Introduction

Electrophoresis is a technique that separates large molecules by size using an applied electrical field and a sieving matrix. DNA, RNA and proteins are the molecules most often studied with this technique; agarose and acrylamide gels are the two most common sieves. The molecules to be separated enter the matrix through a well at one end and are pulled through the matrix when a current is applied across it. The larger molecules get entwined in the matrix and retarded; the smaller molecules wind through the matrix more easily and travel further from the well. Molecules of the same size and charge migrate the same distance from the well and collect into a band.

"Electrophoresis image removed due to copyright reasons."

DNA and RNA are negatively charged molecules due to their phosphate backbone, and they naturally travel toward the positive charge at the far end of the gel. They are typically examined with agarose gels. Proteins are composed of amino acids that can be positively, negatively or uncharged. To give proteins a uniformly negative charge, they are coated with a detergent, SDS, prior to running them on a gel. Protein samples are also boiled to remove any secondary structure that might make two molecules of the same size migrate differently. Polyacrylamide is the matrix commonly used to separate proteins. These gels are typically run vertically while agarose gels are run horizontally but gravity has nothing to do with the separation.

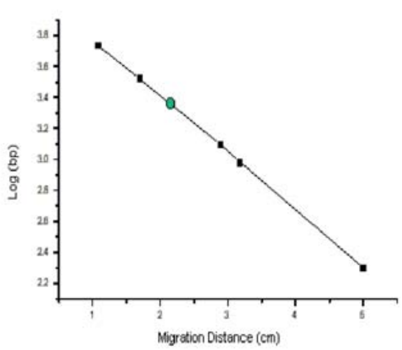
"Image removed due to copyright reasons."

Today you will separate DNA fragments using an agarose matrix. Agarose is a polymer that comes from seaweed and if you've ever made Jell-O™, then you already have all the skills for pouring an agarose gel. To prepare these gels, agarose and buffer are microwaved until the agarose is melted. The molten agar is then poured into a horizontal casting tray, and a comb is added. Once the agar has solidified, the comb is removed, leaving wells into which the DNA sample can be loaded.

The distance a DNA fragment travels is inversely proportional to its length. Over time fragments of similar length accumulate into "bands" in the gel. Higher concentrations of agarose can be used to resolve smaller DNA fragments. The figure below (far right) shows the same DNA fragments resolved with three agarose concentrations. The 1000 base pair fragment is indicated in each.

"Image of scanning EM of agarose polymer removed due to copyright reasons."

Scanning EM image of agarose polymer



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Ethidium Bromide is a fluorescent dye that is commonly added to agarose gels. This dye intercalates between the bases of DNA, allowing DNA fragments to be located in the gel under UV light and photographed. The intensity of the band reflects the concentration of molecules that size, although there are upper and lower limits to the sensitivity of dyes. Because of its interaction with DNA, **ethidium bromide is a powerful mutagen** and will interact with the DNA in your body just as it does with any DNA on a gel. You should always handle all gels and gel equipment with gloves. Agarose gels with Ethidium Bromide must be disposed as hazardous waste.

Today you will run your digested plasmid backbone and the digested PCR product on an agarose gel. You will use a razor to cut the bands of DNA out of your gel and then you will purify them from the agarose. Next time you will mix them in a ligation reaction.