripping" onto your skin or clothing.
   1._____
   2._____

Exercise 3: Making Serial Dilutions

1. Using correct pipette technique, make a 1:2 serial dilution of "1X blue dye" into water. The final volume of your dilution should be 4 ml, and you should make the dilution in a small glass test tube.

2. Perform two more 1:2 serial dilutions from this new "stock" (for a total of three 1:2 serial dilutions). What is the concentration of blue dye in your final dilution? _____

3. **SAVE** these dilutions for the "Using the Spectrophotometer" exercise in Station 3.

--GO TO STATION III—

Station III: Using a Spectrophotometer

Background information:

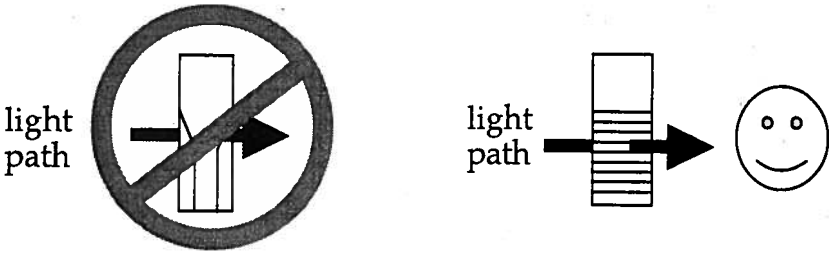
A spectrophotometer (or "spec" for short) is an instrument that measures the amount of light absorbed by a sample. The amount of light absorbed by a sample is directly proportional to the concentration of that sample, which makes the spec a very useful research tool! The amount of light absorbed by a sample is reported as a number followed by the units "OD" ("optical density") and the wavelength of light used (e.g. 0.253 OD600).

Spectrophotometers work by shining light of a particular wavelength into a sample and measuring how much light passes through the sample to a detector. Samples whose absorbance is to be measured are placed in *cuvettes;* these are placed in a holder between the light source and the detector. For each sample whose absorbance is to be measured, one also needs a "blank"—that is, a sample whose absorbance can be used to "zero" the machine.

In 7.02, you will be using the spectrophotometer to measure the absorbance of different things, including bacterial samples and colored dyes. Since each sample will absorb light at a different wavelength, you will need to adjust the spectrophotometer so that the machine provides light of the appropriate wavelength. We can also use the spectrophotometer to follow an increase in the number of bacteria in liquid culture by measuring light scattering of the culture. Basic instructions on how to use our specs—including how to change the wavelength—are provided below:

Operating Instructions for the Milton Roy Spectronic 601 Spectrophotometer

1. Turn power on (switch is in back right corner).
2. Await completion of SELF-TEST.
3. Enter desired wavelength (i.e. 550 nm)
4. Press **<SECOND FUNCTION>** then **<GO TO λ>**
5. Be sure that the display reads "A T" or "A B" If not, press **<T%/A/C>** until this mode (absorbance mode) is reached.
6. Open sample compartment door and insert blank into the sample holder. Be sure that the path length is 1 cm:



7. Close sample compartment door, and press **<SECOND FUNCTION> <100%T/ZERO A>.**
8. Open sample door and remove the blank.
9. Insert sample into sample holder, close sample compartment door, and read the value that is displayed.

Tips for Using a Spectrophotometer

- Mix the contents of the cuvette thoroughly
- Do not touch the clear side of the cuvette with your fingers, or allow it to become dirty. Use a lint-free wipe to clean the sides of the cuvette before inserting it into the spectrophotometer.
- The spectrophotometer's linear range is generally between 0.1-1.0. Values outside this range should be recorded, but not used in calculations.

Station III Exercise:

In station II, you made a set of 1:2 serial dilutions of "1X blue dye." In this exercise, you will measure the absorbance of these samples at 600 nm, and analyze your data.

1. Using a Pipetman, transfer 1 ml of each dilution into an appropriately labeled disposable plastic cuvette. Also, create a "blank" by pipetting 1 ml of water into another cuvette.

2. Following the directions above, set the spectrophotometer to 600 nm, and "blank" the spectrophotometer using the cuvette containing water.

3. Read the OD660 of your samples, and record your data in the table below:

	Initial "blue dye" stock solution	Dilution #1	Dilution #2	Dilution #3
Blue dye concentration	1X			
OD600				

If your serial dilutions were made correctly, how should the OD600s vary with each subsequent dilution? Did your data fit this pattern?

If not, list **two** things you might have done wrong experimentally which might explain your data.

Imagine you have only one pipette tip remaining, and you need to do step 1 in the procedure (transferring samples into cuvettes). In which order would you fill the cuvettes, and **why**?

Station IV: Working with Bacteria

Background information:

Growing bacteria

Bacteria are grown in the laboratory in one of two ways: on "plates" or in "liquid culture." Plates used for growing bacteria consist of media ("bacterial food") mixed with agar (a substance isolated from seaweed that solidifies to form a gel-like substance). This mixture is poured into sterile plastic Petri dishes for use in the laboratory. Bacteria can also be grown in liquid media in sterile test tubes. You will learn more about different types of media and their properties in the Microbial Genetics module.

Purifying Bacterial Strains

In order to study the properties of a given bacterial strain, it is absolutely necessary to work with a genetically homogeneous population of bacteria. A bacterial culture that contains only the descendants of a single cell is such a population; it is referred to as a "*clone*." An easy method for obtaining a pure bacterial strain is to first place the tip of a sterile (autoclaved) wooden toothpick into a bacterial culture. Then one "streaks" the point of the toothpick on an agar surface capable of supporting the growth of the bacteria. In the course of streaking, the needle deposits bacterial cells on the agar; with each streak, the number of bacteria decreases until (after repeated streaks) single cells are deposited. Upon incubation, each single cell grows into a visible "colony." With a little practice, it is possible to streak so that many isolated colonies grow on the plates. Thus "streaking" is a quick method of "diluting" a heavy suspension of bacteria to single cells. You will practice this technique in Exercise 1 below.

Sterile technique

Sterile technique includes a variety of manipulations designed to eliminate the contamination of laboratory cultures, both by each other and by extraneous bacteria and molds. All culture media and all pipettes, Pipetman tips, and vessels (flasks, test tubes, Eppendorf tubes) used to incubate and transfer bacteria are sterilized, usually by heating in an autoclave with steam at 15 lbs. psi for 15 minutes, or in a hot 170°C oven for several hours. Plastic Petri dishes come presterilized from the factory.

In the 7.02 laboratory, you will be using both sterile and non-sterile Pipetman tips and Eppendorf tubes. Boxes containing **sterile** tips or tubes will have "indicator" tape on them (black stripes on a white background). To ensure that the contents of these boxes remain sterile, be sure to keep the tip/tube boxes closed when you are not removing items from them.

Glass pipettes are sterilized in cans, tip down, so that the tops protrude when the lid is removed. Pipette cans should be stored horizontally to minimize chipping pipette tips and to reduce the risk of airborne contamination. To keep pipettes sterile when removing them from a can, grasp the top end of the pipette on the top of the stack; lift and slide along the upper surface of the can. Pull it out handling only the top of the pipette. Once a pipette has been *laid down or touched* it is no longer sterile!

To open a sterile test tube or a flask, remove the cotton plug or cap and hold it so that the surface of the cap or plug faces downward and does not touch *anything*. Briefly flame the lip of the tube or flask in the Bunsen burner before removing any contents. After removing contents, briefly flame the lip of the tube/flask again, and carefully replace the cap or plug.

Station IV Exercises:

Exercise 1: Purifying Bacterial Strains

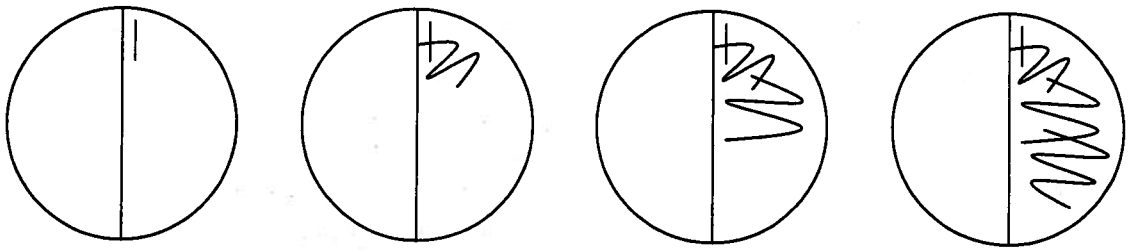
Your goal in this exercise is to "streak" a bacterial strain to obtain individual, isolated colonies ("single colonies") on an LB agar plate.

1. You will be provided with one LB agar plate on which you will attempt to purify two bacterial strains. Use a Sharpie marker to draw a line on the bottom of the plate, dividing it into two sectors.

2. Obtain a Petri plate containing bacteria, which should be on the bench in your working area. (If not, ask the nearest staff person.) Write the name of each strain you are attempting to purify on the back of your LB agar plate (one strain/sector).

3. Using a toothpick, lightly touch one well-isolated colony from one of your bacterial strains. (This toothpick now has millions of cells on it.) Gently touch this toothpick to your own agar plate, making a small line approximately 1/4 inch long (see diagram below). Dispose of this used toothpick in a "picks" can.

4. Using a "stick," cross over your first line (the inoculation) **ONCE**, and make a series of zigzags. Dispose of the stick (stick #1) in the "sticks" can. Repeat this step with two more sterile sticks, as shown in the diagram below:



Inoculate with one toothpick | Stick #1 | Stick #2 | Stick #3

5. Repeat steps 3 and 4 on the other half of your plate using another bacterial strain.

6. Write your last name in big letters (and first name in little letters) on the bottom of your LB plate, and give it to the nearest staff person. He or she will incubate the plate at 37°C overnight to allow the bacterial cells to grow.

Exercise 2: Plating Bacterial Cultures

It is often necessary to spread a small amount of a liquid bacterial culture evenly onto the agar surface of a Petri plate. This is called "plating" a culture. In this exercise, you will practice removing a sample from a liquid culture using sterile technique, and will plate the culture on the surface of an LB agar plate.

1. At your bench, you will find the following materials:
    1) small glass test tube containing a liquid bacterial culture;
    2) LB plate;
    3) bunsen burner and striker;
    4) glass jar containing approximately 1 inch of ethanol (alcohol), with lid;
    5) glass "spreader" (a piece of glass rod bent to form an equilateral triangle with the handle attached at a slight angle to one apex);
    6) Pipetmen and sterile tips.

2. Light the bunsen burner with the striker, taking care to keep the flame away from any ignitable materials (hair, clothing, paper).

3. Using sterile technique, remove 100 μl of the culture from the test tube and transfer it onto the center of the surface of the LB plate.

4. Sterilize the glass spreader by dipping it into the jar filled with ethanol, and then passing it quickly through the Bunsen burner flame. The ethanol on the spreader will ignite, then burn out. Be careful not to ignite the alcohol in the jar—if the alcohol in the jar does catch fire, extinguish the flame by covering the jar!

5. Touch the spreader to the agar in the Petri plate away from where the bacteria were placed. (This step "cools" the spreader before it touches the bacteria.)

6. Move the cooled spreader over the agar surface in order to evenly distribute the culture on the LB plate. One way to do this is to move the spreader back and forth while rotating the Petri plate. (Touch only the sides of the plate so you don't contaminate the agar surface with your fingertips!). Be sure to "spread to dryness" (see note below).

> **Note:** It is important to "spread to dryness" when plating bacterial cultures—that is, leaving no "puddles" of liquid on the plate. There are two clues that will tell you when you have succeeded: 1) as the culture spreads on the plate and seeps into the agar, the resistance you feel when spreading will increase; and 2) the plate will look "drier"—that is, it won't look like there's a film of liquid on top.

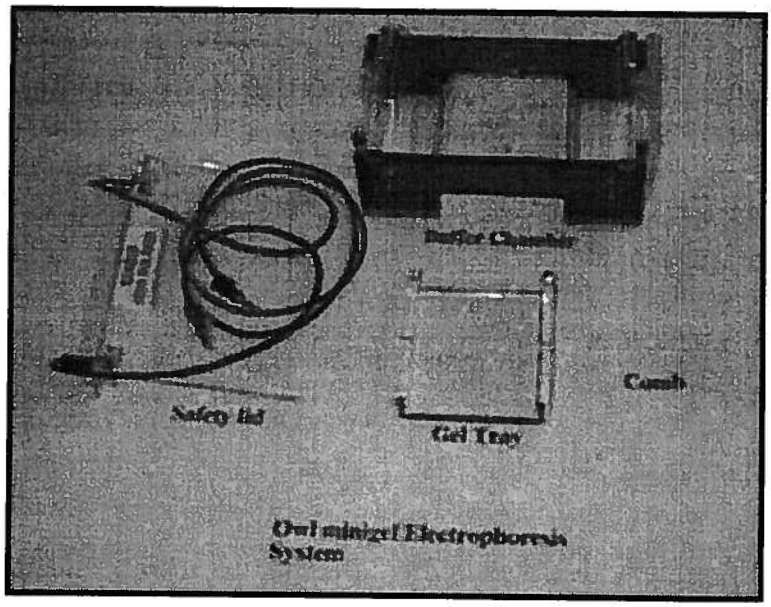
7. After plating your culture, write your last name (in big letters) and first name (in small letters) on the bottom of the LB plate, and give your plate to the nearest staff person. He or she will incubate the plate at 37°C overnight to allow the bacterial cells to grow.

Station V: Loading an Agarose Gel

Background information:

In the Recombinant DNA Methods modules of 7.02, you will use agarose gel electrophoresis to separate nucleic acids (DNA) of different sizes and shapes. Agarose is an inert (non-reactive) polymer isolated from seaweed. When dissolved in liquid and heated, the agarose goes into solution. As the agarose solution is cooled, it solidifies into a "gel" with a matrix of pores in it. Altering the amount of agarose used to make the gel alters the size of the pores (more agarose leads to smaller pores). When a biological sample is loaded into "wells" in the gel and an electrical current applied, the components of that biological sample are "sieved" by the pores of the gel and are separated from one another.

On your bench, you will notice an "agarose gel electrophoresis apparatus" that consists of four parts—a tray, a comb, the gel box (buffer chamber), and the safety lid. These are pictured below, and a description of their functions follows:



(image from: http://www.bio-link.org/vlab/DNAGelBox.html)

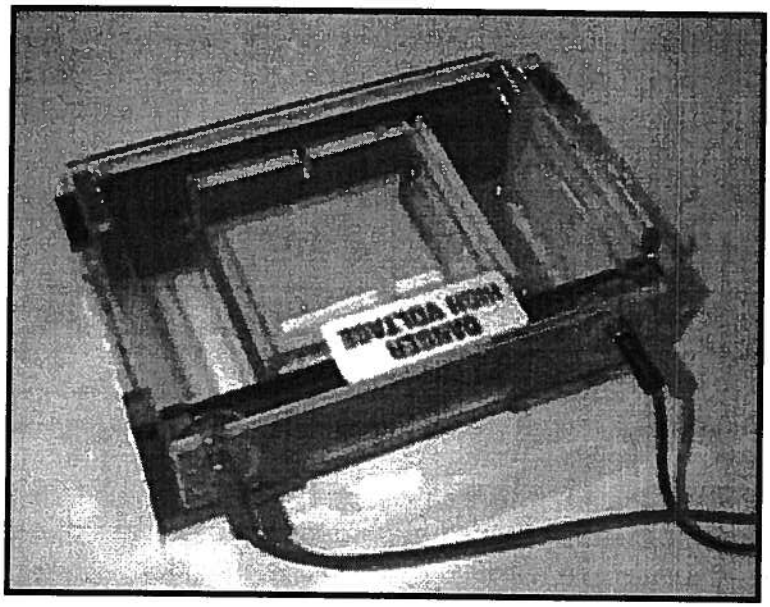
Gel tray: The "tray" is the part of the apparatus into which the molten agarose is poured and allowed to harden into a gel.

Comb: When pouring a gel, a "comb" is placed at one end of the tray. This comb consists of hard plastic "teeth" connected by a plastic bar. When molten agarose is poured into the tray, it surrounds the teeth of the comb and solidifies. When the comb is removed, "wells" are left behind where the teeth once were. Samples to be fractionated by electrophoresis are loaded into the wells.

Gel box: The "gel box" consists of two "buffer chambers" connected by a thin wire. The ends of the wire are connected to the positive and negative electrodes of a power supply. When the power supply is turned on, an electrical current flows between the buffer chambers via the wire.

Safety lid: The safety lid covers the gel box, preventing the researcher from accidentally touching the buffer when the current is flowing. Power cords (or "leads") connect the ends of the wire in the gel box to the electrodes of the power supply.

The figure below shows how the pieces of the apparatus fit together. Note that for electrophoresis, the comb is removed to create the "wells" into which samples are loaded.



(image from: http://www.bio-link.org/vlab/DNAGelBox.html)

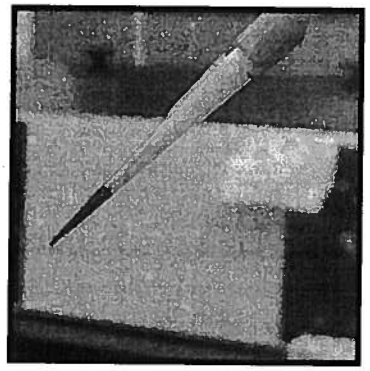
To perform electrophoresis (also known as "running the gel"), the chambers are filled with "running buffer," which is an aqueous solution of salts and a pH stabilizing mix of chemicals (i.e. Tris and acetate or Tris and borate). The samples are loaded into the wells of the gel, which sits on the "shelf" between the two buffer chambers and is itself covered with buffer. When an electrical current is applied, the molecules in the sample move toward the appropriate electrode based on their charge, and are separated from each other due to the "sieving" action of the gel matrix. In agarose gel electrophoresis, smaller molecules move quickly through the pores of the gel, whereas larger molecules take a longer time to "snake" through the pores, and travel less distance in a given amount of time. One can observe the progress of the electrophoresis by including a "loading dye" with your samples; this "dye" is visible to the eye, and allows you to see how quickly your samples are moving through the pores of the gel matrix.

Station 5 Exercise: Loading an agarose gel

In this exercise, you will practice loading an agarose gel by adding a sample of blue "loading dye" to one well of a pre-poured agarose gel. This technique is best "learned by doing," but we have provided some images and tips to help you get started.

Note: the images below are taken from the Virtual Lab Book at the College of Charleston (http://www.cofc.edu/~delliss/virtuallabbook/), an excellent resource for the student in the molecular biology laboratory.

1. The teaching staff has poured agarose gels for you to load. These have been placed in the gel apparatus, the comb has been removed, and the gel has been covered with running buffer.



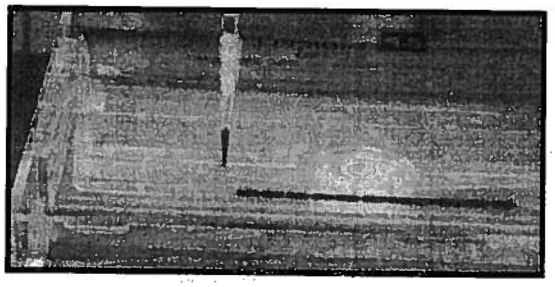
2. Using the appropriate Pipetman, pull up 10 μl of "loading dye" and check to be sure that there are no bubbles in the tip, as shown in the figure to the left.

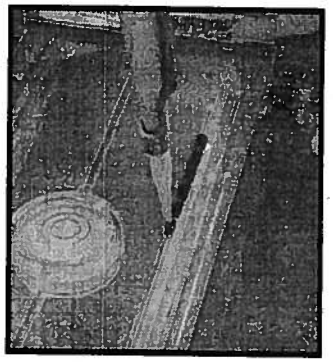
(If you load a bubble along with the sample into the well of the gel, it may "bump" your sample out of the well if it pops!)

3. Carefully load the sample into the well of the gel, as shown in the set of pictures below:

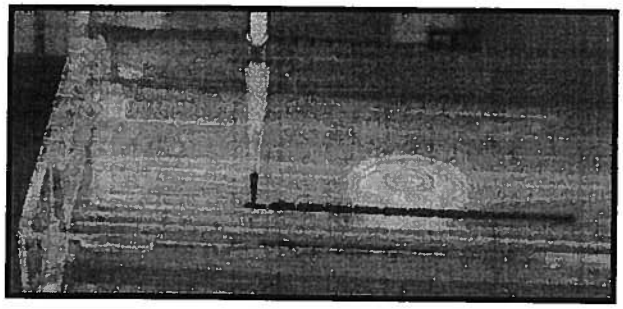
a. First, place the tip containing your sample into the buffer, directly above the well into which you would like to load your sample. Be careful not to put the tip too deep into the well, as you may poke a hole in the bottom of the well!

Tip: Use your free hand to support the hand holding the Pipetman to keep it steady!





b. Using **gentle** and **consistent** pressure, depress the plunger of the Pipetman to the FIRST stop, releasing your sample into the well. DO NOT press through to the second stop, as you will create a bubble!



c. Keeping the Pipetman plunger depressed, **gently** lift the tip out of the buffer. After removing the tip from the buffer, release the plunger. Note that there will be a tiny amount of sample remaining in the tip.