Bi 1X, Spring 2011

Week 4

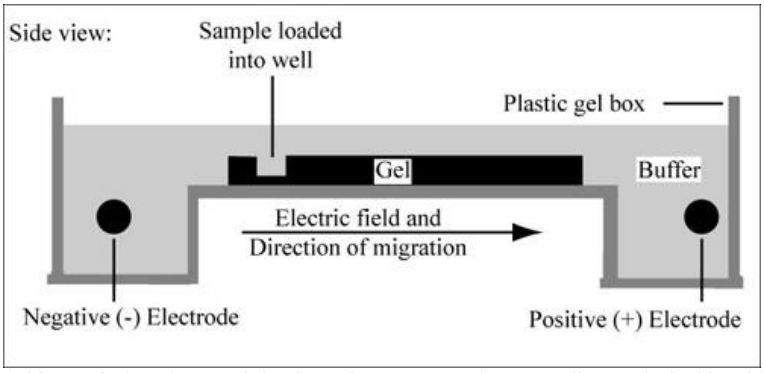
- **Agarose Gel Electrophoresis**

Materials (per group of two):

- Your PCR reactions from last week
- Your digestion reactions from last week
- 1 kbp and 100 bp ladders
- 1X TAE Buffer
- 5X DNA loading dye

Background:

Agarose gel electrophoresis is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes. The agarose gel consists of microscopic pores that act as a molecular sieve. Samples of DNA are loaded into wells made in the gel during casting, as shown in the figure below.



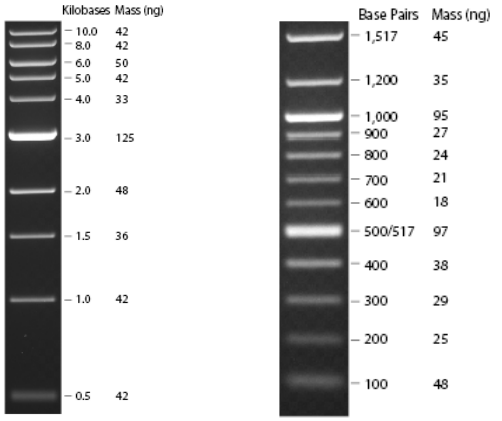
http://greenfield.mit.edu/oeit/OcwWeb/Biological-Engineering/20-109Fall-2007/Labs/detail/mod1_2.htm

Since DNA has a strong negative charge at neutral pH, it migrates through the gel towards the positive electrode during electrophoresis. The DNA molecules are separated in the gel according to their size and shape. Linear DNA molecules are separated according to their size. The smaller the linear fragment, the faster it migrates. If the sizes of two fragments are similar or identical, they will migrate together in the gel. If DNA is cleaved many times, the wide range of fragments produced will appear as a smear after electrophoresis.

Circular DNAs such as plasmids are supercoiled. Supercoiled DNA has a more compact and entangled shape (like a twisted rubber band) than its corresponding non-supercoiled forms (linear, nicked and relaxed circles). When supercoiled DNA is cleaved by a restriction enzyme just once it unravels to its linear form. If supercoiled DNA is nicked (a phosphate bond is broken anywhere in the molecule, in either strand) it completely unravels to form a circle. Under the electrophoresis conditions being used in this experiment, supercoiled DNA migrates faster than its linear form and linear DNA migrates faster than its nicked circular form. (EDVOTEK EDVO-kit #102 Manual)

In order to determine the size of your DNA from the migration distance, you will use "DNA ladders," which are molecular weight standards that consist of DNA fragments of known sizes. The ladders you will use today are shown below:

a) **b)**



DNA Ladders
a) Example of 1 kb DNA ladder on a 0.8% agarose gel, stained with EtBr.
b) Example of the 100bp DNA ladder on a 1.3% agarose gel, stained with EtBr.

Protocol:

1. **Set the reactions up <u>in the order</u> the reagents are listed. Mix by pipetting up and down or flicking the bottom of the tube with your index finger.**

   *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.*

   DNA Ladder

Reagent:	**Amount:**
Sterile water	3 µL
DNA ladder	1 µL
5X DNA loading dye	1 µL
Total:	5 µL

   PCR Reactions

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

   Digestion Reactions

Reagent:	**Amount:**
Sterile water	2 µL
Digested DNA	10 µL
5X DNA loading dye	3 µL
Total:	15 µL

2. **Spin down your samples briefly.**
3. **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	100 bp 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	PCR product from person #2
6	PCR no template control from person #2
7	PCR no primer control from person #2
8	EcoRI/HindIII lambda DNA double digest
9	pZE21-LacZ – HindIII single digest
10	pZE21-LacZ – KpnI/HindIII double digest
11	No enzymes digest control
12	1 kbp DNA ladder

   a. For **ladders** and **PCR** reactions, **<u>load all of</u>** sample/dye mixture.
   b. For **digestion** reactions, **<u>load 10 µL</u>** of sample/dye mixture.
   c. To load the gel, place your pipette beneath the buffer surface layer, part-way into your target well. <u>Very slowly</u> 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.)*
4. **After loading all of your samples, ask your TA to help you setting up the power supply. Run the gel at ~110V for 60min.**

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