DNA DETECTIVES OR "WHO DUNNIT?"

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Introduction: Many of the revolutionary changes that have occurred in biology over the past fifteen years can be attributed to the ability to manipulate DNA in defined ways. The principal tools for the recombinant DNA technology are enzymes that can "cut and paste" DNA. Restriction enzymes are the "chemical scissors" of the molecular biologist; these enzymes cut DNA at specific nucleotide sequences. A sample of someone's DNA, incubated with restriction enzymes, is reduced to millions of DNA fragments of varying sizes. A DNA sample from a different person would have a different nucleotide sequence and would thus be enzymatically "chopped up" into a very different collection of fragments. Because no two people (except identical twins) have exactly the same DNA, a person's DNA fingerprint is unique and can be used for the purposes of identification. We have been asked to apply DNA fingerprinting to determine which suspect should be charged with a crime perpetrated in our city.

WHO DUNNIT?

A murder has been committed, and police discover evidence of a struggle and blood traces at the scene of the crime. Ian, a UPS delivery man, is found dead in his truck on Rt. 221 just west of Forest Middle School. Autopsy has shown that Ian was strangled to death, but there is no blood from the victim on the scene. Packages are missing from the truck, and no witnesses can be found. Suspects X, Y, and Z are arrested and will go through DNA tests to determine if they were at the scene of the crime. ***What DNA sources other than blood might be found at the crime scene?*** All of the suspects proclaim their innocence adamantly, and all want to see their lawyers. At their indictments, it is learned that:

- Suspect X - Bob Smith is a man in his middle thirties with prior convictions for armed robbery. Bob was apprehended shortly after the murder in Bedford driving recklessly on an expired license. No contraband was found in his possession but his hands are cut in several places. He says it's because he works construction.
- Suspect Y - Jim Dale is a man in his late forties. He is suspected of being romantically involved with Ian's wife, Pam. Unexplained scratches were found on the back of his neck.
- Suspect Z - Pam, wife of Ian. She says she was with Jim the entire day. Several cuts on both hands are suspicious. She claims she got them while picking blackberries with Ian.

You are the lab worker who has been handed the DNA samples from the three suspects involved, plus the DNA from the blood at the crime scene. Using molecular biology techniques, your job is to determine which of the suspects might have been at the crime scene. The court awaits your findings.

Purpose: To prepare and analyze a DNA fingerprint, the student will:

1. Prepare and load an agarose gel with enzyme-cut DNA samples.
2. Conduct gel electrophoresis to sort out the DNA fragments in the samples.
3. Stain the gel to visualize the DNA fragments.
4. Analyze the resulting banding pattern or "DNA fingerprint" to solve a crime.

Materials:

- electrophoresis chamber
- casting tray
- comb
- agarose solution
- power supply
- plastic tray for storing gel
- DNA from suspects and crime scene
- micropipette and pipette tips
- electrophoresis buffer (1X SB)
- racks for 1.5 ml microcentrifuge tubes
- 1.5-ml microcentrifuge tubes
- staining solution
- gloves
- light box
- plastic wrap

Procedures:

1. Prepare the casting tray by inserting the metal buffer dams. Insert the comb into the tray.
2. Weigh the flask containing the agarose solution and heat the flask until the agarose is completely into solution. Cool flask briefly, then reweigh it.
3. Slowly add distilled water to the flask until the total mass equals the mass you started with. (This will replace any water last during the heating and prevent your gel from becoming too dense.)
4. After the gel solution has cooled to 50-60°C, carefully pour the agarose into the casting tray. DO NOT jar or move the casting tray as the gel solidifies. As the agarose sets or gels, it changes from clear to slightly opaque.
5. After the gel has set, carefully remove the comb and casting gates.
6. Pour enough buffer into the gel box so that the gel is completely covered, with no "dimpling" above the wells.
7. Load the entire contents (10 µl) of each sample tube into separate wells in the gel. Be sure that the micropipettor tip is below the surface of the buffer and just above the center of each well that you load. CHANGE PIPETTE TIPS BETWEEN SAMPLES TO AVOID CONTAMINATION! Leaving an empty lane on both sides, load in the order shown below:

X	Y	Z	E	L

X = Bob Smith, former thief
Y = Jim Dale, boyfriend
Z = Pam, wife of victim
E = evidence DNA found at crime scene
L = ladder DNA (standardized control sample)

8. Once the wells are loaded, put the top on the gel box and connect it to the power supply. Plug in the power supply and turn the unit to the desired voltage. Run until the loading dye nears of the bottom of the gel. At this point, the current can be turned off and the leads (wires) disconnected.

   The term "electrophoresis' literally means "to carry with electricity." It is a technique for separating and analyzing mixtures of charged molecules. When placed in an electric field, pieces of DNA (because they are ionized and negatively charged) migrate toward the positive electrode (anode); small pieces of DNA experience less resistance and move faster (farther) than larger pieces.

9. Remove the casting tray from the gel box. **Carefully** slide your gel off the casting tray and into its plastic container.
10. Wearing gloves, pour the methylene blue staining solution into the plastic container and allow it to sit for 15-20 minutes, rocking the container periodically.
11. Pour the stain carefully back into the beaker (do not throw away) and gently rinse your gel with water for 5 minutes. You may need to let your gel soak in several changes of water to increase contrast. **Do not** use large volumes of water. The water should just cover the gel. It is better to use small volumes of water and change it frequently than to flood the gel in a large volume!
12. Place a piece of plastic wrap on your light box and examine your stained, rinsed gel by placing it on the plastic wrap. Gently place a transparency over your gel and trace the bands with a permanent marker.
13. Store your gel in a labeled plastic bag in the refrigerator.

Upon completion of the lab

- dispose of designated materials in the appropriate places.
- leave equipment as you found it.
- check that your work station is in order.
- wash your hands.

Analysis:

Compare the fingerprints of all the suspects in this case to the DNA profile of the DNA isolated from the blood droplets at the crime scene. By comparing the banding patterns of the DNA samples you should be able to determine who the murderer was. Which suspect's blood was found at the site of the murder?

Unfortunately, most courts will not accept your preliminary results as being conclusive and will expect a more detailed analysis.

DNA is made up of a series of base pairs (guanine-cytosine, adenine-thymine, cytosine-guanine, thymine-adenine).

G - C
A - T
C - G
T - A

Every individual has a unique series of base pairs in their DNA.

The DNA samples that were used had been treated with a restriction enzyme which seeks out specific DNA base pair (bp) sequences and cuts the DNA at that point. Since the DNA samples were all different, they were all cut at different spots, which resulted in different size pieces of DNA for each sample.

A T C C T G C | C G G A A G T C C G A T C | C G G T A
T A G G A C G G C | C T T C A G G C T A G G C | C A T

After the DNA is cut, the restriction fragments are separated from each other by using electrophoresis. Electrophoresis will separate fragments based on their size and charge. The phosphate groups, which make up part of the backbone of the DNA, carry a negative charge, so they will move through the gel toward the positive pole (red). The smaller fragments will

move faster than the larger ones. After staining, the bands of DNA which represent different size fragments, can be analyzed. By calculating the size of the fragments from different samples, it is possible to determine guilt more definitively.

Analysis Procedure

1. First, look at the lane containing the 1 kb (kilobase) DNA ladder. To figure out which band is which, find the two bands that represent 1650 and 2000 base pairs (bp). These two bands are separated from the other bands and are rather easy to find.

2. Find these two bands on the stained gel and measure their migration from their point of origin in the gel. Measure from the bottom of the well to the foremost edge of the stained band. Be certain to measure each from the same point, e.g., from the bottom of the well each time, not the bottom one time and the top on the well the next. Record the base pair size of the band and it's migration distance.

3. Working up (toward larger DNA fragments) and down (toward smaller DNA fragments) from the 1650 and 2000 bp bands, record the migration of the other bands in the DNA ladder.

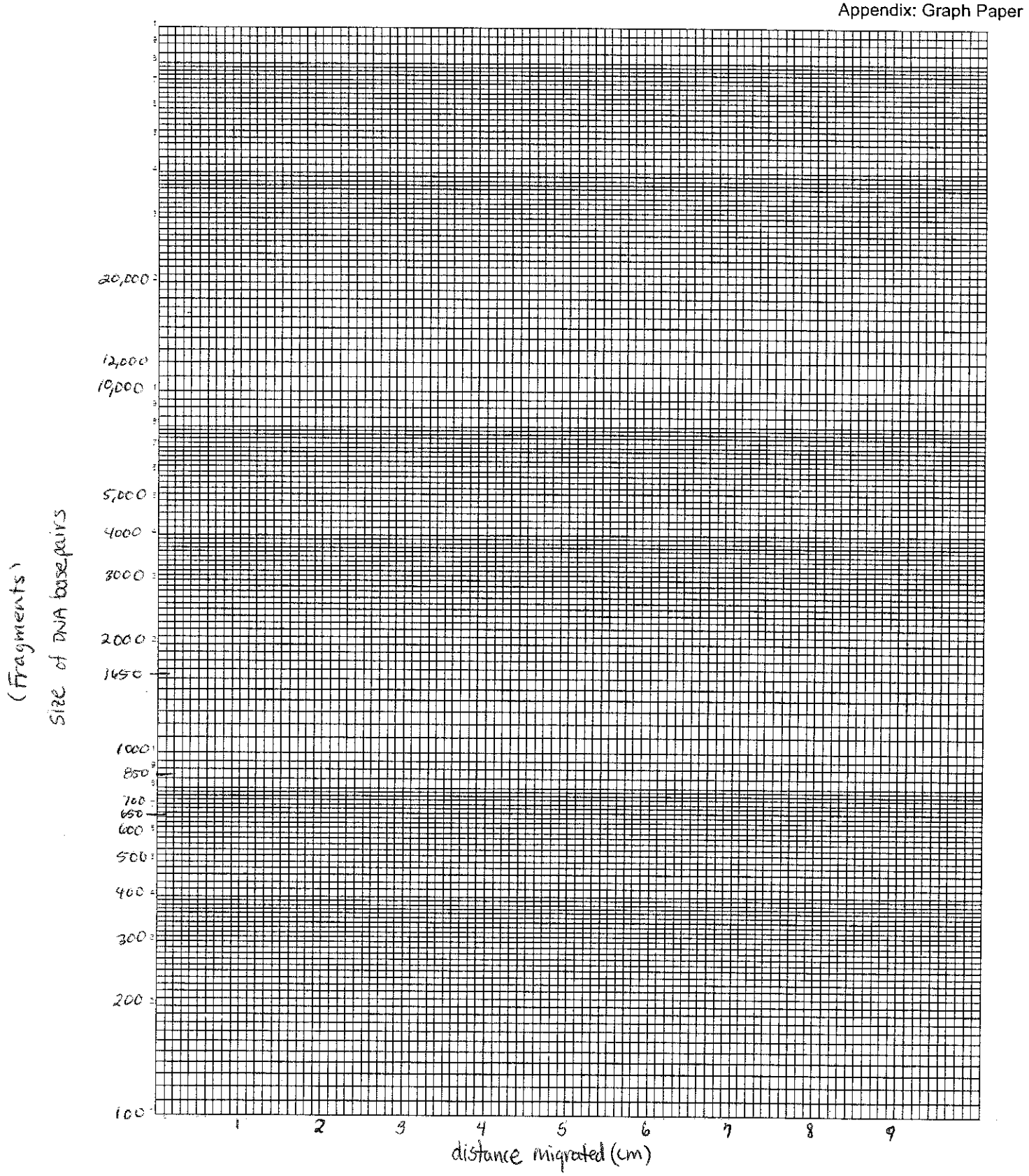
4. Measure the migration of the bands in the experimental lanes and record the migration distances.

5. Create a standard curve using the data from the 1 kb DNA ladder. Graph the migration distance of the DNA fragments (x-axis) against the size of the DNA fragments (y-axis) on semi-log graph paper.

6. Draw a straight "line of best fit," which comes as close as possible to each point.

7. To determine the size of an enzyme-digested DNA fragment, find where the migration distance of the DNA fragment intersects the standard curve. Draw a line from this point to the y-axis. Where this line meets the y-axis is the size of the fragment.

8. Using your calculated bp values, determine which suspect should be charged with the crime.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distance migrated (cm)
Size of DNA basepairs
(Fragments)