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Lab Report 5: DNA Fingerprinting

Abstract

The purpose of this experiment was to allow students familiarize themselves with the use of micropipettes, and understand the different techniques used to identify DNA patterns and genetic inheritance. This lab was divided into two weeks. The first week covered the techniques of pipetting and measurement with the practice of setting restriction enzyme with their corresponding volume and tube. The second week covered the techniques used to make gel electrophoresis of DNA digests and the analysis of the banding pattern between the suspect DNA samples and the DNA found at the crime scene. The use of gel electrophoresis will separate the DNA into fragments and allow a better visualization and comparison with the other DNA fragments.

Introduction

DNA fingerprinting is a type of technique used to examine polymorphism in DNA samples. It enables forensic science to use DNA fingerprinting as evidence and identify potential suspects that were involved in a crime scene. Other methods, such as restriction fragment length polymorphisms (RFLP) are used to assess genetic variation and determine differences in banding pattern. As professor Blair stated, this method is applied in this experiment as it uses specific enzymes to cut DNA into fragments of various lengths. Restriction enzymes are specific proteins that cut DNA at specific base sequence resulting in various lengths of DNA fragments. The mixture of DNA sample with the restriction digests are then placed into gel electrophoresis to allow a visualization of the DNA banding patterns.

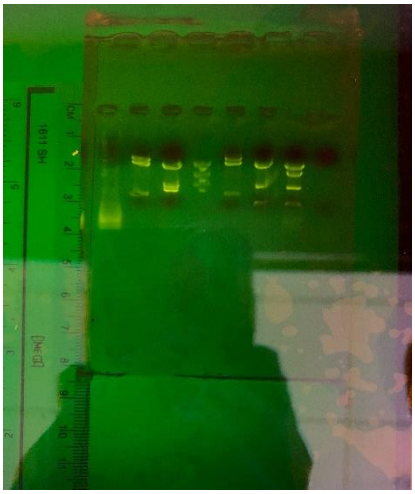
Methods

Students in groups of four prepared 1% agarose gel electrophoresis. Students combined 50 mL of TBE buffer with 0.5 g of powdered agarose. The mixture was swirled and heated in the microwave for approximately 30 seconds to 1 minute. The mixture was heated until all the agarose has dissolved in the buffer solution with a clear looking appearance. The flask was then removed from the microwave and 5 uL of SYBR safe solution was added to the flask and swirled. The SYBR will stain the DNA and help see the DNA in the gel under UV light. The solution was left to cool down for approximately 5 minutes, until it was no longer hot. The solution was gently poured into a gel casting tray and two small plastic combs were placed at each end of the gel. The gel tray was placed in the refrigerator for approximately 20 minutes to allow the gel to solidify. After the gel solidified, the combs were removed, leaving small wells behind. The gel was placed to an electrophoresis chamber, with the wells of the gel facing near the black electrode. Then, chamber was filled with enough buffer to cover the gel. Restriction enzymes digests that were prepared from the previous week, were taken out from the refrigerator and vortex to ensure there are no bubbles inside the tubes. With the pipette, 5 uL of loading dye was added to each sample. Each tube was ensured to contain a total of 25 uL (10 uL of DNA + 10 uL of restriction enzyme + 5 uL of loading dye). Different pipette tip was used for each sample after loading it into the designated well of the gel. After loading, a lid was placed on the electrophoresis chamber, and plugged with the correct electrodes into the power supply and the gel rig. The power supply was turned on and was set to 100 V. The samples were left to electrophorese for 30-45 minutes. After electrophoresis, the gel was placed under UV light for visualization.

Results

Based on the gel electrophoresis, the standard suspect (S) was placed in the first well then, the crime scene DNA (CS), then suspect 1 (S1), suspect 2 (S2), suspect 3 (S3), suspect 4 (S4), suspect 5 (S5), and lastly the negative control. It is evident that suspect 3 (fifth well) matches with the crime scene (second well). Three banding patterns are identical as shown in Figure I. The first banding pattern is 2,000 bp; the second banding pattern is 1,650 bp; and the third banding pattern is 650 bp.

Figure I.



Discussion

In gel electrophoresis, the DNA samples are loaded towards the negative charged side since DNA fragments are negative charged. The DNA is separated based on their size. Smaller fragments of DNA will run faster than larger fragments. Restriction enzymes were used to cleave DNA into fragments and run into the gel electrophoresis. A DNA ladder or marker was used as a standard reference to compare the size of the DNA fragments. Matching DNA is indicated based on same sized fragments and base pairs.

References

Blair, Christopher, (2018). Bio 2450L Genetics Laboratory Manual., pg. 70-84.