Bi 1X, Spring 2013

Week 1

- **Session 1: Introduction on Lab Techniques**

I. Lab Safety

Review the lab safety guidelines attached at the end of the handout with your TAs.

Exercise: Wearing gloves

1. Choose a matching pair of gloves to wear on your hands (only half of the class should wear gloves for this exercise).
2. Rub your hands with some plastic simulated glowing germs offered by your TAs
3. Take off your gloves as demoed by your TAs and examine your hands under the UV keychain light.
4. Wash your hands thoroughly with soap and check again under the UV keychain light.

II. Pipetting

One of the most important instruments in a biology lab is the pipettor, a device that allows highly accurate handling of small quantities of liquid. There are several brands of pipettors in the lab, but they all operate in the same manner, relying on air displacement to move liquid in and out of disposable plastic tips.

Pipettors are manufactured to handle different volume ranges, and it is important to choose the appropriate pipettor for the volume of liquid you wish to deliver to ensure accurate results. You will have been provided with a set of micropipettors consisting of a combination of the following list of volume ranges:

Pipettor model	Recommended Volume Range	Tip
P-2	0.2 – 2.0 µL	Clear
P-10	0.5 – 10.0 µL	Clear
P-20	2 – 20 µL	Yellow
P-50	5 – 50 µL	Yellow
P-100	10 –100 µL	Yellow
P-200	20 – 200 µL	Yellow
P-1000	100 – 1000 µL	Blue

Filter tips

Throughout the course, you will work with purified DNA. To minimize sample contamination, you will use filter tips that can trap aerosols and airborne contaminants. Samples that come in contact with the filter can still be dispensed for further use.

Serological pipettes

These long, disposable, sterile, plastic pipettes are designed for large volume samples on the order of milliliters. The type of pipettor for these pipettes differs from group to group: we will use an electronic transfer pipettor (aka: Pipette Aid) in Bi 1X.

Using a Micropipettor

1. Select the model of pipettor that is appropriate for the volume you wish to dispense (consult the above table, if necessary).
2. Set the volume by rotating the plunger button until the desired volume appears in the display window. Remember that regardless of the volume, pipettors have three digit displays—the digits represent different volume increments.
3. Attach a fresh tip by pressing the bottom of the pipettor shaft down into the top of the tip. Do not apply excessive force!
4. Press the plunger down **to the first stop.**
5. Keeping your pipettor vertical, immerse the tip 2-4 mm into the liquid.
6. Slowly allow the plunger to return to the up position.
7. Wait 1 or more seconds to allow the entire volume of liquid to flow into the tip (you will have to wait longer for more viscous fluids).
8. Withdraw the tip from the sample.
9. To dispense the sample, dip the tip into the recipient solution and slowly depress the tip to the first stop, mix (if necessary) by moving the plunger up and down, then depress to the second stop. This will "blow out" any remaining liquid in the tip. **If you are pipetting into an empty tube, touch the tip to the side of the wall while dispensing.**
10. Withdraw the tip from the sample, and eject it into the appropriate waste container by pushing the eject button.

Using a Pipette Aid

1. Unwrap and attach a plastic transfer pipet to the handle. Save the plastic wrap for later.
2. Place the tip of the pipette into the liquid you wish to aspirate.
3. Push the "aspirate" (top) button to withdraw the liquid into the pipet (note: how far in you push the button controls pipetting speed)
4. Withdraw the pipet from the source solution
5. Place the pipet into the receptacle and push the "expel" (lower) button to eject the liquid
6. Dispose of the pipet into original plastic wrap

Guidelines

Pipettors are expensive, precision instruments and must be treated gently! Before you begin pipetting, please familiarize yourself with the following guidelines:

- **Never, ever** dial to a volume outside of the limits of the pipettor (either too high or too low). This may result in damage to the internal mechanism.
- **Always** use a fresh pipet tip for every pipetting action to minimize sample contamination (tips are cheap, contamination isn't).
- **Do not** allow liquid to enter into the pipettor shaft. You can avoid this circumstance by remembering to:

- **Always** keep the pipettor vertical at all times that liquid is in the tip
- **Never** let the plunger snap back up quickly when you are withdrawing liquid
- **Always** use the pipettor with a disposable tip of the correct size. Be sure to apply the tip with enough force to ensure an airtight seal, but not enough force that you strain the pipettor shaft.

Exercises:

Pipetting Practice:

You have been supplied with tubes of water, glycerol solution, and Triton X-100 (a detergent) so that you can practice pipetting under different sample conditions. Water is the easiest of the three to pipet. Glycerol is more viscious and must be handled more carefully to ensure accurate dispensing. Finally, Triton X-100 is not only more viscous than water, but it also has a reduced surface tension. It has a tendency to creep in and out of pipet tips, and bubble while dispensed.

Use these three solutions to try dispensing under different sample conditions. Try pipetting into empty tubes, sample-filled tubes, and onto Parafilm so that you can practice repeatable and accurate pipetting.

Restriction Digest:

Once you feel confident in your ability to pipette, you will perform a restriction digest. This means that you will mix DNA with buffer and DNA-cutting enzymes called *restriction endonucleases*. You will be provided with two types of DNA. The first is bacterial non-chromosomal DNA called a plasmid. The second is a circular chromosome from the bacteria-eating virus called *lambda phage*. The DNA will be cut at specific sites and sheared into fragments of known length. For now, we are only providing cursory information as the intention is for you to practice accurate pipetting. We will revisit this lab technique later in the week.

You will perform 4 reactions:

1. A no enzyme control of the plasmid pZE21-lacZ
2. A HindIII digest of the plasmid pZE21-lacZ
3. A KpnI/HindIII double digest of the plasmid pZE21-lacZ
4. An EcoRI digest of HindIII predigested lambda phage DNA

Label 4 eppendorf tubes and mix the reactions above according to the tables below. Add reagents in order. Restriction enzymes are supplied in a viscous, glycerol containing solution. Your TAs will pass around the enzymes on ice to be added after you set up all of the reactions except adding the enzymes. Avoid touching the bottom of the enzyme tube with your hand to prevent denaturation. Pipette with care!

After you have assembled your reactions, you should mix them, spin them down briefly, and place them at 37oC for the remainder of the lab session (~2 hours). Your TAs will freeze them afterwards for use later in the week.

Remember to change tips after every pipetting operation!

No Enzyme Control of Lambda DNA:

Reagent:	**Amount:**
Sterile water	calc.
Plasmid DNA (100 ng/ul)	300 ng (calc.)
NEB Buffer (10X)	calc.
BSA (10X)	calc.
Total:	30ul

HindIII Single Digest of pZE21-lacZ plasmid DNA:

Reagent:	**Amount:**
Sterile water	calc.
Plasmid DNA (100 ng/ul)	300 ng (calc.)
NEB Buffer 2 (10X)	calc.
BSA (10X)	calc.
HindIII (10 units/ul)	1 ul
Total:	30 ul

KpnI/ HindIII Double Digest of pZE21-lacZ plasmid DNA:

Reagent:	**Amount:**
Sterile water	calc.
Plasmid DNA (100 ng/ul)	300 ng (calc.)
NEB Buffer 2 (10X)	calc.
BSA (10X)	calc.
KpnI (10 units/ul)	1 ul
HindIII (10 units/ul)	1 ul
Total:	30 ul

EcoRI Digest of HindIII digested lambda DNA:

Reagent:	**Amount:**
Sterile water	calc.
Predigested lambda DNA (0.5 ug/ul)	1.5 ug (calc.)
NEB EcoRI buffer (10X)	calc.
BSA (10X)	calc.
EcoRI (10 units/ul)	1 ul
Total:	30 ul

III. Working with bacteria & sterile technique

In Bi 1X, you will often be using cultures of various organisms, primarily *E. coli*, and it is essential that your cultures remain pure. However, bacteria are found everywhere—on your skin, on your lab bench, and even in the air. Fortunately, by practicing sterile technique, you can easily minimize the opportunity for contamination.

In the first part of today's lab session, you will become acquainted with bacteria and sterile technique by performing several exercises.

Materials

- LB agar plates
- One LB agar plus kanamycin plate
- Liquid LB medium
- Inoculating loops
- *E. coli* liquid culture
- *E. coli* agar plate
- Empty culture tubes

*NOTE: LB is Luria-Bertani broth, a very common media used to grow *E. coli*.

Exercise 1: Bacteria in the air

1. Label one of your LB agar plates, take the top off, and set it right side up on your bench. Leave this plate open for the rest of today's activities. This plate will test for microorganisms that are present on dust particles floating in the air.
2. As a *negative control*, label another plate and place it next to first, only with the top on. This plate will ensure that any results you see on the plate from (1) are from external particles, and not some sort of contaminated media.

Exercise 2: Antibiotics

1. Draw a line on the back of one plain LB plate and one LB-antibiotic plate to divide them each into two halves. Denote one half per plate as "before cleansing" and one half per plate as "after

cleansing." Moisten your fingers with some tap water and place them on the before half of the LB plate and on the "before" half of the LB-antibiotic plate.
2. Wash your hands with soap and water (taking care not to touch the faucet knobs with your fingers) and repeat step 1 using the "after cleansing" sides of your plates.
3. Allow your plates to stand right side up, with their tops on, for the remainder of the class period.

Exercise 3: Microbial ecology of your mouth (just for fun!)

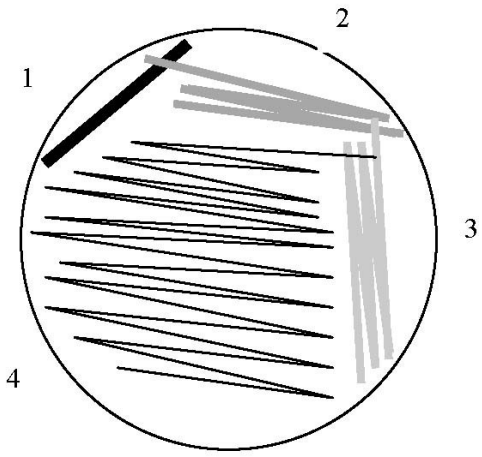
1. Label a fresh LB agar plate. Remove the top, and spit on the surface of the agar.
2. Allow the plate to stand right side up, with its top on, for the remainder of the class period.

Exercise 4: Streaking

Streaking an agar plate allows you to obtain well-isolated, discreet bacterial colonies by sequential dilution of a starting sample.

1. Examine the plate of colonies you have been given, and choose two you wish to streak out.
2. Label the recipient plate.
3. Using a fresh, disposable inoculating loop or fresh pipet tip, pick up a small amount of the colony you wish to streak.
4. Immediately streak this material onto the side of the plate (see figure below), then discard the loop or tip.
5. Using a fresh loop or tip, streak from the first area into a new area of the plate by making a series of straight lines away from (but overlapping) the edge of the previously inoculated area.
6. Streak from the second area into a third, new area using a new loop or tip.
7. Streak from the third area into a fourth, new area—this area should cover most of the remaining space of the plate—using a new loop or tip.
8. Try streaking a second LB agar plate.

Streak as follows:



Exercise 5: Innoculating a liquid culture using sterile technique

1. Start your burner by turning on the gas, and then lighting the flame with a striker.
2. Transfer 5 ml of LB media into each of four tubes, using sterile technique as demonstrated by your TAs.

3. Using a clean loop or pipet tip, transfer some material from one of your supplied colonies into a tube. Repeat for a second tube.
4. Dip a clean loop or pipet tip into a third tube. Repeat for a fourth tube. These will be your control tubes.

Be sure you have labeled all your cultures with your name and the date!

IV. Spectrophotometry to measure cell density

Spectrophotometry is a process by which the ability of a sample to absorb light can be examined quantitatively. You will use a spectrophotometer to obtain the spectrum of chlorophyll and to determine the density of cells in a bacterial culture.

Spectrophotometry of chlorophyll (crude spinach extract)

1. Extract chlorophyll from a small sample of spinach by incubating the spinach in ~5-10 ml of 100% ethanol. After 5-15 minutes, the aqueous phase will turn green (and will contain the chlorophyll) and be suitable for use in your spectrophotometric study.
2. Turn on your spectrophotometer and prepare it for an absorbance scan from 350-750 nm. Each spectrophotometer is different, so you will need to ask your TA for specific instructions.
3. Blank your instrument using a cuvette filled with 1.0 ml of 100% ethanol.
4. Perform the scan on a cuvette filled with 1.0 ml of your chlorophyll extract.
5. Copy the results into your lab notebook as best you can. Why does chlorophyll appear green?

Spectrophotometry to measure optical density of a cell culture

It is often useful to determine how many cells one has in a culture—spectrophotometry is one technique than can be used to do this. As visible light passes through a liquid sample of cells, it will be scattered; the amount of scattering is related to the density of cells in the suspension. At 600 nm, an optical density (OD) reading of 1.0 means there are approximately 10^9 cells in an *E. coli* culture.

1. Create five, 1 ml dilutions of your cell suspension using sterile technique. Do this by transferring varying amounts of cell culture into cuvettes and adding fresh LB media to bring the volume to 1.0 ml.
2. Set your spectrophotometer to measure optical density at 600 nm and blank it using 1 ml of fresh LB media in a cuvette.
3. Insert your sample cuvettes into the spectrophotometer and record the results.

References

1. Bertani, L. E. “Cell Biology Laboratory Manual, 2003/04” Caltech.
2. Ausubel, F. M. et al, “Short Protocols in Molecular Biology” Wiley John & Sons Inc; 4 edition (April 19, 1999)