Name:_____ **Section:**_____

Biology 100
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DNA Crime Scene Lab Protocol

BACKGROUND:

Restriction endonucleases are enzymes that recognize specific sequences of bases within double-stranded DNA called **restriction sites** and cut within that sequence. For example, the two enzymes that we will be using in today's lab, *EcoRI* and *HindIII*, recognize the following restriction sites:

EcoRI

```
      i
5' G-A-A-T-T-C 3'
3' C-T-T-A-A-G 5'
      h
```

HindIII

```
      i
5'A-A-G-C-T-T3'
3'T-T-C-G-A-A5'
      h
```

The arrows indicate the positions where the enzyme will cleave, or cut, the DNA to produce fragments that look like this:

EcoRI

```
G          A-A-T-T-C
C-T-T-A-A        G
```

HindIII

```
A          A-G-C-T-T
T-T-C-G-A        A
```

Depending on the number and location of restriction sites present, cutting a DNA sample with a restriction enzyme will generate a certain number of differently-sized fragments. The number and location of cleavage sites, and hence the number and size of the DNA fragments, are unique to each individual. For example, DNA from two different people may contain a different number of *EcoRI* restriction sites. (For simplicity, only one DNA strand is shown for each person.):

Person 1: 5'ATGGCGATAG**GAATTC**ATTCCAT**GAATTC**ATATGTGCCAC 3'

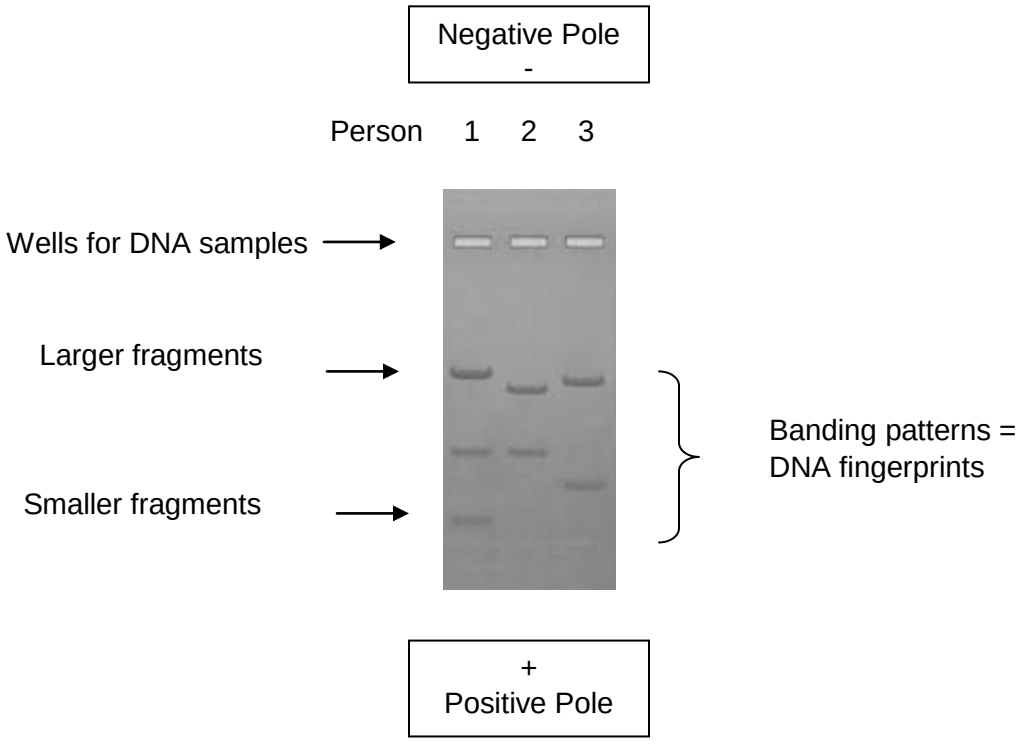
ATGGCGATAGG | AATTCATTCCATG | AATTCATATGTGCCAC

Person 2: 5' ATGGC**GAATTC**AGGATTACATTCCATGATACCATATGTGC 3'

ATGGCG | AATTCAGGATTACATTCATGATACCATATGTGC

Once the DNA has been cut with the enzyme the fragments of DNA can be separated and then later visualized using a technique called **gel electrophoresis**. Gel electrophoresis is possible

because DNA has a negative charge. The DNA fragments of varying length are placed in a gelled substance called agarose, which is like (an expensive) Jell-O. The gelatin-like mold containing the DNA is then placed in a buffer solution that can conduct electrical current. When external voltage is applied the charged fragments of DNA will migrate towards the positive pole. The smaller the fragment, the farther the fragment will migrate; the larger the fragment the shorter the migration. What results from gel electrophoresis is a vertical pattern of **"bands"** representing different-sized DNA fragments that can be seen under ultraviolet light. This vertical banding pattern is a "**DNA fingerprint"**:



LAB SETUP:

Each table will have four DNA samples that you will analyze as if you were a team of investigators inspecting DNA evidence from a murder investigation. The four DNA samples are: DNA from the victim, DNA from the crime scene, and DNA from two suspects. Each table will get the same DNA samples but will be assigned only one of the two restriction enzymes, either EcoRI or HindIII. This is so that you can compare your group's results with others' to see which suspect's DNA matches that found at the crime scene.

MATERIALS AND METHODS:

Step I: Practice Pipetting

Since we will be dealing with very small volumes of fluid, before starting the experiment, you will practice your micro-pipetting. Each person should practice pipetting 1µl, 2µl, 5µl 10µl and 20µl of the PRACTICE tracking dye. Using the fine pipette tip, pipette the practice dye onto the small pieces of the supplied plastic wrap.

Step II: Preparing and Cutting the DNA Samples

PROCEDURE:

Prepare the restriction digest and cut the DNA:

1. Make a digestion mixture by adding 100 μL of 1X reaction buffer to the microfuge tube containing 5μL of restriction enzyme (either HindIII or EcoRI).

Which restriction endonuclease is your group using? _____

2. Close the top of the microcentrifuge tube. Mix the components in the microfuge tube by flicking the tube with your finger. Then tap the tube on the counter or centrifuge it in the minifuge for 1-3 seconds to force the entire sample to the bottom of the tube.
3. Add 20 μL of your digestion mixture to each of the four DNA sample tubes. These tubes are labeled V (victim), S1 (suspect 1), S2 (suspect 2) and CS (crime scene). Mix the components as in step 2.

Be sure to use a fresh pipette tip for each sample and pipette accurately. You don't want to run out of digestion mixture. Try to keep the digestion mixture as cold as possible by keeping it in your minifreezer rather than holding it between your fingers.

Final restriction digestion volume (μL digestion mixture + μL DNA) = _____

4. Remove the tube and place it in the floating racks in the water bath. Incubate at 37°C for 30 minutes.

Sample Preparation:

1. After digesting the DNA, remove the microfuge tubes from the water bath. Mix and tap or spin the tubes as before.

Step III: Practice Loading into Gels

1. While the DNA is being digested by the restriction enzyme, practice loading the blue tracking dye into the mini dishes provided.
2. Flood the surface of the practice dish with enough water to cover the wells. Using the fine pipette tips, pipette 20μl of the PRACTICE tracking dye into each well. Care must be used to assure that the pipette tip is positioned at the opening of the well not inside the well. That may tear the medium and distort your results.

Step IV: Loading into Gels

Your instructor will demonstrate the technique by loading a DNA ladder in the gel. A DNA ladder is digested DNA with known molecular weight fragments. It provides a basis of comparison between your sample bands and the bands created by the ladder after running the gel.

1. Load 20µl of your DNA sample into a well. Steady the tip above the well and just break the surface of the buffer solution. Do not put the tip in the well bottom or push the tip into the gel.

Check to make sure the wells are at the negative end of the chamber. DNA will run to the red.

Step IV: Gel Electrophoresis

1. The instructor will turn on the gel electrophoresis apparatus and run the gel for a prescribed period of time. You will view the gel at a later date.

RESULTS AND ANALYSIS:

View the electrophoresis gel under ultraviolet light. The bands will appear in vertical lanes. Also recognize that larger bands appear closer to the wells and the starting point, while progressively smaller bands appear farther away. Locate your lanes and observe the pattern of the bands. Compare the bands corresponding with the crime scene and the suspects' DNA samples. By looking at the banding patterns of the DNA samples on the gel you should be able to determine which of the two suspect's DNA matches that of the crime scene. Who appears to be the guilty party, or at least found to be present at the crime scene? Now compare the pattern of bands between the other groups of students to determine whether you all arrived at the same conclusion.

LAB WRITE-UP:

You are part of an investigative team hired by the prosecution in a trial to try and identify which of two suspects was present at a (fictitious) murder crime scene. In your write-up, you will creatively establish the murder scenario and then give your testimony as a DNA expert called in to evaluate the DNA evidence that your investigative team collected. Your write-up should consist of 3 parts:

1. Describe the situation in which the (fictitious) murder took place. Come up with a creative and entertaining story.

2. Describe to the jury how you arrived at your conclusion. Explain to them, *in everyday terms*, how restriction enzymes and electrophoresis of DNA fragments work to yield unique DNA fingerprints. Don't use complicated terms like "endonuclease" or "electrophoresis." (Read this part of your write-up to someone who hasn't had BIOL 100 and see if they can understand how DNA fingerprints are made from your description. This will be a good indication of how thorough your description is.)

3. Then, tell the jury why it is you believe one or both suspects may be involved in the crime, or at least were present at the crime scene. Use the results from the DNA gel to support your conclusion. In your analysis, describe how your investigative team's data compares with the data gathered by the other investigative teams. Did the teams who used the same restriction enzyme reach the same conclusion as your team? Did the teams who used the other restriction enzyme reach the same conclusion as well? In your expert opinion, would you recommend to the jury that they find a suspect guilty of the murder based on the DNA evidence available?