Name:

Case of the Broken Beaker

A DNA Restriction Analysis Laboratory Activity

Adapted from *Case of the Crown Jewels* written by Donald A. DeRosa and B. Leslie Wolfe

BREAK IN ON THE MdBioLab!

Your instructors returned to the MdBioLab on Monday to find the door unlocked, a gruesome crime scene, and a lot of missing laboratory equipment and supplies. Approximately $20,000 worth of equipment is missing, including 6 thermal cyclers, 4 spectrophotometers, 12 microscopes, 4 UV light boxes, and 20 micropipettes.

http://www.flickr.com/photos/rozmania/386544424/

A crime scene investigator (CSI) examined the crime scene and discovered a broken piece of glassware. Apparently, the burglar, in fleeing the scene, crashed into a beaker on the lab bench and cut his or herself, leaving blood on the shattered pieces of glass. The CSI has obtained these pieces of glass and brought them to your forensic laboratory for analysis. She informs you that the Police Department would like you, a forensic scientist, to conduct a genetic analysis in an attempt to place a suspect at the crime scene.

The Police have identified four suspects. The first suspect is the director of a competing mobile laboratory. The second suspect is a teacher from a nearby middle school whose physical description fits the accounts of eyewitnesses. The third suspect is a member of a team of black market laboratory equipment dealers known on the street as the “Science Thugs.” The fourth suspect is YOU! All indications are that a single person carried out this heist.

A lab technician performed a DNA extraction from the blood present at the crime scene. This included adding a soap or detergent solution to break open the cell membranes and release the DNA. Once released, the addition of ice-cold alcohol provides an interface in which DNA can be spooled.

As a forensic scientist, you will use restriction enzymes and agarose gel electrophoresis to create a DNA profile for each of the suspects to assist the District Attorney’s prosecution of the case.

IDENTIFY THE PROBLEM

What is the problem you are trying to solve?

RESTRICTION ANALYSIS

DNA molecules can be identified by their unique sequence of bases. Restriction enzymes, which are naturally produced by bacteria, cut DNA at specific base sequences.

When a specific restriction enzyme is used to cut different DNA molecules, the size and number of fragments generated will be unique to each molecule. In this experiment, you will be using *Hind*III, a restriction enzyme isolated from the bacteria *Haemophilus influenzae,* a pneumonia-causing agent. The unique-sized fragments can be separated using gel electrophoresis.

Gel electrophoresis is a technique used to separate molecules based on the differential movement of charged particles through a matrix when subjected to an electric field. DNA is negatively charged –when in the presence of an electric current, DNA will travel according to size (smallest pieces first) towards the positive electrode. By comparing the resulting pattern of DNA fragments on the gel, the DNA strands can be differentiated.

EXPERIMENT

You will need the following materials and equipment:

Crime Scene DNA	Micropipette and tips
Suspect 1 DNA	Electrophoresis equipment
Suspect 2 DNA	Microcentrifuge
Suspect 3 DNA	Restriction Enzyme Mix- Hind III
Suspect 4 DNA	Gloves

PART ONE: RESTRICTION DIGEST

You will be performing restriction digests on five DNA samples to create a DNA profile. These results will be analyzed using agarose gel electrophoresis, paying specific attention to the unique banding pattern for each suspect based on fragment number and approximate size (number of bases).

☐ 1. Locate the five DNA samples in your tube rack: Crime Scene DNA, Suspect 1, Suspect 2, Suspect 3, and Suspect 4. Digest each of the samples with the restriction enzyme *Hind*III.

☐ 2. Add 10 µL of Hind III Restriction Enzyme Mix to each of your DNA sample tubes.

☐ 3. Centrifuge samples for two seconds to collect the contents at the bottom of the tube. Make sure to balance your sample tubes in the centrifuge!

☐ 4. Incubate samples at 37 °C for 5 minutes using the incubator. This period of incubation allows the enzymes to digest the DNA.

What is significant about 37 °C? (Hint: Convert to Degrees Fahrenheit). ____________________________

Why would this be the optimal temperature for restriction enzyme activity?

What do the restriction enzymes do to the DNA samples?

PART TWO: SAMPLE PREPARATION & AGAROSE GEL ELECTROPHORESIS

☐ 5. After incubation, add 5 µL of loading dye to all sample tubes. The loading dye is an agarose gel loading buffer that contains blue dye to color the sample and glycerol to keep the sample in the wells of the agarose gel.

☐ 6. Centrifuge samples for two (2) seconds to collect the contents at the bottom of the tube. Make sure you balance your centrifuge!

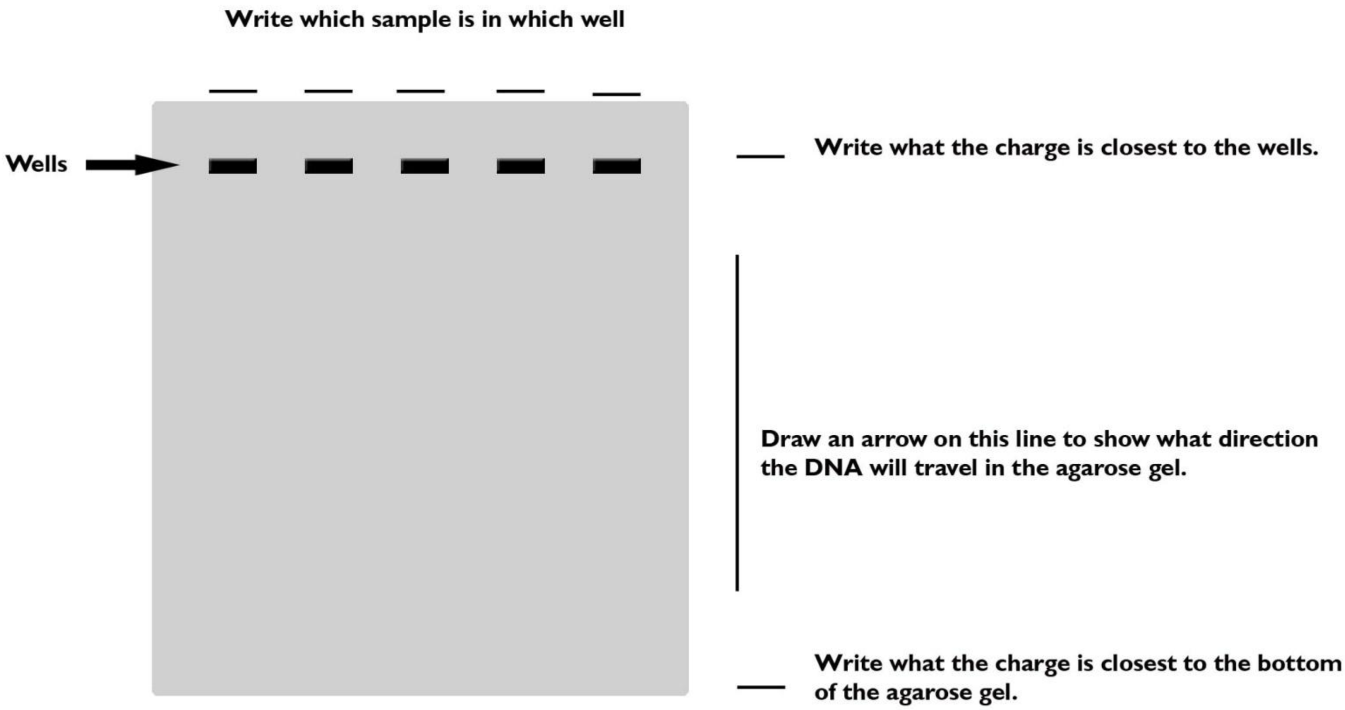
☐ 7. Put the samples back into your colored tube rack. Your instructor will assign you a gel box and a set of well numbers. Write your well numbers in the table:

Sample	Sample Identification Code	Well Number
Crime Scene		
Sample 1		
Sample 2		
Sample 3		
Sample 4		

QUICK CHECK: Compare the DNA samples to each other. Can you tell a difference between them?

SAFETY FIRST! Your agarose gel contains a suspected mutagen called ethidium bromide. This chemical associates itself with the backbone of DNA. Wear your gloves at all times, respect the agarose gel, your equipment and your other classmates.

- ☐ 8. Load 20μL of each sample into the appropriate wells. Be sure to keep track of which wells were used and to fill out the chart above. Notify an instructor when you have finished loading your gel. They will instruct you on how to finish setting up the electrophoresis box.
- ☐ 9. Locate the bottle labeled "electrophoresis buffer." Slowly pour the entire bottle into the bottom chamber of the electrophoresis box until the liquid flows over the gel and fills the upper chamber. This buffer is a salt solution.
- ☐ 10. Put the lid onto the box (black to black, red to red) and plug the other ends of the wires into the power supply. Run the gel at 200 volts for at least 10 minutes.



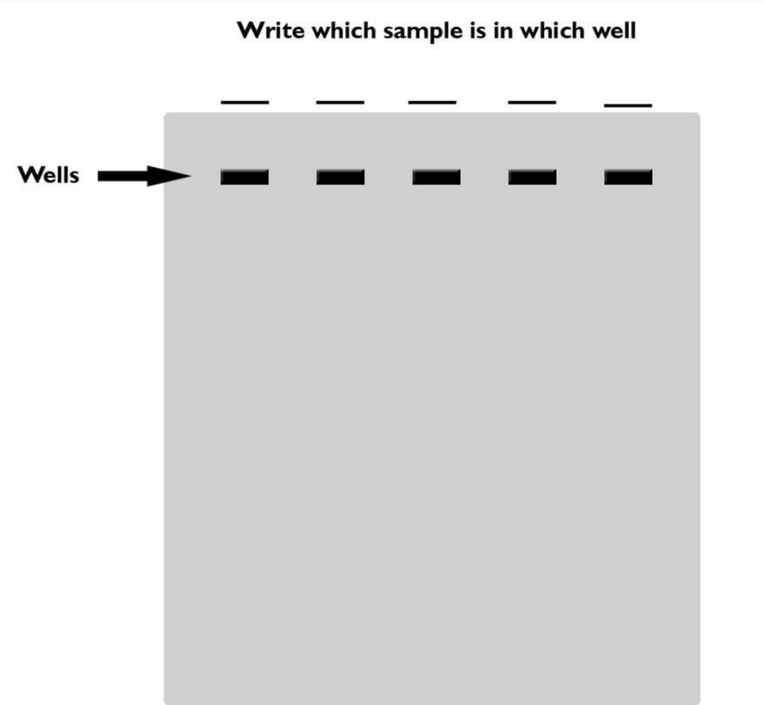
How are you using the agarose gel to compare the DNA samples?

What is the comb used for?

Why did you add electrophoresis buffer to the electrophoresis box?

What direction does the loading dye migrate? Why?

☐ 11. Your instructor has added ethidium bromide to your gel. This is a DNA stain that associates itself with the DNA backbone; it glows orange when exposed to UV light. Follow your instructor's guidance to place your gel on the UV light box and view the DNA fragments. Draw your results below. Be sure to label which samples you drew in which wells, the direction the samples traveled, and the charge at the top and bottom of the gel:



DATA ANALYSIS

It is time to analyze the results of your test. Observe the banding patterns on your gel. Do you see differences or similarities between the Crime Scene DNA and the suspect samples?

CONCLUSION

Think about what conclusions you can make from the experiment. When making a conclusion, scientists have to interpret the results of the test. You have compared the DNA banding patterns of the three suspect samples to that of the DNA found at the scene of the crime. Based on the results of your experiment, could you place any of the three suspects at the scene of the crime? Explain.

Make your own conclusion. Write a statement about the crime scene DNA when compared to that of the suspects. Include your determination for the Police Force and an explanation of your results. Suggest further analysis if necessary (What are the limitations of your analysis?)