The Scenario

“OMG! Is that blood?” Laurel nearly broke Marcus’s arm as she tried to push past him into the classroom.

Marcus grabbed the sleeve of her cardigan and yanked her back. “Don’t! Can’t you see the glass?” Laurel tried knocking his hand free, but the 6’4” varsity basketball captain held tight. He made her settle for looking from under his armpit.

Not that what she saw would make any sense. Their AP Biology lab looked like a riot scene. Four chairs and a potted plant were overturned in the center of the room, and broken pieces of glass were scattered across the floor along with several wet red drops. Plink ... plink ... plink. Marcus’s eyes were drawn to the teacher’s desk where droplets of brownish liquid fell from a paper cup and collected in a puddle on the linoleum.

“What happened?” Laurel asked. “Did somebody get hurt?” Laurel and her classmates had gathered in front of the door and strained to see inside Room 102.

Marcus inspected the scene and raised his right arm above his head, his fingers spread apart as if taking a shot from the free throw line. “Stay back!”

“Where’s Ms. Mason?” Laurel said. “She told me I could meet her before class to review for the quiz.”

“Okay, folks, keep it down.” Mr. Gladson, the teacher in the classroom next door, came into the hall. His white lab coat was streaked with several rust-colored stains. The pungent odor of formaldehyde permeated the corridor. “In case you haven’t noticed, the bell has rung.” He wiped his nose with a tissue and then tossed it into a nearby trash can. A girl’s fake shriek from inside the anatomy lab rose above the buzz of Marcus’s classmates.

“What’s going on?” Bobby’s high-pitched whine was unmistakable—and so was the scent of his bubble gum.

“I think something might’ve happened to Ms. Mason,” Marcus said. He dug around in his backpack and pulled out a magnifying glass. “We’ve got a crime scene to process.”

“Go figure,” Laurel said. “Sherlock Holmes in a varsity jacket.”

For the next hour, Marcus and Laurel searched the classroom and discovered several pieces of “evidence” that Marcus described in his biology notebook:

- Ten small drops on floor confirmed by Kastle-Meyer test to be blood
- Shard of glass from a broken 500-mL Erlenmeyer flask, edge smeared with a reddish stain
- Paper cup with lipstick stains, presumed to be Ms. Mason’s, found on her desk
- Wad of bubble gum stuck underneath overturned chair
- Mr. Gladson’s discarded tissue recovered from trash can in hall outside Room 102
- Bobby’s test on photosynthesis with large “F” scrawled in red ink on first page
- Copy of email from Mr. Gladson to Ms. Mason asking her to give up position as department chair

Marcus’s new game was afoot!

Safety Precautions:

Never handle gels with your bare hands. An electrophoresis apparatus can be dangerous because it is filled with a highly conductive salt solution and uses DC current at a voltage strong enough to cause a small shock. Always turn the power supply switch “OFF” and wait 10 seconds before making any connection. Connect BOTH supply leads to the power supply (black to black and red to red, just like when you jump-start a car battery) BEFORE turning on the power supply. Your teacher will tell you for how long and at how many volts (usually 50 or 135 volts) you should run your gel. After use, turn off the power supply, and then disconnect BOTH leads from the power supply. Remember, power supply on last ... and off first.
Materials

Your Workstation
- 20 uL vials of DNA fragments prepared used restriction enzymes (3-4 Different vials)
- 3-4 plastic bulb transfer pipettes (or 1 micropipette with sterile tips)
- permanent marker
- gel electrophoresis chamber
- power supply
- plastic staining tray
- 0.8% agarose gel in gel tray prepared for by teacher

Common Workstation
- 1x TAE (tris-acetate-EDTA) buffer
- Methylene blue stain

Procedure

Casting the Gel (this step may have already been completed by your teacher)
1. Seal the ends of the gel-casting tray with the blue rubber dams provided. Insert the well-forming comb along the negative line (black). Place the gel-casting tray on the side so the agarose poured in the next step can set undisturbed.
2. Carefully pour the liquid gel into the casting tray to a depth of 5-6mm. The gel should cover only about one-half the height of the comb teeth. While the gel is still liquid, use the tip of a pipette to remove any bubbles.
3. The gel will become cloudy as it solidifies (15–20 minutes). Do not disturb or touch the gel while it is solidifying!

Loading the Gel
Make sure you record the order in which you load the samples. Be sure to use a fresh loading device (either plastic micropipette or other type of pipette) for each sample. Be sure you know how to use the pipette properly. When in doubt, ask your teacher. Take care not to puncture the bottom of the well with the pipette tip when you load your samples.

1. Obtain a pre-made gel tray and place it in the electrophoresis gel box so that the comb is at the negative (black) end. Since you are sharing chambers, place only one gel tray for now.
2. Obtain approx. 550 ml of TAE Buffer. Carefully fill the box with 1x TAE buffer, to a level that just covers the entire surface of the gel.
3. Gently remove the comb by lifting the comb straight up, taking care not to rip the wells. Make sure that the sample wells left by the comb are completely submerged in the buffer.
4. The gel is now ready to be loaded with your DNA samples.
5. Obtain micropipette and sterile tip. Fix measurement amount on top of micropipette to 15μL.
6. Load 15μL of each sample of DNA into a separate well in the gel. You can load consecutive wells or leave empty wells in between.
7. Slowly draw up the contents of the first sample tube into the pipette.
8. Using two hands, steady the pipette over the well you are going to load.
9. Expel any air in the end of the pipette before loading the DNA sample.
10. Dip the pipette tip through the surface of the buffer, position it just inside the well, and slowly expel the mixture. Sucrose in the loading dye weighs down the sample, causing it to sink to the bottom of the well. Be careful not to puncture the bottom of the well with the pipette tip or reaspirate your sample up into the pipette.
11. Draw the pipette tip out of the buffer.
12. Remove and trash used tip. Using a clean loading device for each sample, load the remaining samples into their wells.
13. Repeat steps 1-12 for the second lab group at your table.
Electrophoresis

CAUTION: Be sure to keep the power OFF until you connect all leads!

1. Close the top of the electrophoresis chamber and then connect the electrical leads to an appropriate power supply, positive (+) electrode to positive (+) electrode (red to red) and negative (-) electrode to negative (-) electrode (black to black). Make sure both electrodes are connected to the same channel of the power supply, just as you would connect leads to jump-start a car battery — red to red and black to black.
2. Turn on the power supply and set the voltage as directed by your teacher. (It is recommended that you “run the gel” at 135 volts for approximately 45 minutes.
3. Shortly after the current is applied, you should see loading dye moving through the gel toward the positive pole of the electrophoresis apparatus. (Note: The purplish-blue band in the loading dye migrates through the gel at the same rate as a DNA fragment approximately 300 base pairs long.)
4. Allow the DNA to electrophorese until the loading dye band is about 1 cm from the end of the gel. Your teacher may monitor the progress of the electrophoresis in your absence if you have to attend another class.
5. Label the plastic staining tray with your class period and lab group and place it on the side for later.
6. While you wait for the electrophoresis, sketch a scale-sized drawing of your gel and answer analysis questions.

CAUTION: When it is finished, be sure to turn the power OFF first!

7. Turn off the power supply, disconnect the leads from the power supply, and remove the lid of the electrophoresis chamber.
8. With gloved hands, carefully remove the casting tray and slide the gel into a staining tray labeled with the name of your group.
   - Measure in centimeters the distance that the purplish-blue loading dye has migrated into the gel. Measure from the front edge of the well to the front edge of the dye band (also called the dye front).
   - Be sure to record your data (in centimeters).
9. Remove the buffer solution and place it back into the “used buffer” container on the materials table.

Staining with Final Stain

Methylene blue stain is added to the gel in order to better visualize the bands (fragments) of DNA as they migrate through the agarose gel during electrophoresis. However, you will still have to do a final stain of the gel.

1. Use the final stain “as is.” Do not dilute it. Following electrophoresis, place the gel in the staining tray, and cover it with just enough stain to submerge the gel. Leave them in for 20 minutes.
2. If you allow the gel to stain for more than an hour, the gel will be difficult to destain.
3. Pour the stain back into the bottle for reuse; stain can be used six to eight times.
4. Destain the gels by covering them with distilled or deionized water. Do not use tap water because the chlorine in tap water will cause the DNA bands to fade. We will destain it for a total of 30 minutes. It is helpful to set a timer for 15 minutes. Change the water two times over the course of 30 minutes. Bands that are not immediately present will become more visible with time. Maximum visibility is reached after five or more hours of destaining. Gels may be left overnight in a small volume (just enough to cover the gels) of distilled or deionized water.
5. Place gel in tray, cover with a small amount of distilled water and then place in a plastic bag. Place Plastic bag in refrigerator. Gels left overnight in a large volume of water may destain too much.
Analysis Questions:
The DNA samples collected from the crime scene have been digested with restriction enzymes to generate smaller pieces of DNA, which will then be used to create DNA profiles of suspects. Restriction enzymes are essential tools for analyzing DNA structure, and more than 200 enzymes are now available commercially. Each restriction enzyme is named for the bacterium in which it was first identified; for example, EcoRI was the first enzyme purified from *Escherichia coli*, and HindIII was the third enzyme isolated from *Haemophilus influenzae*. Scientists have hypothesized that bacteria use these enzymes during DNA repair and as a defense against their infection by bacteriophages. Molecular biologists use restriction enzymes to manipulate and analyze DNA sequences (Johnson 2009).

How do restriction enzymes work? These enzymes digest DNA by cutting the molecule at specific locations called restriction sites. Many restriction enzymes recognize a 4- to 10-nucleotide base pair (bp) palindrome, a sequence of DNA nucleotides that reads the same from either direction. Some restriction enzymes cut (or “cleave”) DNA strands exactly in the center of the restriction site (or “cleavage site”), creating blunt ends, whereas others cut the backbone in two places, so that the pieces have single-stranded overhanging or “sticky” ends of unpaired nucleotides.

You have a piece of DNA with the following template strand:

5’-AAAGTCGCTGGAATTCACTGCATCGAATTCCCGGGGCTATATATGGAATTCGA-3’

1. Write down the DNA template strand above. What is the sequence of the complementary DNA strand? Draw it directly below the template strand.

2. Assume you cut this fragment with the restriction enzyme EcoRI. The restriction site for EcoRI is 5’-GAATTC-3’, and the enzyme makes a staggered (“sticky end”) cut between G and A on both strands of the DNA molecule. Based on this information, draw an illustration showing how the DNA fragment is cut by EcoRI and the resulting products.

Two pieces of DNA that are cut with the same restriction enzyme, creating either sticky ends or blunt ends, can be “pasted” together using DNA ligase by reconnecting bonds, even if the segments originated from different organisms. An example of combining two “sticky end” sequences from different sources is shown in Figure 1. The ability of enzymes to “cut and paste” DNA fragments from different sources to make recombinant DNA molecules is the basis of biotechnology.
One application of restriction enzymes is restriction mapping. Restriction mapping is the process of cutting DNA at specific sequences with restriction enzymes, separating the fragments from each other by a process called gel electrophoresis (without pasting any fragments together), and then estimating the size of those fragments. The size and number of DNA fragments provide information about the structure of the original pieces of DNA from which they were cut. Restriction mapping enables scientists to create a genetic signature or DNA "fingerprint" that is unique to each organism. The unique fragments, called restriction fragment length polymorphisms (RFLPs), can, for instance, be used to confirm that a mutation is present in one fragment of DNA but not in another, to determine the size of an unknown DNA fragment that was inserted into a plasmid, to compare the genomes of different species and determine evolutionary relationships, and to compare DNA samples from different individuals within a population. This latter application is widely used in crime scene investigations.

Consider your classmates. More than 99% of your DNA is the same as their DNA. The small difference is attributed to differences in your genetic makeup, with each person having a genetic profile or "fingerprint" as unique as the ridges, arches, loops, and grooves at the ends of his or her fingers.

3. Based on this information, can you make a prediction about the products of DNA from different sources cut with the same restriction enzymes? Will the RFLP patterns produced by gel electrophoresis produced by DNA mapping be the same or different if you use just one restriction enzyme? Do you have to use many restriction enzymes to find differences between individuals? Justify your prediction.

4. Can you make a prediction about the RFLP patterns of identical twins cut with the same restriction enzymes? How about the RFLP patterns of fraternal twins or triplets?
Calculating the Sizes of Restriction Fragment Length Polymorphisms

Mathematical formulas have been developed for describing the relationship between the molecular weight of a DNA fragment and its mobility (i.e., how far it migrates in the gel). In general, DNA fragments, like the ones in your evidence samples, migrate at rates inversely proportional to the \( \log_{10} \) of their molecular weights. For simplicity’s sake, base pair length (bp) is substituted for molecular weight when determining the size of DNA fragments. Thus, the size in base pair length of a DNA fragment can be calculated using the distance the fragment travels through the gel. To calculate the base pair length, a DNA standard, composed of DNA fragments of known base pair length, is run on the same gel as the unknown fragments and is then used to create a standard curve. The standard curve, in this case a straight line, is created by graphing the distance each fragment traveled through the gel versus the \( \log_{10} \) of its base pair length.

Creating the Standard Curve

As explained above, base pair (bp) length is substituted for molecular weight. Note that in plotting the standard curve, calculating the \( \log_{10} \) of the base pair length of each fragment is unnecessary because the base pair size is plotted on the logarithmic axis of semi-log paper. Examine your stained gel on a light box or other surface that helps visualize the bands.

- What observations can you make?
- What quantitative measurements can you make?

1. Examine the “ideal” or mock gel shown in Figure 5 that includes DNA samples that have been cut with three restriction enzymes, \textit{BamHI}, \textit{EcoRI}, and \textit{HindIII}, to produce RFLPs (fragments). Sample D is DNA that has not been cut with enzyme(s). DNA cut with \textit{HindIII} provides a set of fragments of known size and serves as a standard for comparison.
2. Using the ideal gel shown in Figure 5, measure the distance (in cm) that each fragment migrated from the origin (the well). **(Hint: For consistency, measure from the front end of each well to the front edge of each band, i.e., the edge farthest from the well.**). Enter the measured distances into Table 1. (See * and ** notes below the table for an explanation for why there are only six bands seen but more fragments.)

<table>
<thead>
<tr>
<th><strong>Table 1. DNA Fragment Migration Distance</strong></th>
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<tbody>
<tr>
<td><strong>HINDIII</strong></td>
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<tr>
<td><strong>Distance Traveled</strong></td>
</tr>
<tr>
<td>*27,491</td>
</tr>
<tr>
<td>*23,130</td>
</tr>
<tr>
<td>9,416</td>
</tr>
<tr>
<td>6,557</td>
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<tr>
<td>4,361</td>
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<td>2,322</td>
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<tr>
<td><strong>564</strong></td>
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<tr>
<td><strong>125</strong></td>
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*For this “ideal” gel, assume that these two bands appear as a single band instead of resolving into separate bands.

** These bands do not appear on the ideal gel and likely will not be seen.
3. Plot the standard curve using the data from the DNA sample cut with *HindIII*. To do this, your teacher might ask you to graph the data directly using Excel with distance traveled as the (arithmetic) x-axis and the base pair (bp) length as the (logarithmic) y-axis. Based on this graph, why must the data be plotted using the log scale? You might want to plot the data again using semi-log paper.

Connect the data point with a best-fit line. However, you should ignore the point plotted for the 27,491bp/23,130 doublet. When using 0.8% agarose gel, these fragments appear as one. Congratulations! Your best-fit line is the standard curve.

4. Now use the standard curve to calculate the approximate sizes of the *EcoRI* and *BamHI* fragments. Using a ruler, how can you use the standard curve to calculate the sizes of unknown fragments?

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**Evaluating Results**

1. What are some possible challenges you had in performing your investigation?

2. What are some possible sources of error in the electrophoresis procedure? How can you minimize any potential sources of error?
Thinking About Your Results

1. There are important social and ethical implications of DNA analysis. Already, DNA testing can reveal the presence of markers of certain genetic diseases, such as Huntington’s. So, who should have access to your genetic profile? Health insurance companies? College admissions offices? Employers? What issues about confidentiality are raised by genetic testing? Who owns your DNA and its information?

2. Suppose a DNA test that predicted your chances of getting a disease, such as cancer, were available. You take the test for cancer, and the results say you have a two in three chance of getting cancer sometime in the next 20 years. Who should have access to this information? Your doctor? Health insurance companies? Employers? Would you want to know this information?

3. The Innocence Project (IP) is an international litigation and public policy organization dedicated to exonerating wrongfully convicted individuals through DNA testing. Three-quarters of DNA exoneration cases involve misidentification by witnesses. To date, nearly 300 people previously convicted of serious crimes in the U.S. have been exonerated by DNA testing. However, not everyone is in favor of the IP. One United States Supreme Court justice expressed concern that DNA testing poses risks to the criminal justice system, in which a person is judged by a jury of peers. What social and ethical issues are raised by using DNA evidence to re-examine old court decisions? What other arguments can you make (or find) against using DNA evidence for court cases?

4. With genetic engineering, biotechnicians can clip out beneficial genes from native plants in foreign countries and insert them into their crop plant relatives here in the United States, with great benefits to the latter — to prevent attack by insects, to increase productivity, or to allow the crops to be grown in colder climates. These benefits can be worth billions of dollars, but if the crops are grown in the United States, should countries where the native plants are located benefit from the bioengineering? Who owns the information in DNA? Who can profit from that information? Investigate this controversy on the Internet with examples drawn from different crops grown here in the U.S.