**An Investigation into DNA Electrophoresis and Quantification**

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**Introduction**

This report explores how size and polarity of fragment size impacts electrophoresis. Electrophoresis is the use of an electric field to move charged molecules through a matrix or solvent (Krane, 2019). Gel electrophoresis, which is electrophoresis through a gel that contains small pores, was the specific type of electrophoresis used during this experiment (Lee, Costumbrado, Hsu, and Kim, 2012). A molecule’s properties, such as net charge and size, impacts the direction and rate it moves through an electric field, which is also known as it’s electrophoretic mobility (Krane, 2019). During gel electrophoresis, the pores is used to separate different molecules because it is harder for larger and less polar molecules to migrate through the pores (Krane, 2019). DNA ladders, which are pre-cut pieces of DNA with known base-pair (bp) lengths, are used to comprehend gel electrophoresis because there is a known linear relationship between the log10 bp length and distance traveled through agarose gel; which can be used determine the bp length of an unknown DNA molecule (Krane, 2019). A retardation factor, commonly known as a Rf value, is a ratio that can be used in conjunction with a table of known dyes and their respective Rf values to determine the identity of an unknown dye (Krane, 2019).

In this experiment we performed gel electrophoresis on both an unknown dye that was compared to a known standard dye, and unknown DNA which was compared to a DNA ladder (Krane, 2019). The hypotheses are, if the dye is more negatively charged, then it will migrate further towards the anode and through the use of Rf values and this information, the dye can be identified; and if a strand of DNA is smaller, then it will migrate further to the anode and through the use of a DNA ladder, the bp lengths of the unknown DNA can be discovered.

**Methods**

The experiments were conducted following the procedures on pages 129-135 of the Bio 1120 laboratory manual (Krane, 2019) with some changes to the procedure. With the changes being step two of the procedures was done first and by the GTA, part seven of step one (in my case) only having one dye appear, and step three having parts four through six excluded.

**Results**

Table 11.1 Electrophoretic mobility of dyes in an agarose gel

|  |  |  |
| --- | --- | --- |
|  | Unknown Dye (Dye 24) | Amido-Black |
| Distance (cm) | -1.3 | 1.5 |
| Color | Blue | Dark Blue |
| Rf Value | -0.87 | 1 |
|  |  |  |
| Calculation | 1.5\*1=-1.3x | x=-0.87 |

Table 11.2 Electrophoresis of standard and unknown DNA fragment in a 1.5% agarose gel

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| λ DNA Fragment Size (bps) | Log10 λ DNA Fragment Size | Distance λ DNA Fragment Moved (cm) | Distance Unknown DNA Fragment Moved (cm) | Log10 Unknown DNA Fragment Size | Log10 Unknown DNA Fragment Size Calculation | Unknown DNA Fragment Size (bp) | Unknown DNA Fragment Size Calculation |
| 23130 | 4.36417563 | 0.95 | 1.3 | 3.69815 | =-1.2645\*D3+5.342 | 4990.56826 | =10^E3 |
| 9416 | 3.97386645 | 1.05 | 1.5 | 3.44525 | =-1.2645\*D4+5.342 | 2787.72545 | =10^E4 |
| 6557 | 3.81670518 | 1.12 | 1.9 | 2.93945 | =-1.2645\*D5+5.342 | 869.861281 | =10^E5 |
| 4361 | 3.63958609 | 1.22 |  |  |  |  |  |
| 2322 | 3.36586222 | 1.55 |  |  |  |  |  |
| 2027 | 3.30685375 | 1.69 |  |  |  |  |  |

Figure 11.1 A graph using a DNA ladder to determine the relationship between the distance

**Conclusion**

The results for step one align with the hypothesis although only one dye was discovered. The only dye discovered in tube twenty-four was brilliant crestyl blue with a Rf value of -0.87. A, if not the only, dye in tube twenty-four is highly likely brilliant cresyl blue because the difference in the experimental and literature value for its Rf value is only 0.02 (Krane, 2019). The negative Rf value of the dye is likely caused by the dye molecule being positively charged and due to the distance traveled, provided that the charge strength of both dyes is roughly equal, the brilliant crestyl blue dye is likely more massive than the amido-black dye. The discrepancy in the literature and experimental Rf value may have been caused by an inaccurate measurement of either or both the unknown dye and amido-black. If the gel and buffer were at a lower pH, the dye may have been pushed by the hydronium ions closer to the amido-black dye; while, if the gel and buffer were at a higher pH, the dye may have been pulled closer to the cathode by the hydroxide ions.

The results for step two also align with the hypothesis as the DNA ladder, in this case the lambda (λ) DNA, was used to determine the bp length of the unknown DNA. The DNA ladder was used to establish a linear relation between the distance a DNA fragment moved and the log10 of its fragment size. Although the DNA ladder managed to establish a relationship, a majority of the data points had a greater than 0.05 difference from the line of best fit. Through the use of the linear relation, it was discovered that as fragment size increased, distance traveled decreased. There were a multitude of potential sources of error in this portion of the experiment; such as, the image provided being at an angle causing the ruler to not be able to measure accurately, the DNA ladder not having all its rungs appear so the relationship between the movement and size of the DNA fragment to not be as accurate, and the simple human error of potentially misreading the ruler in the image provided. If the agarose gel was less dense, the different segments of DNA both from the DNA ladder and from the unknown DNA would have been separated more while if the agarose gel was denser, the segments would have separated less.

**References**

Krane, D. (2019). *Bio 1120: A Laboratory Perspective*. Cincinnati, OH: Van-Griner Publishing.

Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. *J Vis Exp*. 2012;(62):3923. Published 2012 Apr 20. doi:10.3791/3923