FORENSICS INSTITUTE OF BRIDGEWATER (F.I.B.)

LAB LARCENY

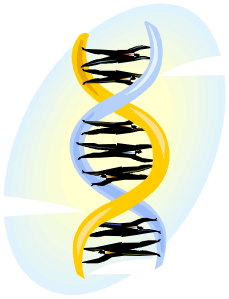
DNA FINGERPRINTING UNCOVERS A BIOTECH LAB'S DARKEST SECRETS

Scientist_____

Date_____

DNA - What is it?

DNA (Deoxyribonucleic acid) is a chemical structure that forms our **chromosomes**. A piece of a chromosome that dictates a particular trait is called a gene.
Structurally, DNA is a double helix: two strands of genetic material spiraled around each other.



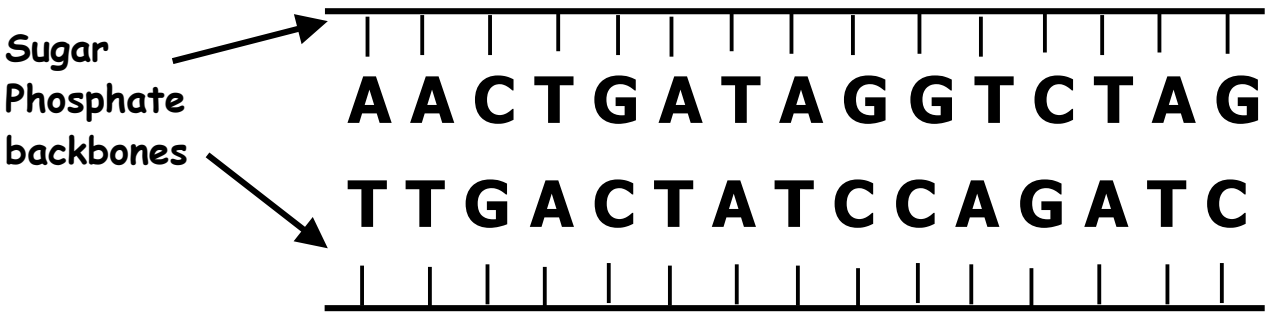
Each strand contains a sequence of nucleotides, comprised of deoxyribose sugar, phosphate and nitrogen-containing bases. A nitrogen-containing base is one of four chemicals (adenine, guanine, cytosine and thymine).
The two strands of DNA are connected at each base. Each base will only bond with one other base, as follows: **Adenine (A)** will only bond with **thymine (T)**, and **guanine (G)** will only bond with **cytosine (C)**. Suppose the sequence of bases in one strand of DNA looks like this:

A A C T G A T A G G T C T A G

The DNA strand bound to it will look like this:

T T G A C T A T C C A G A TC

Together, this section of DNA would be represented like this:



Introduction to DNA Fingerprinting using RFLP.

RFLP stands for **restriction fragment length polymorphism**. This method of DNA fingerprinting uses special types of protein called restriction endonucleases (a.k.a. restriction enzymes) which recognize and cut DNA at very specific sites. Restriction enzymes were originally discovered in bacteria, where they serve in defense systems against invading bacterial viruses. Purified restriction enzyme proteins from a bacterial species will cut DNA only at a specific recognition site.

For example the restriction enzyme EcoRI (found in *Escherichia coli* bacteria) will cut DNA only at the very specific sequence of nucleotides:

G A A T T C

C T T A A G

The EcoRI protein will cut the DNA whenever this sequence called its recognition site is found within a longer piece of DNA.

After treatment with EcoRI, there are now three pieces of DNA.

However, the cut DNA still does not look any different in a test tube when compared to the original uncut DNA. To detect the difference, the DNA fragments must be sorted by size. This can be accomplished by gel electrophoresis. In this technique, negatively charged DNA fragments are driven by an electric field through an agarose gel matrix. The agarose acts like a sieve to sort the pieces of DNA by size as they migrate to the positive electrode. The smaller the DNA, the faster it will pass through the gel.

Therefore, in a given time, smaller fragments will travel further along the gel than do larger fragments.

F.I.B. has developed a **DNA fingerprinting procedure** that detects individual variations, in the number and position of restriction sites in a 50,000 base pair long piece of one particular human chromosome. When this region of human chromosome number 9 is analyzed, it can be used to compare DNA from different sources. In forensic work, the DNA isolated from blood cells or hair cells found at a crime scene can be compared to DNA samples taken from the victims or from suspected perpetrators of the crime.

Entire Procedure in Brief

DNA Fingerprinting for forensic purposes is a multi-step process beginning with the collection of DNA-containing samples at a crime scene. For the RFLP method used at F.I.B., the following steps are performed.

1. Isolate DNA from forensic samples (any cell that has a nucleus-- ex. white blood cells, hair follicles, skin cells).

2. Amplify the DNA from a specific region of human chromosome 9 using the Polymerase Chain Reaction (PCR).

3. Cut the amplified DNA using specific restriction enzymes.

4. Perform gel electrophoresis on these cut DNA's to separate the different fragments of DNA by size.

5. Create a permanent record of the results by staining the gel with a DNA specific dye and photographing the results.

6. Compare the patterns of DNA fragments on the gel from suspects, victims and DNA isolated from cells found at the crime scene.

In our laboratory, the first two steps are automated and are carried out by the DNA Isolation and PCR lab within our company. As new F.I. B. employees, you have been assigned to the RFLP lab and will be responsible for performing **steps 3 thru 6** of the procedure above.

MEMO: To all F.I.B. Employees

Subject: CRIME ALERT!

Dr. Jenna Mendell, Forensic Institute of Bridgewater's Employee of the year for 2001 failed to show up for work yesterday. The first employees, arriving for work on this day found broken glass, ripped notebook pages, overturned furniture and red stains in the area of the laminar flow hood where Mendell usually works. Dr. Mendell, one of the founding members of our company, had been working on a highly promising and lucrative forensic technique that would easily and quickly detect human blood in stained materials.

You may have heard the rumors about industrial espionage. Last week, one of our new hires, Dr. Jeff Williams, reported that he overheard cell phone conversations between Mendell and the CEO of an unidentified Biotechnology company. Dr. Williams claims that Mendell was planning to leave F.I.B. and take with her the project that she was working on. He also claims that Mendell negotiated a figure in excess of $1,000.000.00 for her cooperation.

The crime scene evidence includes several interesting exhibits that appear to be bloodstains. In cooperation with the Massachusetts State Police Crime Lab, we have confirmed that both of these samples contain human blood.

As you may have heard rumored, we have two prime suspects. Both are F.I.B employees. Therefore, in cooperation with the State Police Crime Lab, we have asked for permission to conduct an in-house analysis of the DNA isolated from these blood samples. We have been given 72 hours to complete the initial investigation and report to the District Attorney.

As members of our investigative team, we would like to show you the evidence that we found and the experiments that we would like your help with. For that you will be asked to visit the scene of the crime, located at F.I.B.'s laboratory within the Bridgewater State College CityLab.

Materials Checklist

- ☐ 25 ml of molten agarose stored in a 55° C waterbath
- ☐ (1) casting deck and casting tray
- ☐ permanent marker pen
- ☐ (1) gel comb
- ☐ (1) Electrophoresis box
- ☐ (1) Electrophoresis power source
- ☐ (1) red and black (positive and negative) cables
- ☐ (1) bottle of Electrophoresis buffer
- ☐ p20 Pipetman
- ☐ Pipet tips
- ☐ Bucket for used pipet tips
- ☐ Ice bucket containing:
  - ☐ Restriction Enzyme
  - ☐ A tube of distilled water
  - ☐ A tube of gel-loading dye
- ☐ All of the following samples

☐ B	☐ B-Enz
☐ C	☐ C-Enz

- ☐ One of the following sample sets

☐ V	**AND**	☐ V-Enz
	OR	
☐ X	**AND**	☐ X-Enz
	OR	
☐ Z	**AND**	☐ Z-Enz

Record Your Sample Here:

_____

- ☐ Waterbath set to 37° C
- ☐ Timer
- ☐ Bottle containing DNA Gel Stain
- ☐ Microcentrifuge

Restriction Enzyme Digestions

☐ 1. Add 5 µl of restriction enzyme from your ice bucket to the DNA in any tubes labeled **B-Enz, C-Enz, V-Enz, X-Enz, and Z-Enz.** Mix carefully by flicking the tube with your fingers.

Remember to change your micropipet tip for each sample.

☐ 2. Add 5 µl of distilled water from your ice bucket to any tubes labeled **B, C, V, X, and Z.** These will be your controls. Mix carefully by flicking the tube with your fingers.

Remember to change your micropipet tip for each sample.

☐ 3. Label your six tubes with your initials using your permanent marker.

☐ 4. Transfer the six tubes to the 37° C waterbath. Set your timer for 30 minutes.

While you are waiting for your samples to incubate, you will pour your gels.

Pouring the Horizontal Gel

☐ 1. The casting deck and tray are used to mold the gel into a rectangular shape as it hardens. Place the casting tray into the casting deck by pushing one end of the tray against the thick foam barrier.

☐ 2. Put the casting tray in its deck on a level surface.

☐ 3. Insert a comb into the slots at one end of the tray. Make sure the smooth side of the comb faces the center of the tray.

☐ 4. Pour 25 ml of molten agarose solution taken from the 55° C waterbath into your casting tray. Be sure that the agarose spreads out over the whole tray and to the other side of the comb.

Once you pick up the molten agarose, work quickly to pour your gel.

☐ 5. Wait for the gel to cool until it is opaque (or cloudy) in color and firm to the touch.

Preparing the Gel Electrophoresis Box

☐ 1. Once the gel has hardened, gently remove the comb from the tray. The depressions left in the gel are referred to as "wells".

☐ 2. Carefully push the casting tray into the thick foam side of the casting deck and pull up on the casting tray until the tray comes out of the deck. Put the deck off to one side.

☐ 3. Place the casting tray with the gel in the electrophoresis box so that the wells are located at the negative (**black electrode**) end of the box.

☐ 4. Slowly pour the contents of the electrophoresis buffer bottle into the electrophoresis box. Fill the electrophoresis box with buffer until the gel is covered with a 2-3 mm layer of buffer. You can pour the buffer directly from the bottle.

Gel Electrophoresis

The restriction enzyme has completed its job of cutting the DNA at specific recognition sites. However, before the DNA is loaded onto the gel, a loading dye must be added to the samples. This buffer contains a viscous solution to aid in the loading process and a tracking dye to help visualize the electrophoresis process (the DNA is itself invisible until stained).

☐ 1. Remove the DNA samples from the 37° C waterbath.

☐ 2. Spin the samples for 5 - 15 seconds in the micro-centrifuge. The centrifuge must be "balanced" by placing a tube directly opposite another tube.

☐ 3. Add 5 µl of gel loading DYE to each sample. Mix by flicking the tube with your fingers.

Remember to change your micropipet tip for each sample.

☐ 4. Spin the samples for 5 - 15 seconds in the micro-centrifuge.

☐ 5. Use the following chart to record the order in which you load your samples. Leave wells #1 and #8 empty.

Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
Blank							Blank

☐ 6. Load 10 microliters (µl) of each of the samples into the appropriate wells.

☐ 7. Connect the red cable to the red electrode on the lid of the electrophoresis box.

☐ 8. Connect the black cable to the black electrode on the lid of the electrophoresis box.

☐ 9. Put the lid on the electrophoresis box, listen for a clicking sound.

☐ 10. Connect the other end of the red cable to the red plug on the power source.

☐ 11. Connect the other end of the black cable to the black plug on the power source.

☐ 12. Before turning on the power, have a senior scientist check your connections and then plug in the power source.

☐ 13. Run the gel for 30 minutes at 105 volts.

☐ 14. Once the gel is finished running, shut off the power supply, and disconnect the Electrophoresis Module. One of the senior scientists will come and help you remove your gel.

Staining the Gel

Faint DNA containing bands may be visible at this point, but most likely the only band that you see will be the tracking dye from the loading buffer. It is necessary to stain the gel for 20 minutes to visualize the DNA containing bands

☐ 1. Gently pour 50 ml of stain into a container.

☐ 2. Carefully insert your gel into the stain.

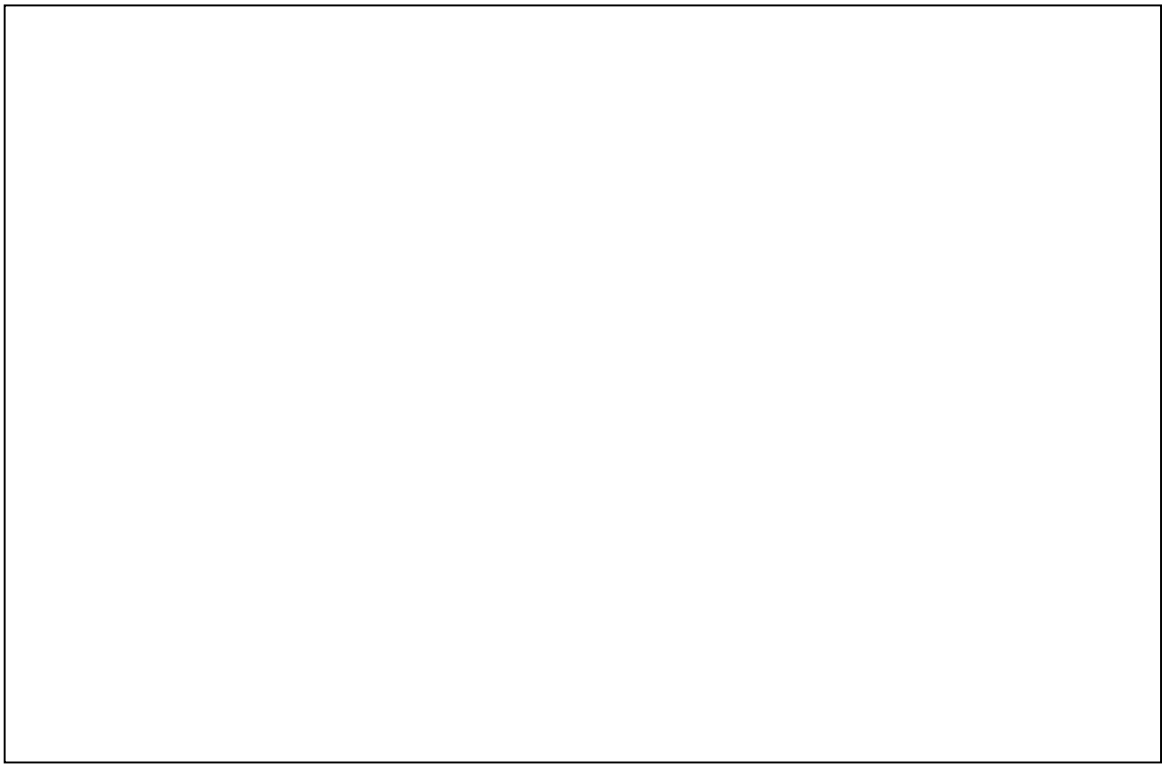
☐ 3. Gently rock the container back and forth a few times each minute for 20 minutes.

☐ 4. Hold the gel gently in the container (wear gloves!) and pour the stain back into its original bottle.

☐ 5. Add distilled water to the container and rock the container back and forth a few times each minute for 10 minutes. Holding the gel carefully, discard the distilled water into a sink. *Repeat step 5 three to four more times.*

Interpreting the Gel

Insert a picture of your Gel here:



Compare the DNA samples treated with restriction endonuclease to those control samples that lack the restriction endonuclease.

What conclusions can you make?_____

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Compare the restriction endonuclease digested samples from the DNA isolated from exhibit B and exhibit C from the crime scene to the DNA isolated from your specially assigned DNA.

What conclusions can you make?_____

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