

Introduction

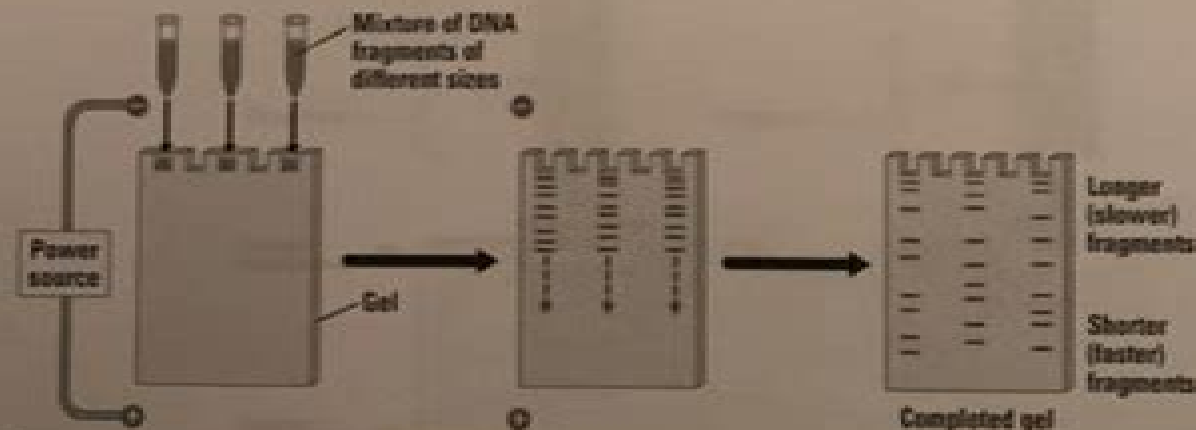
Gel electrophoresis is a basic biotechnology technique that separates macromolecules according to their charge and size. It is frequently used to analyze and manipulate samples of DNA, RNA, or proteins. In this laboratory activity, agarose gel electrophoresis will be used to separate and characterize colored dye molecules of various sizes and charges.

In gel electrophoresis, samples to be separated are applied to a porous gel medium made of a material such as agarose. Agarose gels are made by pouring a molten solution of agarose and buffer into a gel mold called a casting tray. Before the agarose solidifies, a comb is placed in the casting tray to create a row of wells into which samples are loaded once the comb is removed from the solidified gel. The casting tray and solidified gel are then placed in an electrophoresis chamber that has wire electrodes at each end. The gel is covered with a buffer that controls the pH of the system and conducts electricity. The comb is then carefully removed from the gel and samples are loaded into the resulting wells using a pipette.

Once all the samples have been loaded into the wells, the chamber is connected to a power supply and an electrical current is applied to the gel. The chamber is designed with a positive electrode (anode) at one end and a negative electrode (cathode) at the other end. Molecules with a net negative charge migrate toward the positive electrode and molecules with a net positive charge migrate toward the negative electrode because opposite charges attract.

The overall charge of a molecule affects the speed at which it travels through the gel. Highly charged molecules migrate more quickly through the gel than weakly charged molecules. The size and shape of the molecule also affects how quickly it travels through the gel. Agarose gels contain a matrix of minuscule pores that acts like a sieve. Small molecules maneuver more easily through the pores of the gel than larger molecules, allowing them to migrate relatively quickly.

Size and net charge are factors that together determine how quickly molecules will travel through the gel, and thus what their migration distance will be. If a molecule is small or highly charged, this will increase its migration rate through the gel. If a molecule is large or weakly charged, this will decrease its migration rate through the gel.



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Carolina Electrophoresis Lab Answers

Brendan G. Carr

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