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Objectives

By the end of the lab, students should:

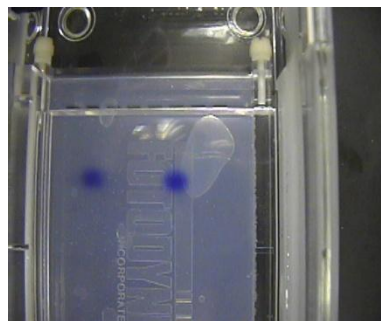
1. Be able to define terms in bold type
2. Have a basic understanding of DNA fingerprinting
3. Be able to identify the culprit in the experiment

Introduction:

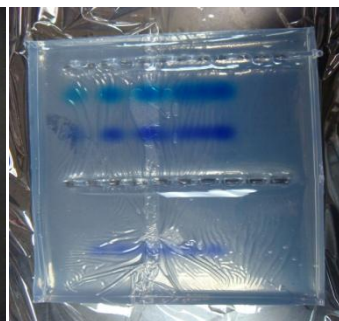
DNA fingerprinting is a technique used to identify individuals based on the unique sequences of nucleotides in a person's genome. In forensic DNA fingerprinting, DNA collected from the crime scene is matched with that of the suspects. Since sequencing entire genomes and then matching them would be a long and arduous process, restriction enzymes are used instead. **Restriction enzymes** are enzymes which cut DNA at specific points along the DNA strand, resulting in fragmentation. In a forensic lab, the DNA collected from a crime scene as well as that collected from the suspects, is cut with multiple restriction enzymes after which, the fragments made by the restriction enzymes are matched. Once cut with restriction enzymes, gel electrophoresis has to be performed. **Gel electrophoresis** is a technique used to separate DNA. Separation occurs based on size and by relying on the negative charge of the DNA to move it towards a positive electrode through the gel. The shorter lengths of DNA will travel more quickly and further towards the positive electrode, while the longer heavier fragments will move much more slowly. A gel plate is first placed into the apparatus. The collected DNA is then loaded into wells (little holes) found at the top of the gel plate. The pictures below show a set up of the gel electrophoresis apparatus (A), a top view of the gel in the apparatus (B), gel after being run with blue spots marking the moved DNA (C)



A

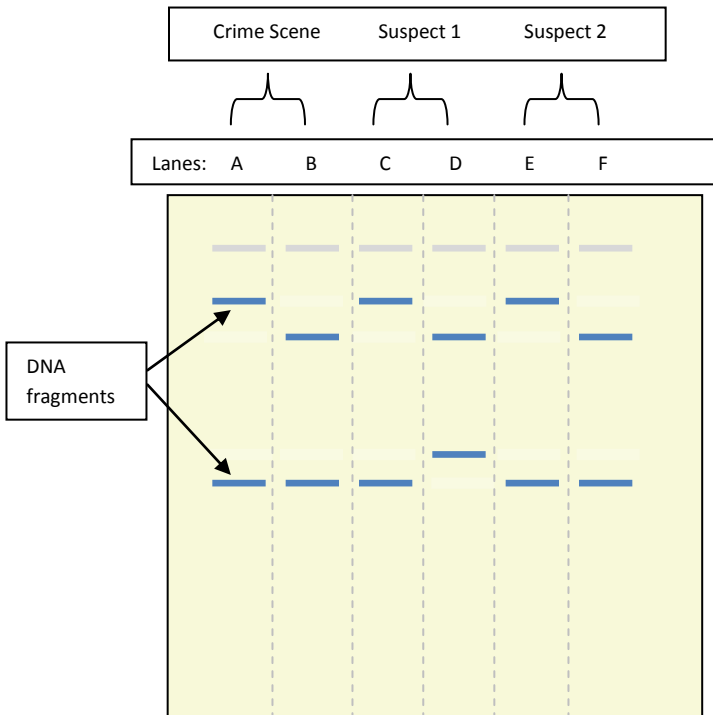


B



C

The example in the figure below shows the result of gel electrophoresis on DNA that has been cut with restriction enzymes.



Key:

Lane A: DNA from **crime scene** cut with **Enzyme 1**

Lane B: DNA from **crime scene** cut with **Enzyme 2**

Lane C: DNA from **Suspect 1** cut with **Enzyme 1**

Lane D: DNA from **Suspect 1** cut with **Enzyme 2**

Lane E: DNA from **Suspect 2** cut with **Enzyme 1**

Lane F: DNA from **Suspect 2** cut with **Enzyme 2**

In order to identify the criminal from the gel above, the bands or fragments produced by the restriction enzymes are matched, i.e. that of the suspects with the one collected at the crime scene. When the DNA samples are cut with restriction enzyme 1 (Lanes A, C, and E), fragments of the same size are produced in all three lanes. On the other hand, when the same DNA samples are cut with restriction enzyme 2 (Lanes B, D, and F), two of the three lanes have fragments of similar sizes (Lanes B and F are the same). Through the use of two restriction enzymes, we can match the DNA collected at the crime scene with that of suspects. In this case, suspect 2 would be the culprit since his DNA, cut with two restriction enzymes, matches exactly to the DNA collected at the crime scene, which is also cut with the same two restriction enzymes.

NOTE: This is a much simplified version of DNA fingerprinting. In forensic labs, multiple restriction enzymes are used which create numerous smeared bands which are sometimes not clearly distinguishable, unlike in the experiment being performed today. However, the fundamentals of the experiment are the same.

Exercise 9.1

DNA Fingerprinting

In this laboratory exercise, you will try to figure out the suspect guilty of the crime based on the DNA samples provided by your instructor. Depending on the amount of DNA available, you may be required to run the gel or may have the instructor run it for you. At the end, you will analyze the bands of DNA and identify the criminal.

Materials per group:

- Gel electrophoresis apparatus
- Practice gel loading solution
- Buffer solution
- Micropipette
- Micropipette tips
- Gloves
- Agarose Gels
- DNA samples
- UV light

Procedure:

1. Before loading the DNA samples into the wells, practice loading using the practice gel loading solution.

IF THE QUANTITIES OF DNA AVAILABLE ARE LIMITED, THEN STEPS 2 THROUGH 7 WILL BE DEMONSTRATED BY YOUR INSTRUCTOR

2. Once you are comfortable enough with the practice solution, load the DNA samples into the wells.
3. Next, cover the apparatus and plug in the color coded wires to the right color (black wire to the black pin and red wire to the red pin)
4. Turn on and set the apparatus to 120V.
5. Let the gel run for about 20min-30min or until the bands are clearly distinguishable
6. Once the gel is run, turn off the apparatus.
7. Slowly remove the gels from the gel holder.
8. Observe the gel under UV light and record the results.

The following is a list of the samples loaded in each lane:

Lane A: DNA from **crime scene** cut with **Enzyme 1**

Lane B: DNA from **crime scene** cut with **Enzyme 2**

Lane C: DNA from **Suspect 1** cut with **Enzyme 1**

Lane D: DNA from **Suspect 1** cut with **Enzyme 2**

Lane E: DNA from **Suspect 2** cut with **Enzyme 1**

Lane F: DNA from **Suspect 2** cut with **Enzyme 2**

****EVEN THOUGH ONLY A SMALL AMOUNT OF ETHIDIUM BROMIDE (A CARCINOGENIC) IS USED, WEAR GLOVES AT ALL TIMES AND DO NOT WEAR SANDALS OR SHORTS TO LAB!!**

This lab was adapted from www.Edvotek.com