# Who took the Madagascar hissing cockroaches????

## Scenario

The Newton Campus biology laboratory manager arrived at work on Tuesday morning to find the non-majors/microbiology laboratory in disarray. Chairs had been tipped over, glassware broken and all of the Madagascar hissing cockroaches were missing!! The perpetrator could not remove the aquarium lid and had apparently in their frustration punched through the rusting mesh lid. Dark brown stains, possibly blood droplets were present on the broken wire mesh fragments extending into the gaping hole leading into the now empty aquarium. Without touching anything, the lab manager left the room, went to her office, and called campus police.

When the police arrived, they ascertained that all of the doors leading into the laboratory space had been locked all night. According to the door swipe, only 3 employees had accessed the prep room space. Cameras located in the hallway confirmed the 3 employees did indeed enter the lab spaces at the times indicated by the swipe access recorder. No one else was seen entering the lab spaces until the lab manager entered the next morning. Therefore, one of the 3 employees was presumably the perpetrator. All 3 employees denied involvement. Two of the employees claimed to have limited their activities to either the prep room or the anatomy lab. One employee did state that they entered the lab in question, but only to check the media in the refrigerator. The lab had not been vandalized at that time according to this employee.

Police removed samples from the wire mesh and forwarded the samples to GBI (Georgia Bureau of Investigation) for DNA fingerprinting. You will be processing the sub-samples of those taken by GSU police today to determine which employee, if any is responsible for liberating the cockroaches.

## Background

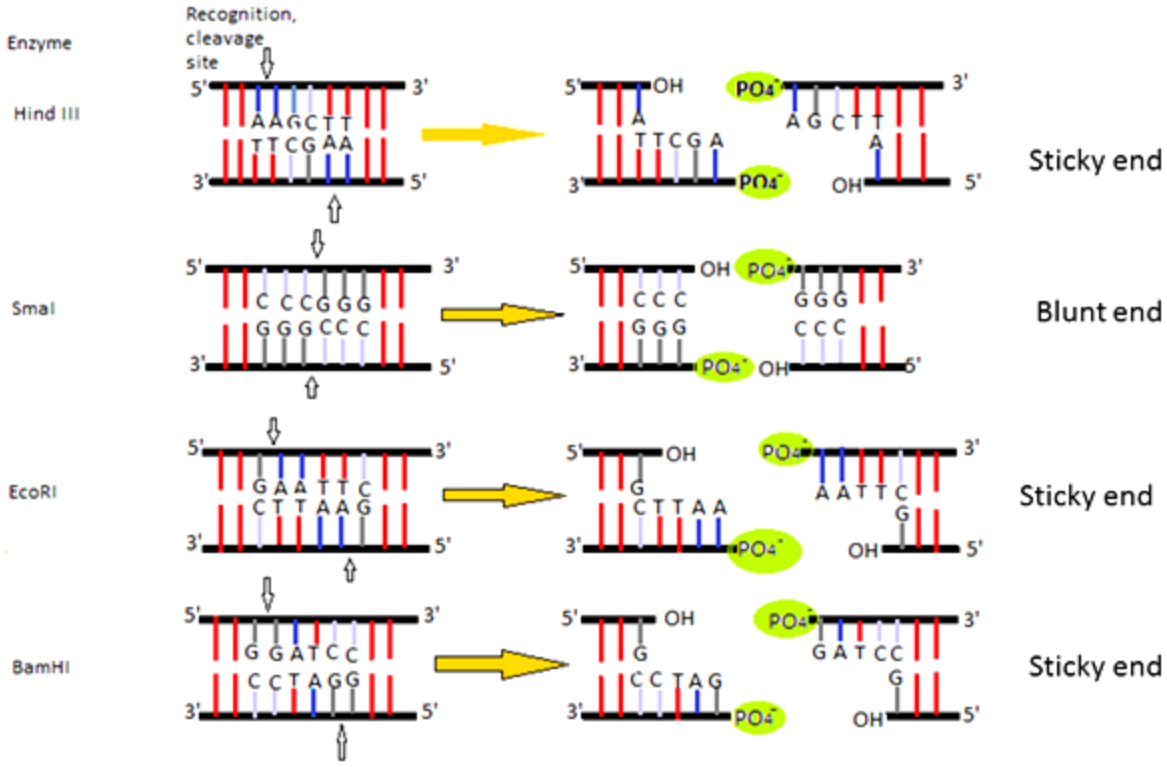
DNA fingerprinting is a widely used laboratory technique in which samples taken from a crime scene are compared to a database or suspect sample. This technique is also used for other purposes such as paternity testing. The test is based on the properties of DNA and the use of special enzymes called endonucleases or restriction enzymes (restriction endonucleases).

## A little about DNA and restriction enzymes

All of your nucleated cells contain DNA. Greater than 99% of the DNA in our cells is identical to everyone else's DNA. The small proportion of bases that is uniquely you is still pretty substantial, about 3 million base pairs. So all the variation we see between individuals can be attributed to these 3 million base pairs.

DNA is a double stranded helical molecule. DNA is a polymer made of nucleotides. Each nucleotide is composed of a sugar (ribose), phosphate, and a nitrogen-containing base (adenine, thymine, cytosine, and guanine). Ribose and phosphate make up the backbone of the molecule. If you think of DNA as a ladder, the sugar and phosphate are the rails of the ladder and the nitrogen-containing bases are the rungs of the ladder. The phosphate groups in the DNA 'rungs' when exposed after digestion give DNA fragments a negative charge.

Restriction enzymes are enzymes that cut DNA. They are also called endonucleases or restriction endonucleases because they cut within (endo) the DNA molecule. Some endonucleases are non-specific, meaning they just make random cuts in the DNA molecules…. they just chop DNA up into smaller and smaller pieces. Other restriction endonucleases are very specific. They will only cut DNA at specific nucleotide sequences. The table below depicts 4 commonly used restriction endonucleases and their restriction sites. HindIII recognizes this sequence, AAGCTT and then cuts between the A and A. EcoR1 recognizes the sequence, GAATTC and cuts between the G and A on each strand. When the strands of DNA separate the negatively charged phosphate groups are exposed and the bases produce what are called a 'sticky ends.' Sticky ends are important in biotechnology and genetic engineering but will not be discussed here. Restriction endonucleases are named for the organisms from which they were isolated. For example, EcoR1, is the first restriction enzyme (R1) isolated from E. coli (Eco).




Each enzyme will cleave a sample of DNA in a specific pattern. Let's pretend the following sentence, " The big red dog ate the small yellow ball." is a stretch of DNA. Enzyme 1 will cut the sentence after every letter 'e'. Enzyme 2 will cut the sentence after every letter o. Enzyme 3 will cut only between ll. What is the result?

Cut with enzyme 1: The big re d dog ate the small ye llow ball.
Cut with enzyme 2: The big red do g ate the small yello w ball.
Cut with enzyme 3: The big red dog ate the smal l yel low bal l.

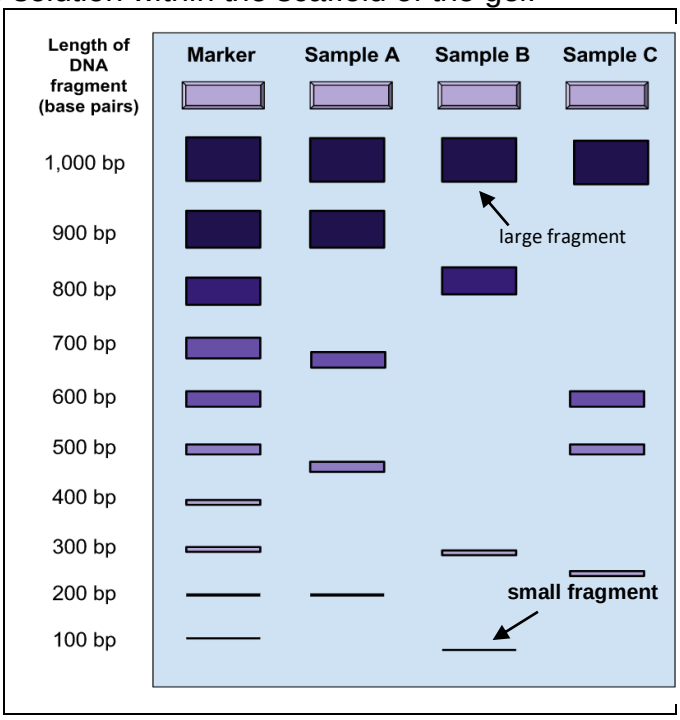
Enzyme 1 cut the sample into 4 pieces. Enzyme 2 cut the sample into 3 pieces and Enzyme 3 cut the sample into 4 pieces but the pieces are very different in size than those produced by enzyme 1. Now that this sentence 'DNA' has been cut into pieces we need a way to separate the pieces, to visualize the DNA. Agarose electrophoresis and

DNA staining allow us to separate the pieces and visualize the pieces. The unique pattern of fragments created by treating the same sample with several different enzymes and then performing the gel electrophoresis is the DNA fingerprint.

## Gel electrophoresis

Gel electrophoresis separates macromolecules within an electrical field based primarily on size. Remember the DNA fragments have a negative charge and will migrate in response to an electrical field. DNA fragments are pretty large and the medium of choice used to separate the fragments is called agarose. Agarose is a purified polysaccharide extracted from seaweed. Agarose gel is made by suspending the dry powder in a buffered solution and boiling the solution. The solution is then tempered to about 55 degrees. When it is still hot/warm, but still liquid it is poured into the casting tray and the comb is added to the end of the tray. The agarose is allowed to cool with the comb in place. When cooled the agarose will be slightly opaque. Agarose is a loose gel. The agarose molecules crosslink to form a molecular scaffold that form the slab of gel. Molecules can travel through the solution within the scaffold of the gel.

The diagram to the right show an example of gel that has been run and stained. Samples were loaded into the wells located at the top of the image. The gel was then run for an appropriate amount of time. The gel was then removed and stained.
(Gel image from creative commons, inserted comments from the author)




To run the gel, the casting baffles are removed and the casting tray is placed in the electrophoresis apparatus with the comb end near/toward the negative electrode (black). Buffer is added to the apparatus and the comb is removed. Wells can now be found at the former location of the comb teeth. The samples are loaded into the wells. The electrodes are attached and the power turned on. Since DNA fragments are negatively charged they move out of the sample well away from the negative electrode toward the positive electrode (red). The smaller the fragment the faster and farther the fragment will move. DNA by itself is uncolored. The DNA must be stained to be visualized.

**Purpose**: To identify who took the Madagascar hissing cockroaches by performing a DNA fingerprint of samples obtained from 3 suspects and the brown spots found on the hissing cockroach enclosure.

**Materials**:

Agarose
TBE Buffer (Tris-borate-EDTA)
6X Sample Loading Buffer
DNA ladder standard
Electrophoresis chamber
Power supply
Gel casting tray and combs
Sybr Gold DNA stain
Staining tray
Gloves
Pipettes and tips
Perp sample
Employee 1
Employee 2
Employee 3

## Procedure

*Preparing the agarose gel*

1. Temper the agarose. It should be about 50-55°C, swirling the flask occasionally to cool evenly.
2. Put on gloves. Minimize handling the casting tray, comb and other materials with your bare hands.
3. Seal the ends of the casting tray with two layers of tape or insert the baffles. Which procedure you use will depend on the model of apparatus you have.
4. Place the comb in the end notch of the gel casting tray. There is a middle notch and an end notch.
5. Slowly pour the melted agarose solution into the casting tray. Cool the agarose until it gels or sets. The gel will appear slightly opaque when cooled and ready to use. It will take 10 minutes or so for the gel to cool.
6. Remove the tape covering the end of the tray or remove or the lower the baffles.
7. Place the gel in the electrophoresis chamber.
8. Add TBE Buffer to the side chambers. Overfill the chamber so that buffer flows over the gel and covers the gel to a depth of 2-3 mm.
9. Carefully pull out the comb by gently wiggling the comb up and down.

*Using micropipettes*

Micropipettes are designed to deliver small volumes of solutions with a high degree of accuracy. The micropipette can deliver a fixed volume or more commonly can be adjusted within a range of values.

*Preparing the samples*

The Employee samples have been pre-digested with a restriction endonuclease.

1. Obtain a microfuge tube containing a sample of digested DNA from Employee 1, Employee 2, Employee 3, Perp, and a sample of the DNA ladder standard.
2. Add 2 µL of loading dye to each microfuge tube. Re-cap the tubes and tap firmly on the benchtop to mix the dye with the sample. Loading dye serves two purposes. It contains glycerol, which makes the samples dense so when dispensed, the sample will sink into the well rather than float into the buffer. The loading dye also contains bromophenol blue which is a small molecule which moves quickly through the gel, typically in front of most of the DNA fragments. It is the marker for front of the electrophoretic line.
3. Change pipet tips.
4. Add 2 µL of Sybr Gold to each microfuge tube. Re-cap the tubes and tap firmly on the benchtop to mix the dye with the sample. Sybr Gold is a fluorescent DNA stain.

*Loading the gel*

1. Use a micropipette with a fresh tip to transfer 20 µL of the DNA ladder standard to the second well.
2. Use a micropipette with a fresh tip to transfer 20 µL of the Employee 1 sample to well 3.
3. Use a micropipette with a fresh tip to transfer 20 µL of the Employee 2 sample to well 4.
4. Use a micropipette with a fresh tip to transfer 20 µL of the Employee 1 sample to well 5.
5. Use a micropipette with a fresh tip to transfer 20 µL of the Perp sample to well 6.

*Running the gel*

1. Place the lid on the gel box.
2. Connect the electrode wires to the power supply, making sure the positive (red) and negative (black) are correctly connected. (Remember – "Run to Red")
3. Turn on the power supply to about 100 volts. Maximum allowed voltage will vary depending on the size of the electrophoresis chamber – **it should not exceed 5 volts/ cm between electrodes!**
4. Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode.
5. Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye – this will take a couple of minutes (it will run in the same direction as the DNA).
6. Let the power run until the blue dye approaches the end of the gel.
7. Turn off the power.
8. Disconnect the wires from the power supply.
9. Remove the lid of the electrophoresis chamber.
10. Using gloves, carefully remove the tray and gel.

*Viewing the gel*

1. Using gloves, remove the gel from the casting tray and place into the staining dish.
2. View the gel in the transilluminator or on the UV box.
3. Record the data while the gel is fresh, very light bands may be difficult to see with time.

Who was the guilty party?

How many fragments were generated in each sample?

Which sample had the largest fragment?

Which sample had the smallest fragment?