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Lab Report #5: DNA Fingerprinting

Abstract:

The purpose of this experiment was to determine the suspect of a crime scene by using DNA fingerprinting analysis. This was accomplished in an elapsed time of two weeks. The first week focused on understanding and performing micropitting as well as the role of restriction enzymes. Two trails of micropipetting was done in order to acquire this skill needed in molecular biology. The second week focused on the making as well as the understanding of gel electrophoresis to analyze DNA. In conclusion, the bands marked from suspect #3 DNA matched with the bands marked from the DNA that was present at the crime scene. This was accomplished through the analysis of DNA fragments by restriction enzymes and gel electrophoresis.

Introduction:

DNA fingerprinting analysis is used specifically in the realm of forensic science to identify organisms as well as to identify any relatedness in a crime scene (Blair, 2018, p.70). DNA fingerprinting allows for the comparison between suspects and the crime scene presented in any case. This is done through the method of gel electrophoresis where the DNA fingerprints are broken down and compared to DNA samples of possible suspects. When using gel electrophoresis to breakdown DNA segments one is able to match the DNA segment through banding patterns. One genetic method that is used in fingerprint analysis is Restriction fragment length polymorphisms (RFLP). In this particular method restriction enzymes are used in other to cleave / cut DNA at specific recognition sites. These restriction enzymes help cut the DNA and form the DNA fragments/ bands in gel electrophoresis. DNA can be reattached once it is been cut by restriction enzymes by DNA ligase. Additionally, plasmid DNA can also be cut with the help of restriction enzyme causing DNA fragment to be inserted into the plasmid. Before loading the DNA into the gel electrophoresis DNA fragments must be loaded with the restriction enzyme in order to cut the DNA segments. Then these digested DNA samples are mixed with a loading dye or stain before loading into the agarose gel. The sain allows for the banding patterns to be seen in the gel. DNA is negative thus the agarose gel runs from negative to positive as well as separates the fragments from largest to smallest. Another method besides RFLP is Southern blotting this is used to compare fragments further in order to determine the DNA.

In this particular lab micropittetors are used to measure an exact amount of DNA or sample during the experiment. It is important to understand how to use a pipette before moving on with the actual experiment. Each pipettor has its own critical values thus the volume range is given as to the capacity of that given pipettor. Things to note when using a pipette they are usually “more accurate in the upper part of their range” (Blair, 2018, 74). Additional, things to remember when using these micropipettes is to not turn the adjuster above or below than the value stated on the pipette. As well as never holding the pipette upside due to i can lead to contamination into the pipette. Micropitting is a skill that is learned and acquired through practice.

Method:

In week 1 the focus was to determine how to use micropitters by preparing 7 dye as illustrated on the table provided from the lab manual. Each dye was prepared with a total volume of 45 ul. Students worked in pairs of two. However, only 10 ul was micropitted from the mixing plate into the appropriate

circles in the target card. During week 1 DNA segments for this crime scene was prepared by adding restriction enzymes. There were 7 colored tubes provided each labeled accordingly. 10 ul of stock DNA was added to the corresponded colored tube as well as 10 ul of the enzyme as well. Once all the tubes were prepared they were incubated for 45 min in a 37 degree Celsius bath. During the 2nd week another 7 colored dye was prepared for the purposes of micropipetting. Also, week 2 constructed of first a making agarose gel. 1.0 grams of agarose was used and 50 ml of buffer this giving a 2% of the gel. Once the gel was solidified, students practiced loading the wells. Once 5ul of dye was added to each colored tube from the previous week the gel was placed in the chamber and covered with enough buffer. Once this was set each well was loaded correspondingly to each colored tube. The chamber was loaded accordingly from negative to positive, this was then turned on and placed to 100 V. The samples were allowed to electrophorese for 45 minutes. Then the gel was placed under UV light in order to determine the bands formed from the DNA segments.

Results:

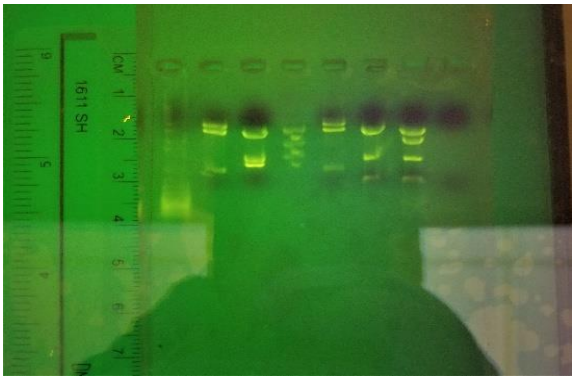

Figure 1: DNA bands after being cleaved with restriction enzymes and loaded into a gel electrophoresis. Starting from left to right the wells read as follows: S (kb ladder), crime scene, suspect 1, suspect 2, suspect 3, suspect 4 and suspect 5, negative control.

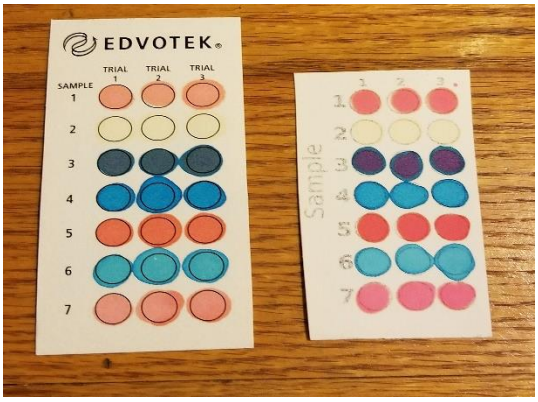

Figure 2&3: 7 colored dye constructed to used during week 1 and 2 for practice micro pipetting

Discussion/ Results:

In conclusion this experiment demonstrates the understanding of DNA analysis through gel electrophoresis. Gel electrophoresis breaks down DNA segments based on their size. A kb ladder is used to determine the distance or measurements of these bands for comparison purposes. Additionally, this lab was able to demonstrate the importance of micropipetting and its accuracy. After performing the gel electrophoresis bands that matched with the DNA at the crime scene was suspect #3 (the 5th well from the left). According to Figure 1 the bands that matched with the DNA at the crime scene measured at 2000 bp and 1650 bp. As well as a slight band was present at 650 bp which appeared on both these DNA segments. Compared to the other DNA segments suspect #3 was the one that matched the best with the crime scene DNA. As far as the micropipetting portion of this lab Figure 2 & 3 show the different colored dyes combined to acquire this skill. In conclusion, this experiment demonstrated a full understanding on micropipetting, restriction enzyme and the process of gel electrophoresis with DNA fingerprinting analysis

References:

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