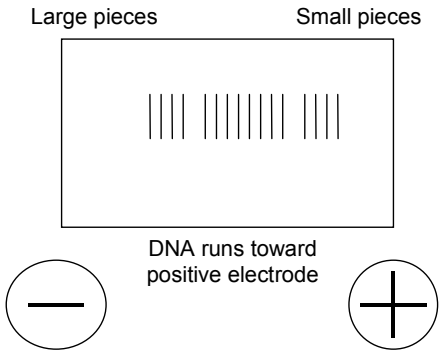
Analysis of Plasmid DNA
2007-08 INS Spring Quarter – Lab 2

Goals: You will enhance your ability to analyze DNA using gel electrophoresis.

Today you will run out a small portion of your DNA plasmid mini preparation on a gel see your results. You should analyze both pGreen and pcDNA on your gel. Get the other plasmid that you did not prepare from someone in the laboratory. At the beginning of class we will post how much plasmid and DNA ladder to load onto your gel. Expect to see RNA as well as your DNA plasmids.

Review your notes from last quarter! Here is some information to remind you about DNA gel electrophoresis.

Agarose is a highly purified carbohydrate obtained from kelp. A 1-2% mixture of agarose creates an easy to handle gel. As the DNA molecules are moved through this gel, the smaller molecules pass more quickly while the larger molecules become hung up on the gel and move more slowly. By combining the driving force of the electric field with the varying drag of the agarose gel, a mixture of DNA fragments will separate out by size.



Protocol:

1. Centrifuge your samples, as per last week's protocol, to pellet your DNA. Remove the supernatant and resuspend it in _____ µL water. Keep your samples on ice until loading them into the wells! Wear gloves!

2. Pour your gel according to the protocol from the winter quarter (look in your lab notebook for details).

3. Add _____ µL of 6X gel loading dye to your samples to make it a 1X final concentration.

4. Load your gel with a ladder and your samples. Run your gel.

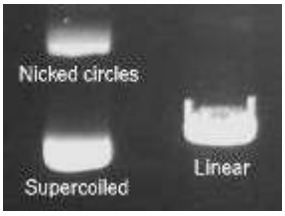
5. Stain your gel for 10 minutes in an ethidium bromide bath and then destain in water for 10 minutes.

SAFETY: Ethidium bromide is a mutagen. Always wear gloves when handling any material containing ethidium bromide and dispose of it in the proper location. Any gloves worn when handling ethidium solutions should also go in the designated waste. Any person in the same room as the UV light should wear UV-filtering eye protection any time the light source is on. Not all safety glasses are approved for UV protection. Check the label on yours or use one of the pairs of goggles provided. Know what you are doing when working with the power sources.

6. Analyze your gel on the UV illuminator.

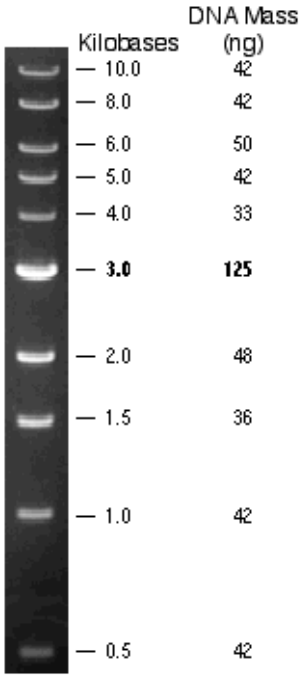
Clean Up
Ethidium bromide solutions and gels should be placed in a designated waste container. Components of the electrophoresis apparatus should be cleaned and well rinsed and returned to their container.

Appendix:
DNA migrates at different molecular weights depending on its morphology. Circular DNA is tightly wound migrates faster than its linear or relaxed circular (nicked circles) forms. Note this when you run your uncut sample.



DNA Standards: We are using a marker referred to as a 1 kb ladder. The company that sells it is New England Biolabs (NEB) and writes this about the the molecular weight standards:
"*A number of proprietary plasmids are digested to completion with appropriate restriction enzymes to yield 10 bands suitable for use as molecular weight standards for agarose gel electrophoresis. The digested DNA includes fragments ranging from 0.5-10.0 kilobases (kb). The 3.0 kb fragment has increased intensity to serve as a reference band. The approximate mass of DNA in each of the bands is provided (assuming a 0.5 µg load) for approximating the mass of DNA in comparably intense samples of similar size.*

Picture Right: 0.5 µg of 1 kb DNA Ladder visualized by ethidium bromide staining on a 0.8% TAE agarose gel."



Pre-lab worksheet

There are many types of restriction enzymes that cleave DNA in a particular location corresponding to the enzyme recognition sequence present in the DNA. Scientists often use restriction maps to designate where particular enzymes cleave. The diagrams to the left show a circular plasmid and a linear DNA map. The plasmid is 48.5 kb in size (very large for a plasmid) and the linear DNA is the same size. The restriction enzymes EcoRI and BamHI will cut the DNA at the locations designated on the map. If you completely digest the DNA with one or both of these enzymes then you will get fragments corresponding to the individual sections of DNA. When these fragments are run out on an agarose gel they separate according to size; the smaller fragments will run much faster through the gel than the larger fragments. In this way we can separate and visualize the fragments of DNA using gel electrophoresis. When we run these digests on a gel, we also include a DNA ladder that has specific DNA fragments of known sizes present in the mix. They will separate as the gel runs, providing us with a reference with which to compare our digested DNA. On the diagram of the gel below, draw the fragments that you would obtain from the following digests:

A) The linear piece of DNA digested with only EcoRI
B) The circular plasmid digested with only EcoRI
C) The circular plasmid digested with only BamHI
D) The circular plasmid digested with both EcorRI and BamHI