GEL ELECTROPHORESIS 101

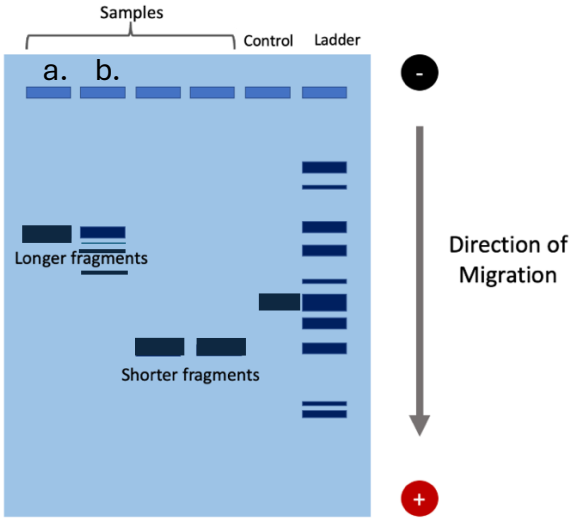
What is Gel Electrophoresis?

Gel electrophoresis is a laboratory technique that can be used to determine the length of DNA, RNA, or protein strands.

In this technique, liquid samples are deposited into "wells" (holes) at the top of a gel plate, shown in the figure below as light blue rectangles. The contents of each sample reflect different experimental conditions. These experimental conditions can vary by the inclusion, exclusion, or alteration of a variety of elements such as DNA or RNA template strands, polymerases, free nucleotides or proteins, proteases, etc.

After these samples are deposited into the gel plate, an electric field is applied. The field polarizes the gel and makes the top of the gel (where the wells are located) negatively charged and the bottom of the gel positively charged. Since DNA and RNA are negatively charged along their phosphate backbone, they will travel towards the positive charge at the end of the plate. **The shorter strands move through the gel matrix more quickly, so they will move further toward the bottom of the gel plate than longer strands.** Therefore, by comparing how far strands travel along a gel plate, the relative size of a strand can be determined.

After the experiment is complete, the gel plate is stained and viewed under UV light. The accumulation of DNA/RNA/protein polymers at a particular location gives rise to a short horizontal line, called a "band." The thickness of each band correlates to the number of polymers within the sample that are the same length at the conclusion of the experiment. The vertical area below each well is called the "lane." The band or bands that correspond to the sample deposited into a given well is visible within that well's lane.



How to Read a Gel Plate

The key to reading data from a gel electrophoresis experiment is to compare the lanes to each other.

In most experiments, there will be some **control condition**, in which no reaction occurred. You should compare the other experimental samples to the control condition to determine whether or not a reaction occurred.

- If no reaction occurred, then the experimental condition will look identical to the control.
- If the reaction resulted in a longer polymer, then the experimental condition will have a band **above** the control (indicated on the figure above as "longer fragments")
- If the reaction resulted in a smaller polymer, then the experimental condition will have a band **below** the control (indicated on the figure above as "shorter fragments")

Some experiments will have a "ladder" lane, which is made up of many fragments of polymer with known lengths. The ladder lane is useful to compare to experimental lanes if you want to know exactly how many nucleotides/amino acids are in the final product. In many of the problems in this class, the length of the strand (typically expressed as the number of nucleotides) will be indicated by the side of the plate rather than showing the full ladder lane.

As mentioned previously, the thickness of each band indicates how many polymers traveled the same distance, and therefore reflects how many polymers are that specific length. One thick band means that the reaction produced many polymers of the exact same length (seen in the lane marked "a"), whereas several faint bands means that the reaction produced polymers with varying lengths (seen in the lane marked "b" and on the ladder).

Why does this matter for CHEM 153B?

This course deals with a variety of mechanisms related to synthesizing or cleaving DNA, RNA, and protein polymers. Gel electrophoresis problems in this course will typically fall into one of two categories:

1. Synthesis problems: a polymer will grow (or not grow) under some conditions
   → Successful synthesis will create a band closer to the top of the gel
2. Cleavage problems: a polymer will be cut (or not cut) under some conditions
   → Successful cleavage will create a band further towards the bottom of the gel