DNA Detectives

Question

Scenario

Police were called to the scene of a homicide. Found at the scene was a large amount of blood. Blood typing revealed that not only was the blood of the victim present at the scene but also blood from another person, assumed to be the guilty party. Blood evidence was collected at the scene. Blood samples from four suspects were also drawn under court order and all samples were found to be of the same blood type as the blood believed to have come from the perpetrator of the crime. The police have turned to you and your fellow lab technicians to employ the process of DNA fingerprinting in building a case against one of the suspects.

Suspect 1: Cunning Connie

Suspect 2: Scheming Steve

Suspect 3: Wily Kyle

Suspect 4: Shady Sharon

Question: Which suspect's blood was found at the crime scene?

Knowledge Probe

Answer the following questions using your prior knowledge and the Background Information in the next Knowledge Probe Bubble:

1. How do DNA polymorphisms help with distinguishing people using DNA?
2. Is the DNA cut by restriction enzymes usable DNA or “junk” DNA?
3. How accurate can DNA fingerprinting be?
4. What do the letters and numbers mean in the names for restriction endonucleases?
5. How does Hind III cut DNA? What does it make?
6. How does Pvu II cut DNA? What does it make?
7. What is a DNA fingerprint?
8. What do the DNA fragments produce after gel electrophoresis?
9. What net charge does DNA have and what is it from? Which direction does DNA flow when an electrical current is applied?
10. Predict what would happen if you placed your gel in the electrophoresis chamber with the wells containing the DNA next to the red (-) electrode instead of the black (+).
11. Why are radioactive probes used (what would happen if they were not)?

12. Below is a DNA molecule. You have just been given the restriction enzyme *Bam*H I. (See picture below as to where *Bam*H I will cut the DNA)

How many DNA fragments would you end up with if you exposed the DNA to the enzyme *Bam*H I? Why?

CATGTGATGGATCCCAGAGTCATTCCGGTACGGGATCCAGTTACGGATCCAGTTCC

GTACACTACCTAGGGTCTCAGTAAGGCCATGCCCTAGGTCAATGCCTAGGTCAAGG

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

11.

12.

Knowledge Probe

Background Information

Of the three billion nucleotides in the human DNA, more than 99% are identical among individuals. The remaining 1%, however, adds up to a significant amount of code variations between individuals, making each person's DNA profile as unique as a fingerprint. Due to the large number of possible variation, no two people (with the exception of identical twins) have the same DNA sequence.

For every 1,000 nucleotides inherited, there is on e site of variation, or polymorphism. DNA polymorphisms change the length of the DNA fragments produced by the digestion of restriction enzymes, so the exact number and size of fragments produced by a specific restriction digestion varies from person to person. The resulting fragments, called Restriction Fragment Length Polymorphisms (RFLPs), can be separated and the size determine, by electrophoresis. More than 900 restriction enzyme have been isolated from more than 230 strains of bacteria.

Most of the DNA in a chromosome is not used for the genetic code; it is uncertain what, if any, use this DNA may have. Because these regions are not essential to an organism's development, it is more likely that changes will be found in these nonessential regions. The regions that contain nucleotide sequences that repeat from 20 to 100 times (e.g. GTCAGTCAGTCAGTCA) are the strands cut by restriction enzymes to create RFLPs.

The difference in fragments can be quantified to create a "DNA fingerprint". Distinct RFLP patterns can be used to trace the inheritance of chromosomal regions with genetic disorders or to identify the origin of a blood sample in a criminal investigation. Scientists have identified more than 3,000 RFLPs in the human genetic code, many of which are highly variable among individuals. It is this large number of variable yet identifiable factors that allows scientists to identify individuals by the number and size of their various RFLPs.

This technique is being used more and more frequently in legal matters. Using DNA fingerprinting, the identity of a person who has committed a violent crime can be determined from minute quantities of DNA left at the scene of the crime in the form of blood, semen, hair or saliva. The DNA fingerprint matched to a suspect can be accurate to within one in 10 billion people, which is almost twice the total population in the world. Certain limitations in the technique prevent two samples from being identified as a "perfect match", yet it is possible to measure the statistical probability of two samples coming from the same individual bases on the number of known RFLPs that exist in a given population.

DNA fingerprinting has many other applications, since half of a person's genome come from each parent, DNA fingerprinting can be used to determine familial relationships. It has a much higher certainty than a blood test when used to determine fatherhood in a paternity suit. DNA fingerprinting can be used to track hereditary diseases passed down family lines, as well as to find the closest possible matches for organ transplants. It can also be used to a certain the level of inbreeding of endangered animals, aiding in the development of breeding programs to increase animals' genetic health and diversity.

Restriction Enzymes

As mentioned previously, restriction enzymes can be used to cut DNA molecules into precisely sized fragments. A more accurate name for this class of enzymes if restriction endonucleases, because they break DNA molecules at internal (endo) positions. Enzymes that degrade DNA by digesting the molecules from the ends of the DNA strand are called exonucleases (exo means outside).

Restriction endonucleases are frequently named using the following convention: the first italicized letter indicates the genus of the organism from which the enzyme was isolated. The second and third italicized letters indicate the species. An additional letter indicates the particular strain used to produce the enzyme. The Roman numerals denote the sequence in which the restriction enzymes from that particular genus, species, and strain of bacteria have been isolated.

Examples of Restriction Endonuclease Names

*Eco*RI *E* = genus *Eshcerichia co* = species *coli* R = strain RY 13

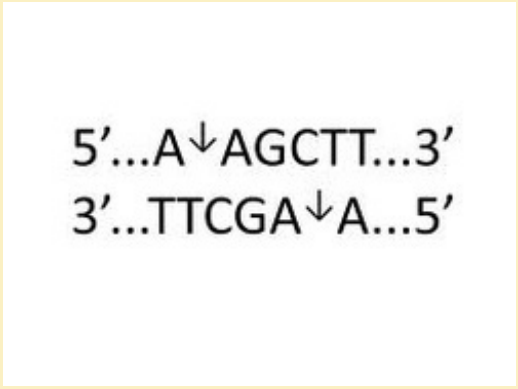
I = first RE isolated from this species

*Bam*HI *B* =genus *Bacillusam* =species *amyloliquefaciens* H = strain H I = first RE isolated from this species

*Hin*d III *H* =genus *Haemophilusin* =species *influenza* d = strain R_d

III = third RE isolated from this species

Some restriction endonucleases cut cleanly through the DNA molecule by cleaving both complementary strands of the DNA molecule at the same nucleotide position within the recognition sequence, which is generally four to six base pairs long. These nucleotide recognition sites are also termed palindromic sequences, because both strands have the same sequence running in opposite directions. The restriction endonuclease scans the length of the DNA molecule only at its particular recognition site. For example, the endonuclease *Hin*dIII will cut a double strand of DNA in the following way: The *Hin*d III enzyme recognizes the sequence AAGCTT and will cut the DNA at a different point within recognition site, resulting in a staggered cut.



A staggered cut exposes single-stranded regions of the molecule, which are known as “sticky ends”; these are especially useful in making recombinant-DNA molecules. The sticky ends produced as a result of the staggered cut made by the restriction enzyme allow complementary regions in the sticky ends to recognize one another and pair up.

Another class of restriction enzymes cuts cleanly through the DNA molecule by cleaving both complementary strands of DNA at the same nucleotide position within the recognition sequence. These enzymes produce a blunt-end cut. For example, the restriction endonuclease *Pvu* II used in this activity will cut a double strand of DNA in the following way, leaving blunt ends.

Because restriction enzymes cut DNA at specific sequence and generate fragments of various sizes, that can be resolved by gel electrophoresis, they are commonly used to generate a “fingerprint” of a particular DNA molecule. This DNA banding pattern on a gel represents a DNA fingerprint, characterizing a particular DNA type, a genomic region, or even a person.

Electrophoresis

Gel electrophoresis is a separation technology that uses gel, a substrate (like gelatin), electricity (“electro”) and movement. “Phoresis” from the Greek verb phoros, means to carry across. Gel electrophoresis, then, refers to the technique in which molecules are forced across a gel by an electrical current with activated electrodes at either end of the gel providing the driving force.

As mentioned previously, an individual’s DNA, when digested with a restriction enzyme, produces fragments can be separated from each other on the basis of size. Once separated, the DNA fragments produce a pattern of bands that visually resemble a bar code – that familiar pattern used to identify consumer products. Each band is a unique “signature” revealing a recorded and identifiable DNA fragment.

In order to separate the DNA molecules on the basis of size, the DNA must be run through a gel. The DNA is mixed in a buffer solution, and electricity is applied to the system. The electrical current from one electrode repels the DNA molecules as the electrical current from the other electrode attracts the molecules. The net negative charge of the phosphate backbone of DNA molecules results in DNA always having a negative charge and thus the samples will always migrate toward the positive pole. The frictional force of the gel acts as a “molecular sieve”, separating the material by size.

The substrate used primarily in the separation of large macromolecules, such as DNA or RNA, is Agarose. Agarose is a natural colloid extracted from seaweed and when melted and re-solidified, forms a matrix of microscopic pores. The size of these pores depends on the concentration of Agarose used in the gel. Typically, the concentration used in electrophoresis varies from 0.5 – 2.0%. The lower the concentration of Agarose in the gel, the larger the pore size and the larger the nucleic acid fragments that can be separated. Once electricity is applied, the DNA molecules wind through the pores in the gel. As pore size decreases (by increasing Agarose concentration), it is harder for longer fragments to orient properly. Smaller DNA fragments can thread their way through the poser more easily, moving more rapidly toward the end of the gel.

DNA Fragment Length Determination and Southern Blotting

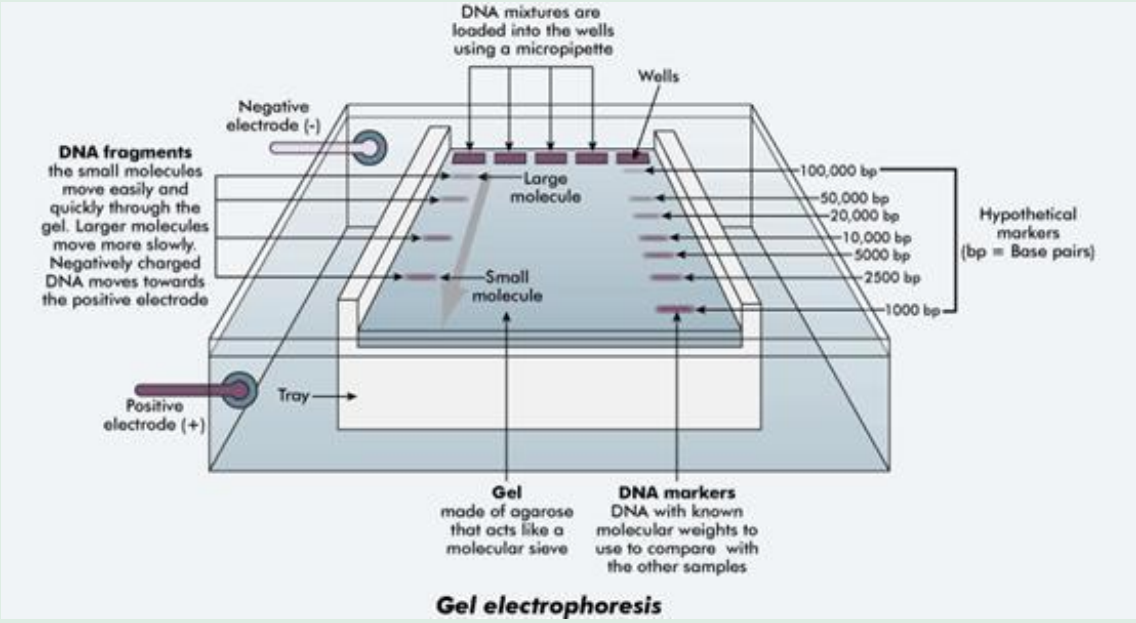
Under a given set of electrophoretic conditions, such as pH, voltage, time, gel concentration, etc., the electrophoretic mobility of a DNA fragment is standard. The length of a given DNA molecule can be determined by comparing the electrophoretic mobility on an Agarose gel with that of a DNA marker

sample of known length. The smaller the DNA fragment, the faster it will move down the gel during electrophoresis.

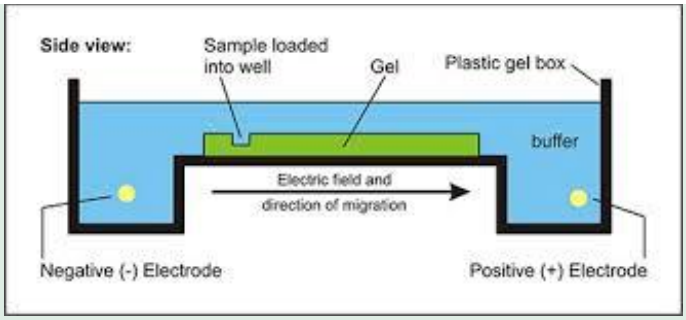
The separation of DNA on an Agarose gel may or may not be enough depending on the DNA sample in question. Since human DNA can be billions and billions of base pairs long, digestion of the DNA with a restriction enzyme often produces tens or hundreds of thousands of DNA fragments. When run electrophoretically on an agarose gel, the sample will often appear as a long smear, with no resolution visible due to the extremely high number of fragments.

To counter this, scientists employ a technique known as Southern blotting. In Southern blotting procedure, the separated fragments (RFLPs) are transferred to a membrane (usually nylon or nitrocellulose) and exposed to alkaline conditions. The fragments, when treated with an alkaline substance, will denature, separating into single-stranded DNA molecules are then labeled with a radioactive probe. A probe is a small piece of single-stranded DNA, with a radioactive marker attached, constructed from a known sequence of DNA. The probe will only bind to the denatured DNA fragments on the membrane if a region complementary to the sequence of the probe is found within a fragment. After exposure to the probe (or, in most cases, several different probes), the membrane is covered with X-ray film and any RFLP to which a probe is bound will develop the film, resulting in a series of distinct bands along the length of the film. The resulting pattern is the DNA fingerprint of the tested sample. Comparing the similarities and differences between both the number of and placement of these bands in various samples allows scientists to determine the degree of similarity or difference between the samples.

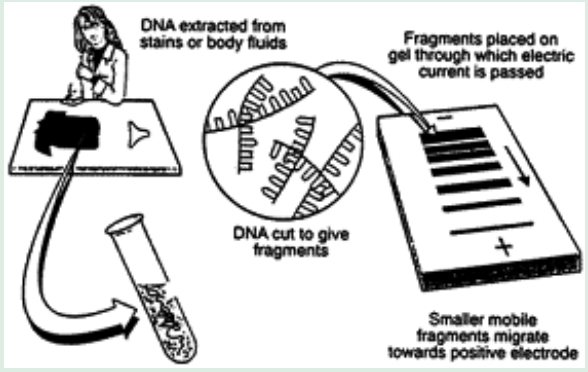
Investigation Plan



Gel electrophoresis



1. Make sure the electrophoresis apparatus is close to the power source. You cannot move it once the samples are loaded.
2. Before loading samples, gently tap the tubes so dye samples are at the bottom of the tubes.
3. Place the tip of a fresh micropipette into the dye sample tube at an angle and withdraw the sample. Gently load the dye sample to the top of the well by slowly and carefully squeezing the bulb of the pipette. Be careful to avoid puncturing the agarose gel. Use a fresh microtip pipette for each sample and continue loading each sample into the wells in consecutive order.
4. After the samples are loaded, carefully snap the cover down onto the electrode terminals. Make sure that the negative (black) and positive (red) indicators on the cover and apparatus are properly oriented.
5. Make sure the power source is plugged in correctly. Insert the black plug (negative input) into the black input of the power source. Insert the red plug into the red input of the power source (positive input).
6. Turn on the power source. Make sure you see bubbles forming at the electrode wires at the bottom ends of the electrophoresis chamber.
7. Conduct electrophoresis until the samples are well separated. (This will take the majority of the hour). Turn off the power sources before the dyes run off the gel.
8. After electrophoresis, dye migrations in the gel will be clearly visible.



Observation

Take a picture of your completed gel and insert it below. Indicate which sample was run in which lane.

Data Analysis

What patterns do you notice?

Are there any bands from one lane that are in the same location as bands in another lane?

Are there any exact matches?

Explanation

Answer the initial question using a claim, evidence, and reasoning.

Claim: Which suspect's blood was found at the scene of the crime?

Evidence: What data supports your claim?

Reasoning: Why does this evidence count as evidence? What does it mean for bands in a DNA fingerprint to match? (Think restriction fragments, probes, etc.)

Evaluation

How confident are you in your results? Why?

What were some possible sources of error?

What could you do next time?

Application

1. You used electrophoresis to perform DNA fingerprinting and identified a guilty suspect involved in a criminal investigation. What is another use for electrophoresis?
2. List 5 sources of DNA evidence that could be obtained from a crime scene.
3. What steps must be taken in the collection of evidence, such as DNA, at a crime scene to ensure the evidence is not compromised?

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