

Lab #2: Agarose Gel Electrophoresis:

Introduction:

According to NCBI, agarose gel electrophoresis is the most effective way of separating DNA fragments according to varying sizes and charges. The gel itself is porous, and the higher the concentration of the agarose the smaller the pore size and vice versa. The separation of proteins occurs fastest with small proteins and slowest in the larger proteins, however, different forms of DNA move through the gel at different rates and the electrophoretic mobility can be calculated

using this equation $\mu = \frac{Ze}{f}$ where z is the charge, e is the applied voltage, and f is the

frictional coefficient. For example, supercoiled plasmid DNA moves through the gel the fastest because it is compact, then linear DNA fragment would move the second fastest, with open circular DNA moving the slowest. In this purification technique, loading dye is used to help track the movement of the DNA through the gel. The negative phosphate backbone fragments will migrate from the cathode or negative end to the anode or the positive end until the fragments reach the end of the gel. The leading model for DNA movement through the agarose gel is known as the biased reptation, this model states that the leading edge moves forward and pulls the rest of the molecule along (Smith et al). From this technique, one should be able to understand to the mechanism by which DNA fragments are separated within a gel matrix, how the conformation of the DNA molecule will determine its mobility through a gel matrix and determine the sizes of separated DNA fragments.

Objective:

The objectives of this experiment is to use the techniques associated with making an agarose gel from scratch for the electrophoresis, being able to load wells carefully with the samples, performing the electrophoresis experiment, and analyzing the results from the electrophoresis experiment.

Procedures:

In preparing the casting tray for the gel, the open ends of a clean dry casting tray were taped with laboratory tape. $\frac{3}{4}$ of an inch-wide tape was then taped over the sides and the bottom edge of the bed, with the extended tape edges folded back onto the sides and the bottom and the contact points pressed firmly in order to form a good seal. A comb was then placed in the set of notches at the top of the casting tray making sure that the comb sits firmly and evenly across the tray.

Next, when casting the agarose gel, a flask or beaker was used to prepare enough gel solution of a 1.0% gel solution in order to make a 0.5 cm gel using the casting tray provided at the lab station. The table below was used to determine how much gel was needed for casting the tray.

| Casting Tray Type/Brand | Dimensions (width x length) in cm |
|--------------------------------|-----------------------------------|
| Fisher Scientific (FB-SB-1320) | 13 x 16 |
| Bio-Rad Ready Sub-Cell GT MINI | 7 x 10 |

For the larger tray, it was determined that 1.04 g of agarose powder was needed to cast the tray and for the small tray it was determined that 0.35 g of agarose powder was needed to cast the

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