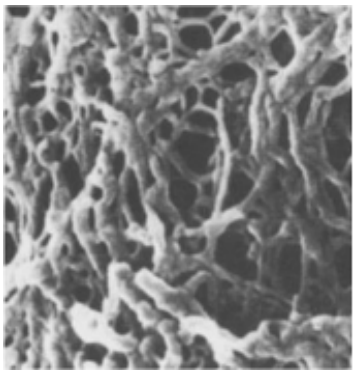
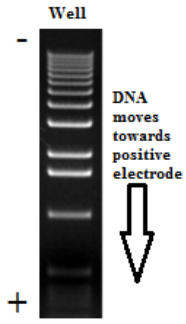
Running an Electrophoresis Gel



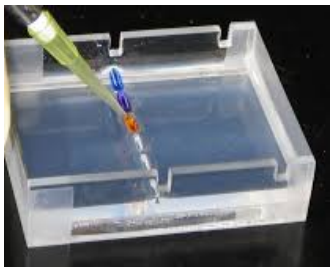
Background: One of the most basic and frequently used tools of the molecular biologist is electrophoresis. In fact, DNA fingerprinting is based on this process. Electrophoresis is a technique used to separate molecules by size and electrical charge, most often fragments of protein or DNA. In this lab, however, we will be using colored dyes. To perform gel electrophoresis three things are needed- a medium in which to separate the molecules, an electrical current, and a buffer solution.



During agarose electrophoresis, molecular fragments migrate through a gel when exposed to electricity. Agarose is a Jell-O-like substance derived from seaweed. The gel has a relatively large pore (hole) size, making it useful for size-separation of molecules. The picture at the right is a microscopic image of what agarose gel looks like. In the gel are several small wells, made by the teeth of a comb placed in the gel before it set. Samples to be tested are placed in the wells with a micropipette. The rate of movement of each sample depends upon molecule size, shape, and degree of electrical charge. In the presence of an electrical current, small molecules move quickly through the porous gel. Large molecules find it more difficult to move, so they travel more slowly. This allows us to take a sample of things of different sizes and separate them and compare them to other samples. This can help us identify similarities and differences, which can be used for things such as forensic science. The direction of movement also depends on the electrical charges (+ or -) of the molecules, and the density of the gel through which the DNA moves. Since DNA molecules all have the same negative charge, they all move in the same direction— towards positive. Today, however, you will be separating different color dyes; some have a positive charge and others a negative charge. The process provides a current of energy to move molecules through an electrophoresis gel made of agarose.

The electrophoresis gel chamber has a positive and negative side to the box. Once the samples are placed into the wells, the gel is placed in the box and filled with a buffer solution so that it covers the wells and makes contact with the electrodes at each end of the gel box. The buffer solution contains ions (like salt) to help allow electrical current to run through the gel and move the sample. The buffer also stops the gel from drying out. Prior to loading the samples in the wells of the gel, the molecules to be separated are mixed with a dense sugar solution so that when the mixture is added to the wells, it sinks to the bottom, taking the test molecules (in this case, dye) with it. Without the sugar, the test molecules would simply float away in the buffer.After the gel has been exposed to the electrical current—in this particular experiment about 20 minutes—the dye can be seen on the gel.

Electrophoresis Gel Loading Procedure:



1. Make sure the buffer level just barly covers the gel (approximatly 2-3 mm above gel).
2. Make sure the wells have their opening on the top of the gel.
3. Set your micropipettes to 15 ul and get the sample out of microfuge Tube A.
4. Steady your elbows on the table and use two hands to steady the pipette. Carefully submerge the tip into the buffer but do NOT touch the gel at all with the tip. Hold the tip directly above the well that you wish to put Sample A into. Slowly press the plunger to release the liquid into the well of the gel. DO NOT press to the second stop, because air bubbles may cause your solution to float out of the wells.
5. Repeat this procedure for all 6 tubes. Make sure that you put them in the wells acording to the order in your diagram.
6. Bring your gel up front. We will turn on the power supply when every group has loaded their gel, and it will run at the level between 110-130 Volts.
7. Make sure you see little bubbles coming up from the electrodes. This tells you that the gel box has been correctly turned on. Let the gell run for 20-25 minutes.