

DNA gel electrophoresis

Introduction

One of the most common methods for separating and visualizing biological molecules in the lab is called gel electrophoresis. The word “electrophoresis” means carried by electricity, and during gel electrophoresis, an electric field propels molecules through a gel. At the molecular level, the gel’s internal structure resembles a web-like mesh through which molecules can move.

Scientists most commonly use gel electrophoresis to separate DNA fragments. Because DNA is negatively charged, it moves towards the positive electrode in an electric field. Smaller DNA fragments fit more easily through the gel’s web and travel faster and farther than larger pieces of DNA. This allows scientists to separate the DNA fragments by length.

DNA gel electrophoresis setup

Agarose gel: DNA gel electrophoresis typically uses an agarose gel (Figure 1). Agarose gels are close to the consistency of firm jello and are made with agarose, a polysaccharide extracted from seaweed. In a gel, agarose forms a web-like mesh with pores of many different sizes (Figure 1, inset).

Making an agarose gel is similar to making jello, and scientists refer to the process as “pouring” or “casting” a gel. First, agarose powder is dissolved by heating it in a buffer solution. Then it is poured into a mold where it will cool and solidify. A casting comb is also placed in the mold. When the gel hardens and the comb is removed, small indentations called wells are left behind. Using a micropipette, scientists load their DNA samples into these wells. When an electric field is applied, the negatively charged DNA will migrate into the gel towards the positive electrode.

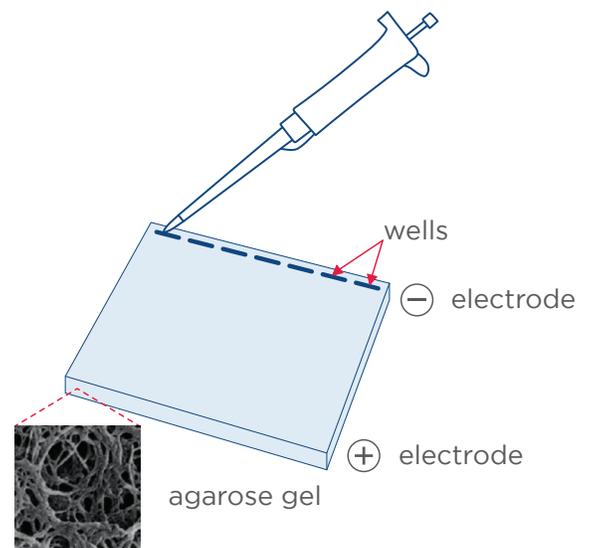


Figure 1. Agarose gels

Electrophoresis chamber: An electrophoresis chamber is a container that holds the agarose gel and electrophoresis buffer. The gel is positioned on a tray within the chamber and covered with a liquid buffer that conducts electricity. Positive and negative electrodes at opposite ends of the chamber create the electric field necessary to move the DNA through the gel.

Visualizing DNA samples during gel electrophoresis

DNA is colorless, so scientists use dyes and stains to visualize their samples during gel electrophoresis.

Loading dye: Scientists add a colored dye to their samples before loading them into the wells of a gel. The loading dye has several practical functions. First, the dye solution is denser than the electrophoresis buffer in which the gel is submerged; this helps the DNA sink to the bottom of the well. Second, because the dye is colored, scientists can check that the sample was successfully loaded into the well. Third, the dye is negatively charged and will migrate towards the positive electrode, providing visual confirmation that electrophoresis is taking place. It is important to note that the loading dye is just a colored liquid in which the DNA is dissolved. It does not allow for visualization of the DNA. A separate DNA stain is used to see the DNA.

DNA stain: Scientists typically use a stain that fluoresces only when bound to DNA. Exposing the gel to light of a specific wavelength causes the stain that is bound to DNA to light up, revealing the presence and location of DNA in the gel.

Interpreting DNA gel electrophoresis results

The DNA molecules loaded into each well will migrate through the gel in straight lanes the width of the well. If enough molecules of the same size are present in the sample, they will appear as bands (Figure 2). Each band contains millions of DNA molecules of the same size that traveled at the same speed through the gel. Shorter DNA fragments that moved quickly will have moved farther through the gel, while longer DNA fragments that moved more slowly will not have traveled as far. When scientists refer to bands of different sizes, they are talking about the size of the individual DNA molecules that make up the bands.

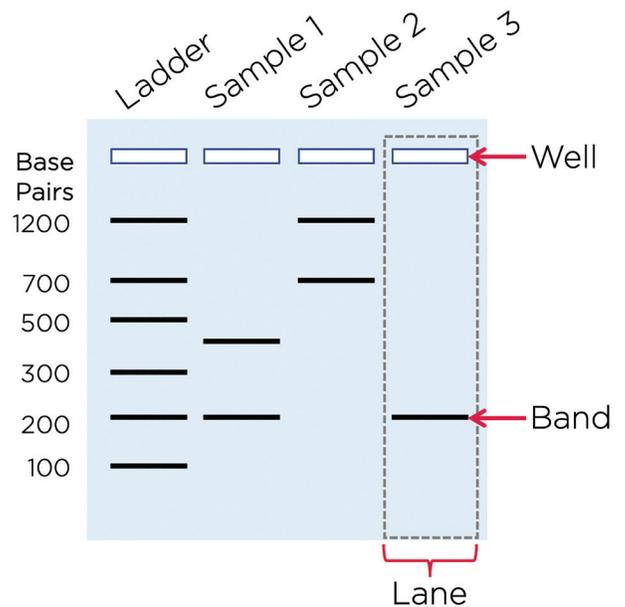


Figure 2. DNA gel electrophoresis

To estimate the size of the DNA fragments that make up each band, scientists use a DNA ladder. A DNA ladder is a mixture of DNA molecules of known sizes. Because we know the sizes of the DNA fragments that make up the ladder, we can estimate the size of unknown DNA bands by comparing them to the ladder, which you can think of as a molecular ruler. For example, in the gel in Figure 2, sample 1 contains a 400 base pair (bp) band and a 200 bp band, while sample 2 includes a 1200 bp band and a 700 bp band. Sample 3 has only a 200 bp band, which means all the DNA fragments in that sample were the same length.

Importance of DNA gel electrophoresis

The ability to separate DNA fragments by length is a powerful tool in molecular biology. Sometimes, visualizing the pattern of bands in a sample is the last step in an experimental protocol. This is the case for applications like paternity testing or forensic DNA analysis, where examining the length of specific DNA fragments is used for genetic identification. In other instances, scientists isolate specific DNA fragments by cutting bands out of the gel, like cutting a brownie out of the middle of the pan. These DNA fragments can then be used in other experiments. With so many different potential applications, DNA gel electrophoresis is a versatile technique that has been an essential lab tool for decades and continues to be a mainstay of molecular biology labs today.