

Case of the Broken Beaker

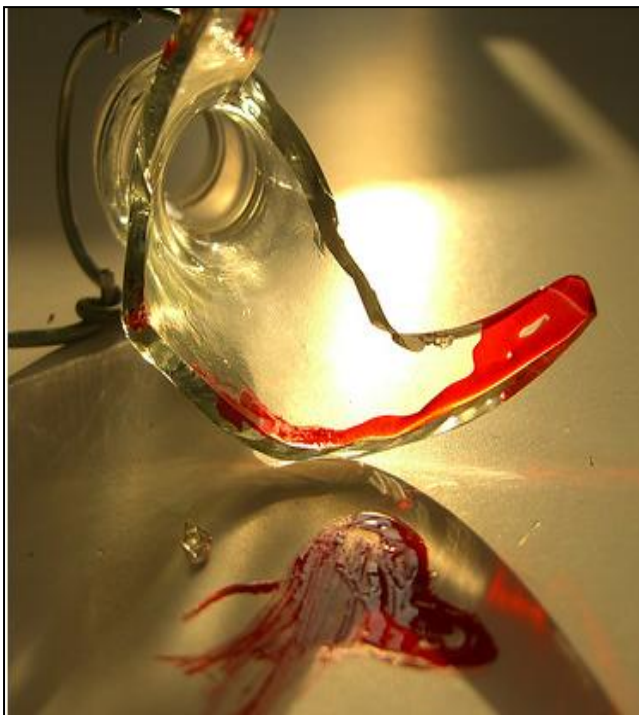
Creating DNA Profiles Using Restriction Analysis

Break-in on the MdBioLab!

This morning, the MdBioLab instructors returned to the truck to find that someone had forced their way onboard the night before. The truck had been torn apart, and over \$20,000 worth of equipment had been stolen. Examination of the security footage confirmed that a single person, whose identity was concealed from the camera, carried out the heist. This suspect remains at large.

Crime Scene Investigators have processed the scene and have retrieved a key piece of evidence. Inside the truck, the investigators found a broken beaker containing what they believe to be the blood of the thief. DNA has been extracted from the blood and is ready for forensic analysis.

Luckily, several witnesses, who observed a person fleeing from the scene the previous night, provided officers with a fairly detailed description of the offender. Using these descriptions, officers have rounded up four suspects that have committed similar crimes. In accordance with a court order, each of the four suspects has submitted their DNA for analysis. The MdBioLab and police department need you to help create a DNA profile for each suspect that will assist the D.A. in prosecuting the case.



To create a genetic profile you will be using restriction enzymes and agarose gel electrophoresis. These restriction enzymes, which are produced naturally by bacteria, “digest” the DNA by cutting it at specific base sequences. Gel electrophoresis can then be used to separate the resulting DNA fragments.

Why can DNA be used reliably to show that someone was at a crime scene?

MATERIALS

Crime Scene DNA
Suspect 1 DNA
Suspect 2 DNA

Suspect 3 DNA
Suspect 4 DNA
Microcentrifuge

HindIII
Micropipette and tips
Electrophoresis equipment

PART I - Agarose gel preparation for gel electrophoresis

- 1. Locate the clear electrophoresis tray and white comb on the laboratory bench.
- 2. Insert the white comb into the tray to form the wells. There are two different slots in the tray for the comb. Be sure to use the slot closest to the end of the tray.
- 3. Remove the cap from the agarose gel tube, and pour its entire contents into the tray. Allow the gel 15-20 minutes to solidify.

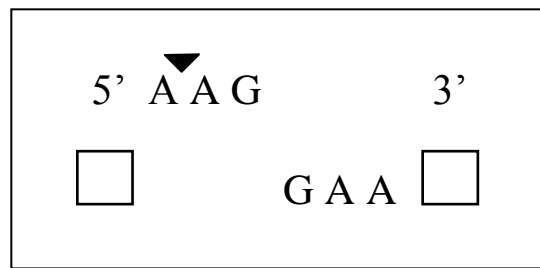
PART II – Restriction digest

- 4. Locate the five DNA samples in your tube rack: Crime Scene DNA, Suspect 1, Suspect 2, Suspect 3, and Suspect 4.
- 5. Centrifuge the HindIII tubes for two (2) seconds to collect the contents at the bottom of the tube. Make sure you balance your centrifuge.
- 6. Add 10 μ L of the restriction enzyme, HindIII, to each of your DNA sample tubes.
- 7. Centrifuge the samples for two (2) seconds to collect the contents at the bottom of the tube, making sure to balance your sample tubes in the centrifuge.
- 8. Incubate the samples at 37 °C for five (5) minutes using the incubator. This period of incubation allows the enzymes to digest the DNA.

QUICK CHECK: Why is 37 °C the optimal temperature for restriction enzyme activity?

What do the restriction enzymes do to the DNA samples?

Fill in the missing information in the diagram below, which shows the DNA sequence of the cutting site of HindIII. Make sure to complete the DNA sequence for both strands, indicate the polarity of the second strand, and mark the cutting site of HindIII on the second strand.



HindIII Recognition Site

PART III – Agarose gel electrophoresis

- 9. After incubation, add 5 μL of loading dye to all the sample tubes.
- 10. Centrifuge the samples for two (2) seconds to collect the contents at the bottom of the tube. Make sure you balance your centrifuge.
- 11. Put the samples back into your colored tube rack. Assign each sample to a different well in your gel. Write the assigned well numbers in the table below:

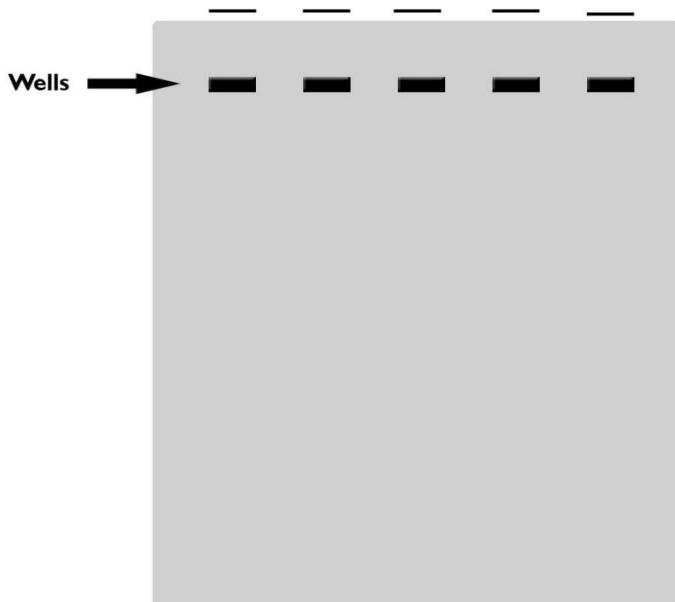
Sample	Tube Label	Well Number
Crime Scene		
Sample 1		
Sample 2		
Sample 3		
Sample 4		

- 12. Load 22 μL of each sample into the well that it was assigned to in the chart above.
- 13. Locate the electrophoresis box, and place your gel into one of the six gel slots. The gel should be completely covered by the electrophoresis buffer. Place the lid on the box, ensuring that the black wire is on top.
- 14. Run the gel at 240 volts for at least 10 minutes.

PART IV – Data analysis

- 15. To visualize your results, slide your gel off of the tray and onto the UV light box. Draw any differences or similarities between the Crime Scene DNA and the suspect samples below.

Write which sample is in which well



— Write what the charge is closest to the wells.

— Draw an arrow on this line to show what direction the DNA will travel in the agarose gel.

— Write what the charge is closest to the bottom of the agarose gel.

What affects the rate at which DNA travels through an agarose gel?

How do restriction enzymes cause the different banding patterns on the gel, and how can this be used as a genetic profile?

How reliable do you think this DNA profile is? Are there any instances in which this profile may not be informative?

PART V- Conclusion

When making a conclusion, scientists have to interpret the results of the test. You have compared the DNA banding patterns of the four suspect samples to that of the DNA found at the scene of the crime. Based on the results of your experiment, can you place any of the four suspects at the scene of the crime?

The D.A. wants to know how sure you are about your conclusion and has inquired about other tests that can be used as confirmation. Explain the limitations of your analysis and suggest another test or piece of information that would accurately confirm your conclusion.