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# Experiment 2: Agarose Gel Electrophoresis of DNA Isoforms

The purpose of experiment 2 was to determine the length of the DNA plasmid we were given by running an electrophoresis gel. In order to accomplish this, we created an agarose gel mold and ran our plasmid DNA in the gel. In the end, we visualized our gel and analyzed our results. With what we learned we modified our methods to improve our results in the future.

We used a sodium borate buffer and 0.8% agarose by mass for our gel. We did this because sodium borate is known to provide high resolution and have a lower specific heat capacity than a TAE buffer. Specifically, this means that the sodium borate buffer could be run at a higher voltage, and thus the gel could be run faster. We chose 0.8% agarose because this percentage is sufficient for the separation of DNA segments of similar size to the given plasmids. We also decided that 100 V would work best for our experiment because of our choice of buffer. Originally, we planned to run the experiment until the plasmids separated sufficiently. Using our buffer solution, we created an agarose gel mold and added the rest to the gel chamber to act as a buffer solution. The ladder can be added to both sides of the gel, but with such few wells this is not entirely necessary for this experiment. We then mixed our samples with 1.0 µL of dye and loaded 3.0 µL of each sample into the wells, making sure to not go too deep and therefore lose samples. After adding the ladder, we ran the machine and ensured that the apparatus worked by spotting the bubbles at the anode and the cathode. When the gel was finished we imaged it in the blue light illuminator

## Methods:

1. Prepare approximately 250 mL of buffer solution using the SB 20 X concentrate (12.5 mL) and DI water (237.5 mL).
2. Add 0.2 g of agarose to 25 mL of buffer in a Falcon tube. Close the lid and swirl. Heat in the microwave until fully dissolved. Let cool for 5 minutes.
3. Place casting gates and comb in the apparatus, then pour the agarose solution into the central compartment. Let solidify for 20 minutes.
4. Remove the comb and add the buffer until it exceeds the height of the gel by 1 mm.
5. Load the ladder in the outermost well.

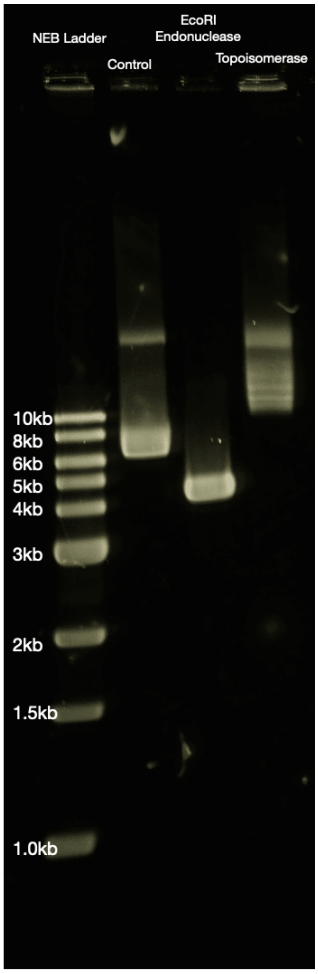
6. Add 1 µL of the loading dye to the provided plasma samples and pipette 3 µL of each sample into their respective wells.
7. Run the gel by plugging in the power supply and set the voltage to 100 V.
8. Observe gas bubbles rising from the cathode and anode.
9. Fill the staining box with 50 mL of the SB buffer and 5 µL of SYBR safe. Mix and then place the gel inside, then let it stain in the dark for 25 min.
10. Rinse the gel with DI water and place it in the blue light transilluminator.
11. Take a photo of the gel for later analysis

Results:


(0.008) agarose x (25 g) solution = 0.2 g agarose

250 mL of 20 X concentrated buffer = 250/20 = 12.5 mL concentrated buffer

250 Total - 12.5 mL concentrated buffer = 237.5 mL DI water




Agarose (0.8%) Gel Electrophoresis (100V 60 min) of PBR322 plasmid DNA (4361 bp)
Using a sodium borate buffer

| | |
|---|---|
| Control | 1st Peak > 10,000 & 2nd Peak ~7,000 |
| 1st | 1 band ~ 4500 bp |
| 2nd linear | Blurry Smudge > 10,000 |

Our linearized DNA (Lane 3) was created by the EcoRI endonuclease and traveled a distance of 1250 and thus was measured to be about 5100 bp and had a migration rate of about 21 pixels/min. The distances for the control are not sufficient for quantitative analysis, yet we still know that the supercoiled plasmid should travel farther than the nicked plasmid.

Discussion:

In the end our experiment did not sufficiently accomplish a majority of our objectives. This can mainly be attributed to a few key errors. The first error was the buffer that we chose. A sodium borate buffer could theoretically work, but as seen from other groups data, the TAE buffer significantly outperformed. This is most likely due to the fact that sodium borate is better suited for resolution of smaller DNA molecules. In order to make our sodium borate buffer solution work we can learn from the other lab groups. The other data set collected under the same conditions appears to have been far superior to our own. All of their samples passed the 10 kb marker, as expected, whereas only our linear DNA and half of our control did. This is most likely due to an unaccounted for variable, such as: too much buffer was added on top of our gel, the plasmid samples were not properly stored or prepared, or the agarose gel was not created appropriately and thus the matrix was too dense. Many other errors could have also been the cause of this. Regardless, it seems very possible to use sodium borate for this experiment, but we also should have slightly increased the agarose percentage to 0.9 or 1.0. Our selection of 0.8% worked, but most of the other experiments that had slightly better bands also had slightly higher agarose concentrations. For our selected buffer and agarose concentration, 100 V for 60 minutes is a fundamentally sound choice. Not only did the other similar data set use this voltage and timing but sodium borate is perfectly suitable for running at this voltage. The dye and DNA concentration of our samples seems to be adequate because the bands are relatively clean and bright. The photograph of the gel is good, the resolution is obviously high and the contrast is suitable. There are a few discontinuities regarding a few splotches of dye throughout the background; however, there are few of them and they do not interfere with the bands. In the topoisomerase lane, the band looks correct. That is, it fades over a few thousand base pairs. This is because the relaxed circular DNA varies greatly in its migration and will separate out. The problem with this lane as well as with the control was that they did not migrate far enough. The potential causes discussed above still apply, but it matters less for this lane because the plasmid spreads out over such a range.

I now have a much greater understanding of plasmid DNA. How it is affected by various enzymes and the various structures it can form. I also now understand how those structures run in a gel electrophoresis experiment and gel electrophoresis as a whole. I have a better understanding of when to choose a more challenging method for potentially better results and when to use an easily reproducible and consistent method.