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Abstract

Deoxyribonucleic acid (DNA) is an integral molecule to all living organisms because its expression confers life through the production of proteins and the expression of traits. Scientific interests in DNA make it a target molecule for many procedures in the lab. However, scientists must extract and separate DNA based on various properties to perform intricate studies and analyses relative to desired results. One of the main techniques for separating DNA molecules following their extraction is gel electrophoresis. Agarose gel electrophoresis derives from the principle of separation of DNA molecules based on size and their interaction with the agarose gel

in a charged medium. The following experiment reviews the process of gel electrophoresis in preparation of the gel matrix, buffer, and sample loading. It performs the electrophoretic run on a sample of known and unknown dyes to provide intricate assessments of the nature of gel electrophoresis and its use to separate and purify DNA molecules based on their size differences.

DNA Separation Through Agarose Gel Electrophoresis

Introduction

Deoxyribonucleic acid (DNA) is the primary component in living organisms that confers the storage of genomic material and its expression to yield various protein products. DNA comprises a primary structure that includes a nitrogenous base bound to a deoxyribose sugar, which then binds to three phosphate groups to form a nucleotide (Aze and Maiorano). The nucleotides bind together to form the secondary structure and occur as double-helical strands that confer the tertiary structure. Given the increased understanding of the role of DNA in a wide variety of conditions and functions in the life of organisms, its use in the laboratory finds considerable importance. Various techniques help scientists purify DNA from cells and separate them according to various sizes or properties. The Agarose gel electrophoresis technique is one of the methods that allow scientists to separate DNA in the laboratory (Lee, John and Hsu).

Agarose gel electrophoresis separates DNA molecules based on their charge. Markedly, the technique exploits the differences between the different sizes of target DNA molecules to immerse them into an agarose-filled plate and run a current through them. The process allows DNA to move towards the anode as they separate based on size and are collected at different times or visualized according to the experiment needs in the lab (Lee, John and Hsu). The current study proposed the null hypothesis that the dyes included in the electrophoretic run will reach the anode of the gel plate at the same time while traveling at equal distances. It also proposed an alternate hypothesis that the dyes will reach the gel plate at different times and travel different differences. The hypotheses derived from the experiment's objective are to study the nature and principles of agarose gel electrophoresis in the separation of DNA molecules.

Materials and Methods

The experiment utilized various materials for different purposes and aspects of preparing and running the electrophoresis procedures. The materials for preparing the agarose gel included 30 grams of agarose made into a solution, 30ml of Borax Buffer, a heat source (microwave), an Erlenmeyer flask, and a beaker for stirring the reagents. The preparation of the gel and its setting into a solid gel involved using a casting tray (gel box), comb, and the electrophoretic chamber. The loading and running of the dye samples into the electrophoretic gel included materials such as the micropipette, power source (electricity), and the electrophoretic chamber. The dye samples used were known, including Bromophenol Blue, Methyl Orange, Xylene Cyclonal, Ponceau G, and unknown dyes.

The experiment began with forming the agarose gel and loading it into the electrophoretic chamber. The process involved measuring 30 grams of agarose and assuming 1 gram of agarose was 1ml. The 30 grams of agarose were then added to the 30ml of the Borax Buffer and placed

in the Erlenmeyer flask, which was then loaded into the microwave and heated until the mixture became clear. Upon removing the hot solution-filled flask from the microwave, care was taken when loading the solution into the casting tray (gel box), and a comb was added alongside the solution to form wells. Once the gel was hard, the cob was removed, creating the wells as sites for sample addition.

The gel was then placed in the electrophoretic chamber and covered by adding Borax Buffer to the top of the hardened gel. Appropriately-sized micropipettes were used for each sample dye to lead them into the wells. The electrophoretic chamber was then connected to the power source and set to the indicated voltage, and the power source was turned on. The electrophoretic gel run was allowed to proceed until the fastest migrating dye reached near the end of the gel (approximately 0.5 cm to the end of the gel). The gel was then removed from the electrophoretic chamber for visualization.

The variables in the experiment included the presence of a known and unknown dye within the electrophoretic run. Markedly, the size of the dye molecules was taken to be the independent variable, and the traveled distances within the gel were taken as the dependent variable. The known dye measurements were taken as the DNA ladder and formed the control variable. The experiment had no replicates because it was only done once.

Results

The distances the dyes migrated are given in the table below in millimeters (mm)

Table 1: Distances traveled by the known and unknown dyes in mm

Lane #	Sample	# of bands	Migration distance

1	Bromophenol Blue	1	2 mm
2	Methyl Orange	1	1 mm
3	Ponceau G	1	5 mm
4	Xylene Cyclonal	1	5 mm
5	Unknown 1; Xylene Cyclonal and Ponceau G	2	5 mm
6	Unknown 2; Bromophenol Blue, Ponceau G, Methyl Orange	3	3 mm

The dyes also produced different bands and marking on the gel that helped in their visualization. These are evident in the picture below;

Figure 1: Dye bands within the gel after the run

Discussion

The distances of the different known and unknown dyes provided insights into the principles and mechanism of operation of agarose gel electrophoresis. In particular, the disparate distances between the unknown dyes indicate that they contained more than one component that

separates based on predefined properties to different lengths within the gel. The known gels also traveled to different distances within the gel, with Ponceau G and Xylene Cyclonal traveling the furthest, followed by Bromophenol Blue, and lastly, Methyl Orange. The results indicate that the dyes have different sizes with charge similarities in charge properties allowing them to move through the dye but differences in size dictating migration distance. The experiment reflects the application of agarose gel electrophoresis in the separation of DNA molecules in the laboratory for different extraction purposes (Lee, John and Hsu). It points to the high resolution of the technique, which informs its application across different research and laboratory procedures that require the separation of DNA according to size.

The experiment also helped reject the null hypothesis provided during the experiment and accept the alternate hypothesis. The observation that the dyes travel along different distances informed the rejection of the null hypothesis that stated that the dyes would travel the same distance. One of the main mistakes occurred in the snapping of the picture of the final electrophoretic run that did not account for the background. In particular, the background shows the gel supported by a human hand, which reduces the ability to visualize the dye. Future runs of the same nature will ensure that the final gel is placed on a bright or dark background depending on the staining properties for improved visualization of the bands.

Works Cited

Aze, A and D Maiorano. "Recent advances in understanding DNA replication: cell type-specific adaptation of the DNA replication program." *F1000Research* (2018). <https://www.researchgate.net/publication/327293518_Recent_advances_in_understanding_DNA_replication_cell_type-specific_adaptation_of_the_DNA_replication_program>.

Lee, Y, et al. "Agarose Gel Electrophoresis for the Separation of DNA Fragments." *Journal of Visualized Experiments* 62 (2012). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4846332/>.