The Nail File Crime
Aka The Rockina Lab

Purpose:
This activity will simulate the use of restriction enzymes and gel electrophoresis in making DNA fingerprints. Some steps have been eliminated to simplify the procedure. However, this lab will allow the positive identification of the guilty suspect.

Objectives:
1. Students will learn how restriction endonucleases cut DNA at specific base sequences and produce DNA fragments of specific and varying lengths.
2. The students will learn how electrophoresis separates different sized fragments of DNA and how these fragments can form a specific, recognizable pattern.
3. The students will learn the principles behind the use of DNA fingerprinting for the identification of people.

Materials:
3 different dark color pens or colored pencils (red, blue, and green)

The Crime:
Late one night, the famous rock star, Rockina, returned to her luxurious penthouse apartment. As she entered the locked apartment, she noticed that everything in her apartment was a mess - drawers had been emptied out onto the floor; the cushions on the couch were ripped open; and the safe that has been hidden behind an oil painting had been opened. She then noticed that the lights were on in her bedroom.

She stormed into the bedroom and surprised a burglar in the process of removing her magnificent (and expensive) jewelry from its hiding place between the mattresses. Indignant, she jumped on the burglar and tried to stab the person with her nail file. While Rockina managed to inflict a small wound, she was no match for the assailant's knife. In the subsequent struggle, Rockina was killed and the murderer escaped with her jewelry.

The Investigation
When the housekeeper, Casperina, entered Rockina’s apartment the next day, she saw the body and immediately called the police. When the police noted there had been no signs of a forced entry, the investigation narrowed down to people who knew Rockina and who had a key to enable them to enter the apartment. The suspects were:

1. Casparina, the housekeeper, who had just had a bitter argument with Rockina over a refused raise in salary.
2. Lucifer, her former boyfriend, whom she had just jilted for another man.
3. Pinky, the leader of Rockina’s weight lifting class, who was her new boyfriend. It was rumored that Pinky was insanely jealous of Rockina’s fame.

When it was established that all three of the suspects had a key to Rockina’s apartment, all had a motive for killing her, and all had no ironclad alibi for the evening that she was killed, the police realized that they had a problem. They consequently decided to hire a world-class forensic company to prove which of the three suspects was guilty of Rockina’s murder.

**The Procedure:**

1. **Use of Restriction Enzymes**

DNA can be isolated from any cells containing chromosomes. This includes skin cells, white blood cells and sperm cells. (Note: Red blood cells lose their nucleus when released into bloodstream. Their life span is 60 days.) This DNA can be isolated from very tiny samples. To analyze the DNA from various samples, scientists make use of enzymes called restriction endonucleases, or more commonly called “restriction enzymes.” Different versions of these enzymes have been isolated from many sources, primarily bacteria. Bacteria use these enzymes to destroy invaded DNA from sources such as viruses. When these enzymes recognize a certain area of a DNA molecule (a specific sequences of bases), they cut the DNA at this point. The cut is uneven, leaving jagged or extra unpaired bases at the ends. This type of cut has been taken advantage in genetic engineering but that’s another story.

For example, the first restriction enzyme isolated from the R strain of *Escherichia coli*, known as Eco RI, cuts DNA at the following sequence:

\[
\text{G} | \text{AATT C} \\
\text{C TTAA} | \text{G}
\]
Mark a similar line as appearing above in the following sequence of DNA each time the 6-base sequence occurs.

TTACGTAGAATCCCTAGAGATGAATTCCCTTTAAA  
AATGCATCTTAAGGGAATCTCTACTTAAGGGAATTT

1. How many sections of DNA result when Eco RI is applied to this particular DNA sequence? ________
2. Determine the length of the sections of the DNA by counting the number of bases in the top line and record those answers (reading left to right):

If you have done this exercise correctly, you should have fragment sizes of 10, 16 and 12. If you can't determine how these sizes were attained, ask for help from your teacher before continuing.

Each restriction enzyme only cuts at one particular sequence of bases. The sequence varies for each restriction enzyme. There are more than 100 restriction enzymes identified and more being found every week.

In real-life, scientists use restriction enzymes on DNA without knowing the sequence of the DNA and obtain restricted (cut up) DNA that can be separated by size by electrophoresis. For this exercise, you will be given sequences of DNA from the different suspects. You will need to identify the restriction sites with the sequences and determine the length of the resulting fragments. In the early days of this technology, there were only a few restriction enzymes identified. To extend the use of these enzymes, the use of two enzymes simultaneously was used. You will use this trick in this simulation.

The two enzymes you will use are the first two common enzymes used:

Recognition sequences:

**Eco RI:**

G|AATT C  
C TTAA|G

**Hin dIII:** (From *Hemophilus influenza* strain D)

A|AGCT T  
T TCGA|A


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Use a blue pen or colored pencil to determine the cutting points and mark the size of the resulting fragments for the DNA sequences of the three suspects when Eco RI is used. Use a red pen or colored pencil to determine the cutting points and mark the size of the resulting fragments for the DNA sequences of the three suspects when Hin dIII is used. Finally, use a green pen or colored pencil to determine the size of the resulting fragments if Eco RI and Hin dIII were used simultaneously.

Section of DNA from each suspect:

Note: In real life the sequences are much longer and the distance between restriction sites can be hundreds of bases long. This is a simplified simulation.

2. Use of Gel Electrophoresis:

After the restriction enzymes have cut the DNA, the fragments in each sample are then separated by size using a technique called "gel electrophoresis." In this sample, a block of agarose (a derivative of agar and similar in appearance and consistency to unflavored gelatin) is created in a chamber. A horizontal line of wells, which are holes that partially penetrates the gel, are formed in the gel at the time the hot, liquid gel is poured into the chamber. Once the gel has set, the chamber is flooded with a particular salty solution that allows an electric current to pass through the gel. This current causes the naturally negatively charged DNA fragments to be attracted to the positive electric wire at the "bottom" of the chamber. (The negative wire is at the top - thus creating the electric field in the chamber.)

Imagine that the whole class has been taken to the cafeteria, which is set up with the tables, as it would be for lunch. Students have been placed in lines with different number of students in each line at the east entrance. The students within a line are expected to old hands as they would to play crack the whip. Their object is to get to the west entrance as fast as possible, finding the shortest path through the tables. However, when the teacher blows her whistle, the students must stop where they are.

3. Which set of students would you expect to be closer to the west entrance, a line of two students, a line of 4 students or a line of 8 students? _________________

4. Why? ____________________________________________________________________________
____________________________________________________________________________________
Casperina's DNA

TTGAGTA
TTCAAGCTTCCGATGGAAATTCGAGAATTCAAGCTTATAGAATTCGCGCTAAGCTTCCC
GAATTCGTTCCATA

AACTCATAAGTTCGAAGGCTACCTTAAGCTCTT AAGTTCA

Lucifer's DNA

TTGAGAATTCAAGCTTCCGATGGAAATTCGAAGCTTGATCGTTATAGAATTCGCGCTAAGCTTCCC
GAATTCGTTCCATA

AACTCTTAAGTTCGAAGGCTACCTTAAGCTTCGAACTAGCAATATCTT AAGCGCGATTCGAA
GGGCCTTAAGCAAGGTAT

Pinky's DNA

TTGAGAATTCAAGCTTCCGATGGAAATTCGAGAATTCAAGCTTATAGAATTCGCGCTAAGCTTCCC
GAATTCGTTCCATA

AACTCATAAGTTCGAAGGCTACCTTAAGCTCTT AAGTTCA

Casperina's DNA

TTGAGAATTCAAGCTTCCGATGGAAATTCGAGAATTCAAGCTTATAGAATTCGCGCTAAGCTTCCC
GAATTCGTTCCATA

AACTCATAAGTTCGAAGGCTACCTTAAGCTCTT AAGTTCA
The same thing happens in the agarose gel. Longer DNA fragments take more time to
threat around the agarose molecules than the shorter fragments. Thus, when the electric
field is removed from the gel at the right time, the DNA fragments appear in the gel
sorted by size. The smallest DNA fragment is near the bottom. The largest DNA
fragment is closest to the well. While in real life, the fragments sort in a log rhythmic
pattern, you will assume a linear pattern.

Using the information of fragment sizes from part I, mark the banding pattern for each
gel. You will note that marker DNA and the nail file DNA has already been marked for you
on each of the three gels. Marker DNA is DNA from a known source, usually viral for
which the DNA fragment sizes are already known. For this simulation, your marker DNA
happens to have fragments that are 5, 10, 15, 20, 25 and 30 bases long. Write the size of
the marker DNA fragments to the left (outside the gel border) of the marker DNA bands.
Use these bands to mark your fragment bands from each of the suspects below their respective wells.

Compare the fragments of the suspects to the nail file DNA. Which suspect(s) have the same pattern? Someone who has the exact same pattern in every gel is probably the guilty person.

4. Who is guilty based on the Eco RI gel? ____________________________________________________________________

5. Who is guilty based on the Hin dIII gel? ____________________________________________________________________

6. Who is guilty based on using both restriction enzymes? __________________

   ____________________________________________________________________
   ____________________________________________________________________

Summary Questions:

1. Why would scientists use more than just one enzyme to analyze the DNA samples before going to court to testify?

2. Suppose a lab technician did a poor job of making the agarose gels and didn’t completely dissolve the agarose before pouring the gels. As a consequence, areas of the gel are denser than other areas. What would the results of the gel look like? Would you use these results in court? Explain.

3. Suppose the restriction enzymes had been stored improperly and were damage as a result. How would you recognize this event based on the gel? Explain.
4. The method of using restriction enzymes and gel electrophoresis is used for more than forensic science (science used as evidence in the court room.) For instance, it is the basis of one of the procedures for identifying genetic diseases in fetuses and for identifying people who are heterozygous (carriers) for genetic diseases. Suppose that the following DNA is part of a normal gene for a certain trait.

```
CGGAATTCTGAAATCCGGCATGAATTCCGGGAGAATTCGC
GCCTTAAGCAATTAGGCCGTACTTAAGGCCCTTTAAGCG
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If Eco RI cut this sequence, how many fragments would be produced? ________

5. Explain how the appearance of the gel could be used to diagnose a genetic disease if this mutation caused that particular genetic disease.

6. A heterozygous person who is normal but has a recessive allele for a genetic disease is called carrier. A carrier can pass the trait on to his or her offspring. Explain how you would use gel electrophoresis data to diagnose a carrier.

7. The bands produced on a gel are frequently designated a DNA fingerprint. How similar must a DNA fingerprint be to be used as identification?

8. What are the 3 most important concepts learned from this lab?

   A. __________________________________________________________
      __________________________________________________________

   B. __________________________________________________________
      __________________________________________________________

   C. __________________________________________________________
      __________________________________________________________