

DNA Fingerprinting

Objectives:

After completing this lab, you should be able to:

1. Define *restriction enzyme* and *RFLP*.
2. Perform agarose gel electrophoresis.
3. Identify a prime suspect based on RFLP analysis of a crime scene sample.

Background:

Restriction enzymes recognize specific short nucleotide sequences in double-stranded DNA and cleave both strands of the molecule. Restriction enzymes are produced naturally in bacteria. More than 300 different restriction enzymes have been isolated and purified for use in DNA research. Each enzyme recognizes a different nucleotide sequence in the DNA. The enzymes are named with three-letter abbreviations for the bacteria from which they were isolated.

DNA cleaved with restriction enzymes produces **restriction fragment length polymorphisms (RFLPs)**. The size and number of the pieces is determined by **agarose gels electrophoresis**. The digested DNA is placed near one end of thin slab of agarose and immersed in a buffer to allow current to flow through the agarose. An electric current is applied to the gel, with electrophoresis buffer to better conduct the current. Each piece of DNA migrates from the negative electrode toward the positive electrode. Shorter DNA fragments migrate faster; larger DNA fragments migrate more slowly. The DNA fragments are visualized by staining the gel with methylene blue or ethidium bromide.

These enzymes can be used to characterize DNA because a specific restriction enzyme will cut a molecule of DNA everywhere a specific base sequence occurs (**Table 1**). When the DNA molecules from two different individuals are cleaved with the same restriction enzyme, the restriction fragments that are produced will be different. These fragments can be separated by electrophoresis. A comparison of the number and sizes of the restriction fragments produced from different individuals provides information about their genetic similarities and differences – the more similar the patterns, the more closely related the individuals are expected to be.

In forensic science, analyses of DNA can be used to determine the perpetrator of a crime. In this case, DNA evidence is collected at the crime scene (blood and semen are the most common types of samples.) DNA samples are also taken from the suspects. The RFLPs are compared to establish if the crime scene sample matches any of the samples taken from the suspects.

During this lab, we will set up restriction enzyme digests for a DNA sample gathered at the crime scene and DNA samples from several suspects to identify our prime suspect. We will use gel electrophoresis to separate the DNA fragments and visualize and compare the RFLPs.

Table 1 – Recognition sequences of some restriction endonucleases

Enzyme	Bacterial Source	Recognition Sequence
<i>EcoRI</i>	<i>Escherichia coli</i>	G↓AATTC CTTAA↑G
<i>BamHI</i>	<i>Bacillus amyloliquefaciens</i>	G↓GATCC CCTAG↑G
<i>HaeIII</i>	<i>Haemophilus aegyptius</i>	GG↓CC CC↑GG

Restriction Enzyme Digest Procedure

Working in pairs or small groups, use the materials and procedures listed below to set up five digests: One from the crime scene sample and one each from the four suspects.

1. Label five microcentrifuge tubes "CS", "1", "2", "3" and "4."
 - a. Be sure to also label each tube with name or initials.
2. Add to each tube: 5 μ l of restriction buffer.
3. Using a new pipette tip, add to each tube: 4 μ l of restriction enzyme.
4. Add to each tube the DNA samples as follows. *Use a different pipette tip for each sample.*
5. Centrifuge the tubes for 1-2 seconds to mix. *Be sure to balance the centrifuge.*
6. Incubate the tubes in a 37° water bath for 45 min – 1 hr.
7. After incubation, the tubes can be frozen if electrophoresis will be done during another lab period.

Tube	DNA
CS	Crime scene – 4 μ l
1	Suspect 1 – 4 μ l
2	Suspect 2 – 4 μ l
3	Suspect 3 – 4 μ l
4	Suspect 4 – 4 μ l

Gel Electrophoresis Procedure

Continue working in pairs or small groups to perform your gel electrophoresis procedure.

1. Place gel tray on benchtop and tighten sides of gel tray into the "up" position.
2. Position the comb in the gel tray at the end where there are slots to hold the comb.
3. Pour melted agarose into the gel tray.
4. If necessary, defrost the tubes of digested DNA by holding them in your hand or placing them in a 37°C water bath.
5. Add 2 μ l tracking dye to each sample. Centrifuge tubes for 1-2 sec. to mix. *Be sure to balance the centrifuge.*
6. Load 10 μ l of each prepared sample into wells. *Use a different pipette tip for each sample.*
7. Pour 1X TAE electrophoresis buffer to your gel box so that it is about 2cm deep over the middle of the gel box. Pour some buffer into each side rather than tilting.
8. Put the lid securely on the gel box and plug the electrodes into the power source. Turn on the power source and run gel at 125 V.
 - a. During the electrophoresis run, the tracking dye will migrate and separate into its two component dyes. Turn off the power before the faster dye runs off the gel.
 - b. Good separation of the DNA fragments occurs when the two dyes have separated by 4-5 cm.
 - c. Run the gel until the dye is near the end of the gel; then turn off the current and remove the gel.
9. To stain the gel, use either step a or step b as follows. Your professor may oversee this step.
 - a. Transfer the gel to ethidium bromide staining tray for 5-10 minutes.
Do not touch ethidium bromide. It is a mutagen.
 - Destain by placing gel in tap water for 5 minutes. (Chlorine in tap water will inactivate residual ethidium.)
 - Place your gel on UV transilluminator and close the plastic lid.**Do not look directly at the UV light without the plastic lid.**
 - b. Transfer gel to methylene blue staining tray for 30 min. – 2 hrs.
 - Destain by placing gel in water for 30 min. – overnight.
 - Place your gel on a light box. Methylene blue that is bound to DNA does not wash off.
10. Photograph your gel using the transilluminator camera or carefully draw the location of the bands.

Name _____

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Questions

1. After studying the results of your gel electrophoresis, which suspect is indicated?
Attach a photograph or drawing of your gel below and label the contents of each lane.

2. You are the forensic science expert witness at a murder trial involving DNA evidence. The defendant, who's DNA matched that found at the crime scene, is arguing he was never near the scene. *Based on the results of your Bio 110 lab experiment, construct an argument for the jury to show the defendant was present at the scene.*

In your brief paragraph explain to the jury:

- What a gene is.
- What an RFLP is.
- What restriction enzyme you used.
- What relevant information you gathered using the restriction enzyme.

3. How would your argument above be strengthened or weakened if you had NOT used a new pipette tip for each sample?