Bi 1X, Spring 2012

Week 5

- **Agarose Gel Electrophoresis**

Materials (per group of two):

- Your PCR reactions from last week
- 1 kbp ladder
- 5X DNA loading dye

Protocol:

1. **Run a gel as you did last week, this time with your PCR reactions to see if they have worked.**

   *Note: DNA loading dye consists of 1) glycerol which will help your solutions sink into the wells of the agarose gel, and 2) reference dyes that help you determine how far your samples have migrated. Be sure to **thoroughly** mix the DNA and loading dye before adding the samples in the gel.*

   PCR Reactions

Reagent:	**Amount:**
Sterile water	3 µL
PCR product	5 µL
5X DNA loading dye	2 µL
Total:	10 µL

2. **Spin down your samples briefly.**
3. **We'll provide you with ladder this time.**
4. **Load your samples on a 1% TAE[1] agarose gel (prepared by your TAs) in the order listed below. Each group will run a single gel. <u>Be sure to write down the sample order in your notebook as well!</u>**

Lane:	**Sample:**
1	1kbp DNA ladder
2	PCR product from person #1
3	PCR no template control from person #1
4	PCR no primer control from person #1
5	1kbp DNA ladder
6	PCR product from person #2
7	PCR no template control from person #2
8	PCR no primer control from person #2

   a. For **PCR** reactions, **<u>load all of</u>** sample/dye mixture.

[1] TAE stands for **T**ris-**a**cetate **E**DTA.

b. To load the gel, place your pipette beneath the buffer surface layer, part-way into your target well. Very slowly depress the pipette plunger to load your sample. As you depress the plunger, you will notice the sample fall into the well. **Do not rush!** This may cause the sample to flow out of the well. (*Loading samples onto agarose gels is tricky! Ask your TA to demonstrate how to load a gel before attempting to do it yourself.)*

5. **After loading all of your samples, ask your TA to help you setting up the power supply. Run the gel at ~110V for 30min.**