

Electrophoresis

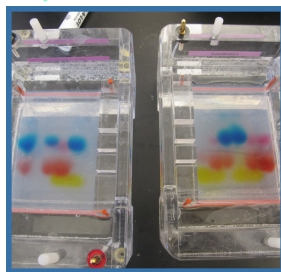
Grade 9

Time: 60 min

How can we separate molecules by size?

Learning Objectives

Gel electrophoresis is used almost daily by many molecular biologists. Students will firsthand make a gel with wells to load, choose colors of food coloring to load the wells with, and load them in with a disposable pipette. With a teacher's help, they will run the colors down the gel using 5 9-Volt batteries, and watch as the colors separate based on molecular size. The same color will always split into the same bands. This separation corresponds to the way scientists separate DNA molecules by size.



Materials

Electrophoresis Gel Box (May be borrowed from the local high school)
5 9-Volt battery
Saltwater solution
Gelatin/agarose
Food coloring
Disposable pipettes
Fork comb or plastic forks
Tape
TAE buffer solution

Procedure

Approximate Cost: \$25 initially, reusable

Combine 50 mL TAE buffer solution with 0.5 grams agarose, heat in the microwave, and pour into the provided gel frame with the fork-comb (If using a plastic fork, wait until the gel is dry and poke holes along one edge of the gel). Once solid, remove the fork-comb. Pour just enough TAE buffer to cover the gel and fill all the wells. Finally, have students load the gel with 1 drop of food coloring in each well using disposable pipettes. For more colors, mix food coloring in advance. Have students guess what colors their lane will split into, and which molecules they think may be heaviest/go the shortest distance. Once the gel is loaded, attach the 5 9-Volt batteries to positive leads, and lay these leads in the gel chamber solution, such that the positive lead is at the bottom (opposite to the wells, and the negative lead is at the top (close to the wells)).

Background



A gel is best thought of as a dense web through which we are running small molecules. These molecules have a slight negative charge, thus the current will pull them toward the positive end of the batteries (toward the positive lead). However, food coloring colors are made up of the primaries (red, blue, and yellow—some food coloring has a bit of pink that separates from the red as well). Each of these colors is a different size, so it moves at a different speed down the gel. Imagine a large molecule trying to move through dense web vs. a small molecule trying to move through the same web. Which will go further?

Scientists use electrophoresis to check the sizes of their DNA, because they cannot visually see how big the DNA molecules are. Usually, scientists know how long a piece they are looking for is, and running a gel can help them understand if the piece they have is approximately the right size.

When scientists run gels using DNA, they also run a ladder in one of the wells, which has a bunch of DNA fragments of different, known sizes. That way, they can compare the band their DNA makes to the bands on the known ladder and estimate the size.

Critical Thinking Questions

What are some applications of gel electrophoresis?

How does the current separate the DNA fragments by size?

If we were using DNA instead of food coloring, it could be clear.

Scientists use a chemical called ethidium bromide in their gels, and then put the gel in a UV chamber to see where the DNA is. What does ethidium bromide do? What might it be dangerous to us?

Why does DNA migrate toward the positive end? (Think about the charge on DNA molecules.)

