Amanda McNussen
Genetics BIO2450 E158 Tuesday
Prof. Michael Gotesman

Lab Report 5: Gel Electrophoresis

Abstract: This lab report discusses an experiment that utilized horizontal agarose gel electrophoresis of digested DNA to compare DNA collected at a crime scene with five samples of DNA.

Introduction: Gel electrophoresis is an assay often used in biochemical experiments to separate molecules based on their charge, size, and shape.[1] For larger biomolecules, such as DNA, the gel acts as sieve effectively creating size exclusion. In genetics, gel electrophoresis can be used to for DNA fingerprinting. By using restriction enzymes, DNA is cleaved at recognition sites creating fragments of DNA, which are unique to each individual person. Larger fragments do not migrate as far as smaller molecules which creates banding patterns that can be visualized. By comparing the visualized bands and looking for similarities, DNA can be used to identify if two DNA samples came from the same source.

Methods:

Week 1

Step 1 - Collect 7 tubes of varying colors labeled CS (crime scene DNA), S1-S5 (suspect DNA), and NC (negative control = dH2O). The tubes provided the contained the corresponding DNA samples preloaded.

Image 1.0

Step2 - Add 10 µL of the enzyme mix EcoRI and PstI to each tube being mindful to use a fresh pipette tip between samples. Use the pipette to mix the sample a few times so that it homogenizes. Further, if there are bubbles in the tube, tap the tube on your bench or briefly vortex the sample.

Step 3 - Incubate the tubes in a dry bath for 45 minutes at 37°C. When complete, transfer tubes to the refrigerator for further testing the following week.

Week 2

Step 4 - Set up gel molds. Prepare 1% gels by combining 50 mL of TBE buffer with 0.5 g agarose and swirl mixture. Then microwave the mixture in 30 second intervals until the agarose is completely dissolved and the solution looks clear.

Step 5 - Add 5 µL of SYBR to the flask and swirl. SYBR helps visualize the DNA in the gel.

Step 6 - Pour solution into gel casting tray and insert two combs. Place mold in refrigerator for ~20 minutes.

Step 7 - Add 5 µL of loading dye to each of the tubes from 7 tubes of DNA/control from the previous week.

Step 8 - *Once* the gel is solidified, remove the combs and casts. Then transfer the gel to the electrophoresis chamber in the correct orientation and fill chamber with enough buffer to completely cover the gel.

Step 9 - Next, load the gel in the following order. Use 10 µL of the standard (S) and 20 µL of the remaining samples.

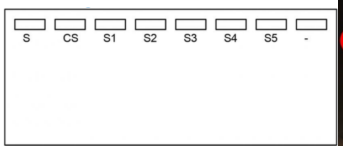


Image 2.0

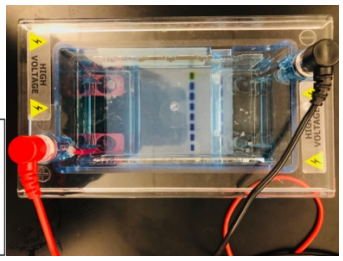
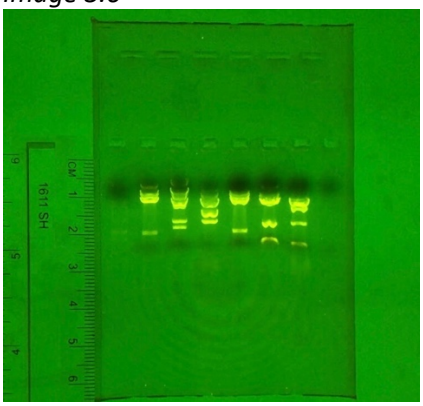


Image 2.1

Step 10: Run the gel at 120 V for about 30 minutes and then view.

Data & Results:

Image 3.0



Discussion and Conclusions: From *Image 3.0* we can determine that the DNA collected from the crime scene best matches the DNA provided by suspect #3 as per the results of the gel electrophoresis. They both have one notable marker approximately 2 cm away from the wells and no other remarkable indicators.

References:

1. Biological Sciences Department. (2019, Spring). BIO3601 Lab Manual. Lab 6. Brooklyn, NY: NYCCT. Retrieved from class website: https://drive.google.com/file/d/1cs9u0B_LqexzjThWJAe3JOHk2kdWh3at/view