Name:_____Period:_____Date:_____
Biotechnology Laboratory at William Floyd High School
Lab activity: Forensic DNA Fingerprinting

Background:

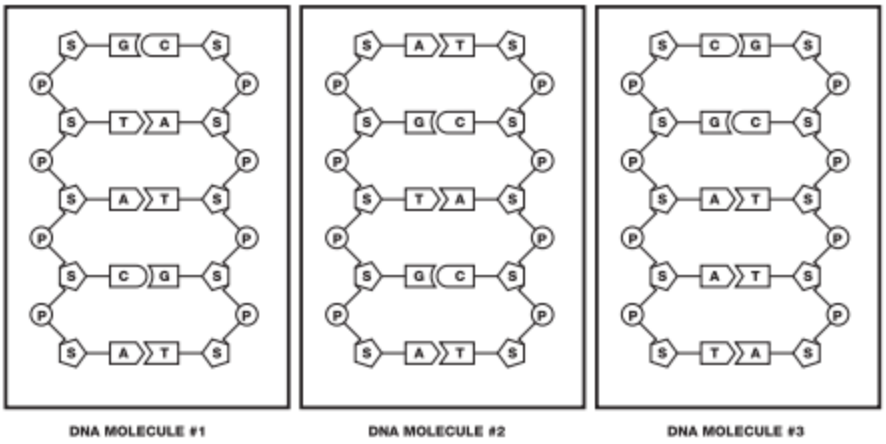
DNA. It's what makes you unique. Unless you have an identical twin, your DNA is different from that of every other person in the world. And that's what makes DNA fingerprinting possible. Experts can use DNA fingerprints for everything from determining a biological mother or father to identifying the suspect of a crime. What, then, is a DNA fingerprint and how is it made? DNA fingerprinting, also called forensic DNA analysis, is considered by many to be the police investigator's secret weapon, a means of building cases or reanalyzing crimes using tiny bits of cryptic evidence. Indeed, since 1986, when DNA evidence first entered the courtroom, the technique has aided in the prosecution or defense of hundreds of cases, and in the exoneration of dozens of people wrongly convicted.

As the acceptance of DNA evidence in the courtroom has grown, so has its importance, for the simple reason that physical evidence linking suspects to crimes is often very sparse. Sometimes the weight of an entire case -- even the life of the individual on trial -- rides on just a few drops of blood or strands of hair. But as the reinvestigation of the 1954 murder of Marilyn Sheppard shows, the quality of evidence can dramatically affect the amount of information that evidence provides.

In general, forensic DNA analysts compare the genetic makeup of tissue samples in search of similarities and differences among them. They do this not by comparing all of the DNA contained in each cell, but instead by marking a small number of segments and then checking for the presence or absence of those segments in each sample.

In this activity the data obtained may allow you to determine if the samples of DNA that you will be provided with are from the same individual or from different individuals. For this experiment it is necessary to review the structure of DNA molecules. DNA consists of a series of nitrogenous base molecules held together weakly in a double helix. These base pairs are in turn bonded to a sugar-phosphate backbone. The four nitrogenous bases are adenine, thymine, guanine, and cytosine (A, T, G, and C). Remember the base-pairing rule is A - T and G - C. Refer to the figure below of a DNA molecule.

The Structure of DNA



The pictures above represent a very small section of DNA from three different individuals. In this representation of DNA the symbol system is as follows: Backbone: S = Five carbon sugar molecule known as deoxyribose P = Phosphate group DNA Nucleotide Bases: A = adenine C = cytosine G = guanine T = thymine Analysis of the three DNA samples above (see next page) might help us detect similarities and differences in samples of DNA from different people.

Pre-Lab Questions:

1. Compare the "backbone" of the sugar-phosphate arrangement in the side chains of all three figures. Are there any differences?

2. In the above figure, do all three samples contain the same pattern of bases? Describe your observations.

3. What will you need to compare between these DNA samples to determine if they are identical or non-identical?

Objectives:

- Successfully perform a restriction enzyme digest of crime scene DNA
- To draw conclusions based on data collected though DNA gel electrophoresis analysis

Part I Restriction Enzyme lesson

How can we detect differences in base sequences?

At first sight, your task might seem rather difficult. You need to determine if the linear base pair sequence in the DNA samples is identical or not! An understanding of some historically important discoveries in recombinant DNA technology might help you to develop a plan. In 1968, Dr. Werner Arber at the University of Basel, Switzerland and Dr. Hamilton Smith at the Johns Hopkins University, Baltimore, discovered a group of enzymes in bacteria, which when added to any DNA will result in the breakage [hydrolysis] of the sugar-phosphate bond between certain specific nucleotide bases [recognition sites]. This causes the double strand of DNA to break along the recognition site and the DNA molecule becomes fractured into two pieces. These molecular scissors or "cutting" enzymes are restriction endonucleases. Two common restriction enzymes (endonucleases) are EcoRI and PstI which will be provided to you in this lab procedure. To better understand how EcoRI and PstI may help you in performing your DNA fingerprinting experiment, first you must understand and visualize the nature of the "cutting" effect of a restriction endonuclease on DNA:

The line through the base pairs represents the sites where bonds will break if the restriction endonuclease EcoRI recognizes the site GAATTC. The following analysis questions refer to how a piece of DNA would be affected if a restriction endonuclease were to "cut" the DNA molecule in the manner shown above.

1. How many pieces of DNA would result from this cut? _____

2. Write the base sequence of the DNA fragments on both the left and right side of the "cut". Left: Right:

Left: **Right:**

3. What differences are there in the two pieces?

4. DNA fragment size can be expressed as the number of base pairs in the fragment. Indicate the size of the fragments [mention any discrepancy you may detect].

   a) The smaller fragment is _____ base pairs (bp).

   b) What is the length of the longer fragment? _____

5. Consider the two samples of DNA shown below - single strands are shown for simplicity:

   Sample #1 C A G T G A T C T C G A A T T C G C T A G T A A C G T T

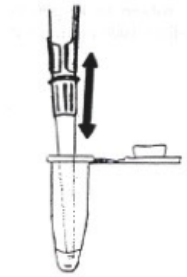
   Sample #2 T C A T G A A T T C C T G G A A T C A G C A A A T G C A

   If both samples are treated with the restriction enzyme EcoRI [recognition sequence GAATTC] then indicate the number of fragments and the size of each fragment from each sample of DNA.

Sample # 1	**Sample # 2**
# of fragments:_____	# of fragments:_____

List fragment size in order: largest ——> smallest

Sample # 1	**Sample # 2**

Important Laboratory Practices	
Add reagents to the bottom of the reaction tube, not to its side. b. Add each additional reagent directly into previously added reagent. c. Do not pipet up and down, as this introduces error. This should only be done only when resuspending the cell pellet and not to mix reagents. d. Make sure contents are all settled into the bottom of the tube and not on the side or cap of tube. A quick spin may be needed to bring contents down. 	Pipet slowly to prevent contaminating the pipette barrel. b. Change pipette tips between each delivery. c. Change the tip even if it is the same reagent being delivered between tubes. Change tip every time the pipette is used! 
Keep reagents on ice. 	Check the box next to each step as you complete it. 

Procedure: Follow the procedure in order as written below

1) Describe the samples of DNA (physical properties).

2) Is there any observable difference between the samples of DNA?

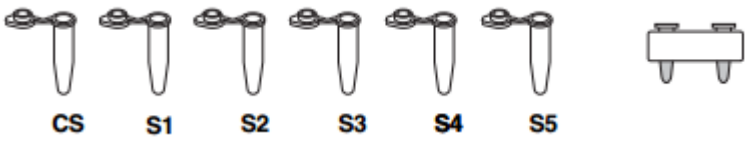
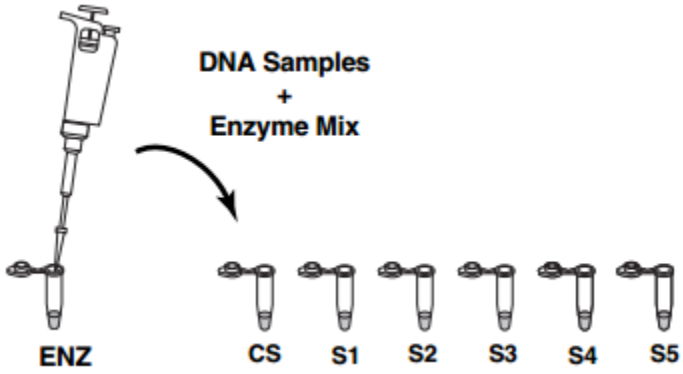
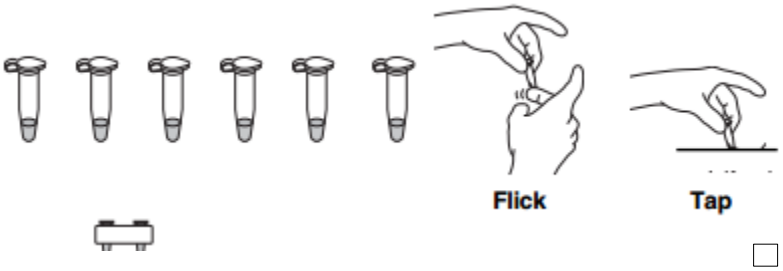
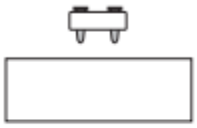
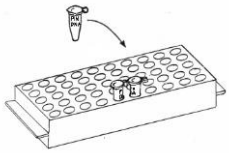
3) Describe the appearance of the restriction endonuclease mix.

4) Combine and react. Using a new pipet tip for each sample, pipet 10 µl of the enzyme mix "ENZ" to each reaction tube as shown below. Pipet up and down carefully to mix well.

Note: Change tips whenever you switch reagents, or, if the tip touches any of the liquid in one of the tubes accidentally. When in doubt, change the tip! DNA goes in the tube before the enzyme. Always add the enzyme last.

Part I: DNA Preparation Using a Restriction Enzyme

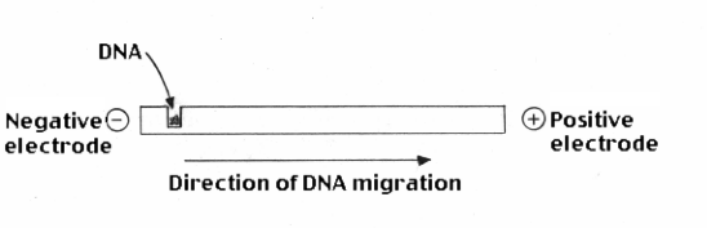
Place a check mark in the box as you complete each step.

1. Label one of each colored microcentrifuge tubes as follows: green tube CS (crime scene) blue tube S1 (suspect 1) orange tube S2 (suspect 2) violet tube S3 (suspect 3) pink tube S4 (suspect 4) yellow tube S5 (suspect 5) with your name, date, and lab period. Place the tubes in your microcentrifuge tube rack.	 ☐
2. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube. Pipet up and down carefully to mix well	 ☐
3. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, pulsespin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.	 ☐
4. Incubate the tubes for 45 min at 37°C or overnight at room temperature in a large volume of water heated to 37°C.	 ☐
5. After the incubation period place the tubes in the refrigerator until the next laboratory period. If there is sufficient time to continue, proceed directly to Part 2.	 ☐

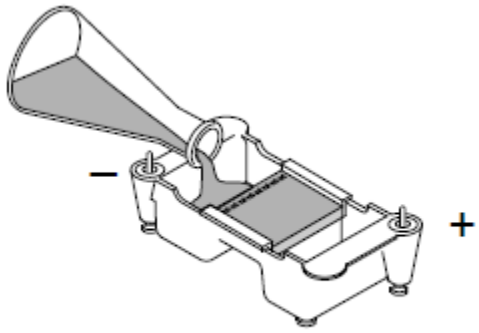
Part II: Gel Electrophoresis

Background: To determine which suspect committed the crime, you will need to "see" the products of your amplification. This will be done using a process called **gel electrophoresis** in which electric current forces the DNA to move through a special gel material. Since DNA is negatively charged, it will migrate in an electric field towards the positive electrode (Figure 2). When moved through a gel, shorter fragments of DNA move at a faster rate than longer ones.

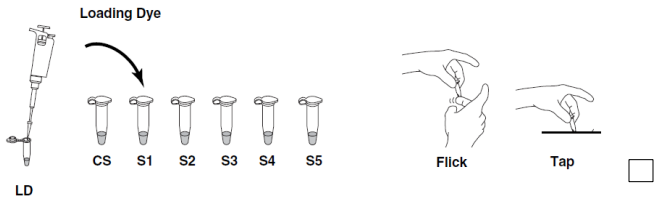
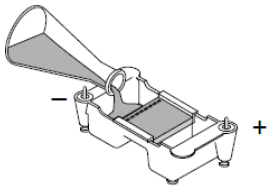
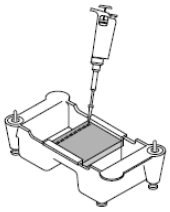
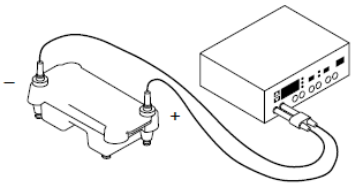
Figure 2. Side view of an agarose gel showing DNA loaded into a well and the direction of DNA fragment migration during electrophoresis.



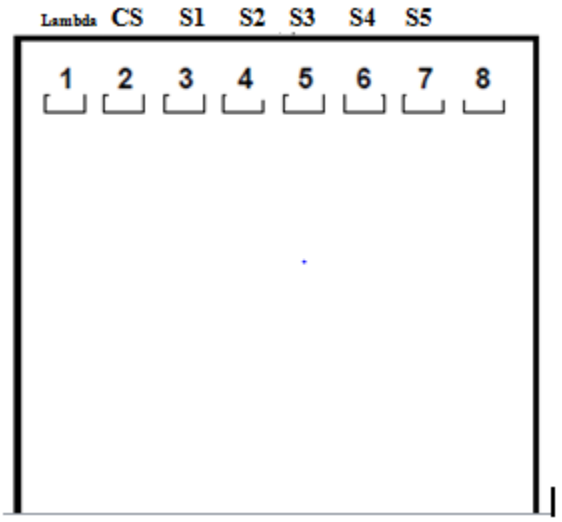
The gel material to be used for this experiment is called **agarose,** a Jell-O™ like substance made from red algae. The gel, in its casting tray, is placed in a buffer chamber connected to a power supply and running buffer is poured into the chamber until the gel is completely submerged. **Loading dye** is a colored, thick liquid containing dyes (making it easy to see) and sucrose or glycerol (making it dense). To a small volume of your digested DNA, you will add loading dye, mix and then put the mixture into one of the wells of your agarose gel. When all wells have been loaded with sample, you will switch on the power supply. The samples should be allowed to run until the dye front (either yellow or blue, depending on the dye used) is 1 to 2 cm from the bottom of the gel. The gel can then be moved, stained and photographed.



Place a check mark in the box as you complete each step.

1. Remove the digested DNA samples from the refrigerator (if applicable).	 ☐
2. Using a new tip for each sample add 5 µl of sample loading dye "LD" to each tube. Tightly cap each tube. Mix the components by gently flicking the tubes with your finger. If a centrifuge is available, pulse-spin the tubes to bring the contents to the bottom of the tube. Otherwise, gently tap the tubes on the table top.	 ☐
3. Place an agarose gel in the electrophoresis apparatus. Check that the wells of the agarose gels are near the black (–) electrode and the base of the gel is near the red (+) electrode.	☐
4. Fill the electrophoresis chamber and cover the gel with 0.25x TAE buffer. This will require ~275 ml of 0.25x buffer.	 ☐
5. Obtain the tube of HindIII lambda digest (DNA standard). The loading dye should already have been added by your instructor.	
6. Using a separate pipet tip for each sample, load your digested DNA samples into the gel. Gels are read from left to right. The first sample is loaded in the well at the left hand corner of the gel. Follow the order on the next page	 ☐
7. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect the electrical leads to the power supply.	 ☐
8. Turn on the power supply and electrophorese your samples at 200 V for 20 minutes.	

Lane 1:	HindIII lambda digest (DNA standards), clear tube, 10 µl
Lane 2:	CS, green tube, 20 µl
Lane 3:	S1, blue tube, 20 µl
Lane 4:	S2, orange tube, 20 µl
Lane 5:	S3, violet tube, 20 µl
Lane 6:	S4, red tube, 20 µl
Lane 7:	S5, yellow tube, 20 µl



For optimum visibility, 10 µl of the HindIII lambda digest (DNA standard), and 20 µl of digested DNA samples should be loaded on each gel.
(You may use a double welled gel)

Staining of Agarose Gels

When electrophoresis is complete, turn off the power and remove the lid from the gel box. Using gloves, carefully remove the gel tray and the gel from the gel box. Be careful, **the gel is very slippery**. Push the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray.

Protocol 1: Quick staining (requires 12–15minutes)
Overnight procedure may be used instead with 1X Fast Blast-See Instructor

a. Add 120 ml of 100x Fast Blast stain into your staining tray (2 gels per tray).

b. Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use. Stain can be reused at least 7 times.

c. Transfer the gels into a large washing container and rinse with warm (40–55°) tap water for approximately 10 seconds.

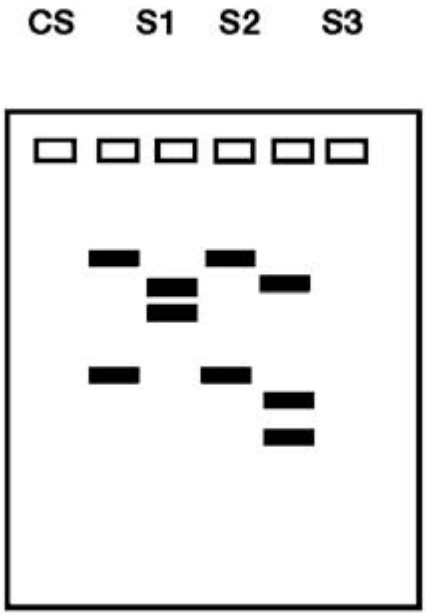
d. Destain by washing **twice** in warm tap water for 5 minutes each with gentle shaking for best results.

e. Place the gel on a light background and record your result. Draw the banding pattern on the gel template on the previous page.

f. With the help of your instructor, determine whether you are homozygous or heterozygous for the Alu insertion.

g. To obtain a permanent record, air-dry the gel on gel support film. Tape the dried gel onto your lab notebook. Avoid exposure of the stained gel to direct light, since it will cause the bands to fade.

Example: May not match your gel banding pattern



Analysis:

By looking at your agarose gel, you will determine whether or not the suspects committed the crime. When you look at the gel, you will notice that not all suspects have the same pattern of bands. Some suspects will have only one band, while others will have two or more. Comparing the banding pattern to the crime scene DNA wil help you to determine which suspect is guilty.

1. Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?

2. What caused the DNA to become fragmented?

3. What determines where a restriction endonuclease will "cut" a DNA molecule?

4. A restriction endonuclease "cuts" two DNA molecules at the same location. What can you assume is identical about the molecules at **that location**?

5. Do any of your suspect samples appear to have EcoRI or PstI recognition sites at the same location as the DNA from the crime scene?

6. Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.