Lab- DNA Typing Macro-DNA: Doing DNA Fingerprinting and Gel Electrophoresis on a Large Scale

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Background: It's hard not to turn on the evening news or open a newspaper without one story or article making reference to DNA "fingerprinting." What are they talking about, does DNA have a distinctive "fingerprint"? Everyone knows that everyone has a distinctive fingerprint on the tips of their fingers, but did you know that everyone's DNA is different as well? In this lab, you are going to simulate using a restriction enzyme to "cut" a piece of DNA into restriction length polymorphisms (RFLPs). A RFLP is simply a piece of DNA that has been cut out of a long strand of DNA. We will then separate the pieces we obtain on a simulated gel electrophoresis, which will allow you to distinguish the various samples of DNA given to you.

Scenario: On Sunday night, at 3:00 am in the morning, a jewel heist occurred at a prominent Manhattan shop with the loss of $2.4 million in gems and jewelry. In the process of breaking the glass display cases to get at the jewels, at least one of the thieves cut themselves on the glass. Two distinct samples of blood were taken from the scene, both O+. Three suspects have been brought in for questioning, and a court order has been issued to check their blood for type and DNA analysis. All have blood type O+ blood, and the DNA taken is enclosed here for your analysis.

Objectives:

- Observe how by using restriction enzymes, DNA fingerprints can be used to identify individuals
- Simulate gel electrophoresis and analyze their results to solve the crime

Materials:

- One set of DNA sequences from evidence 1&2 and three suspects
- One Gel Electrophoresis sheet

FOLYR1: Recognizes the following site and cuts between TT

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

DNA Probe for Huntington's disease

3' ATTCTTT 5'

5' TAAGAAA 3'

3' AAAGAAT 5'

Procedure:

1) Gel set up: Place a large red positive sign near the positive end of the gel and place a large black negative sign at the negative side of the gel
2) Analyze the DNA sequences:
    a. Look at enzyme FOLY R1 and the sequence where it cuts.
    b. Cut the DNA Evidence #1 where the enzyme cuts. (See Evidence #1A has lines drawn where it cuts)
    c. Tape the pieces to the gel according to their length
3) Repeat for evidence #2, Suspect #1, Suspect #2, and Suspect #3.
4) Analyze the DNA for Huntington's disease using the probe.
    a. Cut the probe sequence out
    b. Color it to make it "radioactive"
    c. Scan through to match the probe with the suspect samples
    d. Tape the probe onto the sequence that it will complementary base pair with
    e. HINT: THE TOP LINE GOES FROM 3'-> 5' AND THE BOTTOM LINE GOES FROM 5'->3'
5) Answer all questions in complete sentences.

Questions (**Answer in complete Sentences**)

1. Compare the samples of DNA fingerprints you have created from the evidence and samples. What differences do you see? What similarities do you see?
Evidence #1A matches suspect 1 and evidence #2B matches suspect 3. The gel from suspect 2 does not match the evidence.
2. Who's blood was found at the crime scene? It seems that suspects' 1 and 2 blood was found.
3. Why is the term "DNA fingerprint" used when we use restriction enzymes to separate DNA in RFLPs?
The term "fingerprint" is used because every person's individual patterns of fragments will be different from another person's.
4. What effect would using a different restriction enzyme have (one that cuts between, say A-A sequence)?
The restriction fragments would be different and the probe for Huntington's disease may not attach.
5. What are some other uses for DNA fingerprinting?
Paternity/maternity tests, criminal identification, detection of inherited diseases
6. Explain what step would be necessary in order for the probe to bind. Explain why that step is necessary.
The restriction enzyme step would be necessary because the probe couldn't attach to the sticky end if that step was skipped.

Multiple choice: Use the following words to match up with their description 7-10

Restriction enzyme, gene cloning, DNA ligase, gel electrophoresis, reverse transcriptase

7. Produces many copies of a gene for basic research or for large scale production of a gene product
gene cloning
8. Enables on to create cDNA from RNA; results in a smaller gene product (RNA processed- no introns) that is more easily translated by bacteria
reverse transcriptase
9. Separates molecules by movement due to size and electrical charge
gel electrophoresis
10. Seals the sticky ends of restriction fragments to make recombinant DNA
DNA ligase
11. Eco R1 and Hin dIII are two different restriction enzymes (endonucleases). If the DNA of different animals were cut with either Eco R1 or Hind III, which cut of DNAs would not join together easily so that they could be sealed with ligase?

a. Human DNA cut with Eco R1 and chimp DNA cut with Eco R1

b. Prokaryotic DNA cut with Hind III and eukaryotic DNA cut with Hind III

c. mouse liver DNA cut with Eco R1 and mouse kidney DNA cut with Eco R1

(d.) E. Coli DNA cut with Eco R1 and mouse DNA cut with Hind III

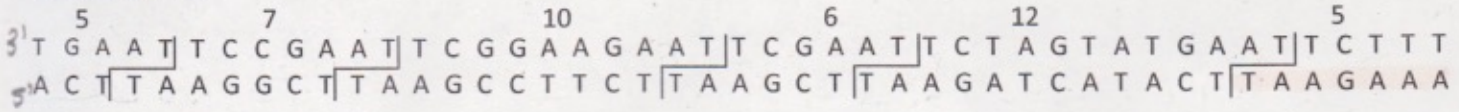
e. mouse DNA cut with Hind III and chimp DNA cut with Hind III

12. The principle problem with inserting an unmodified mammalian gene into a bacteria and getting proper expression is

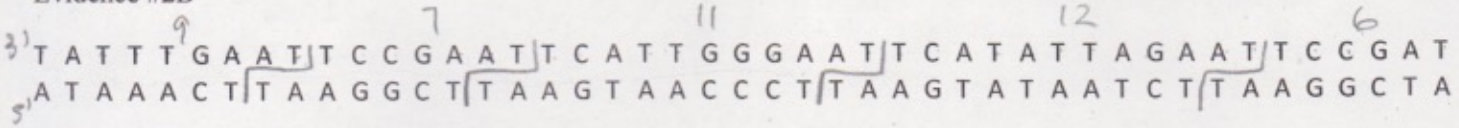
a. Prokaryotes use a different genetic code
(b.) bacteria can not remove introns
c. bacteria polymerase cannot make RNA complementary to mammalian DNA
d. bacteria DNA is not found in a nucleus and is therefore incompatible with mammalian DNA

DNA Samples:

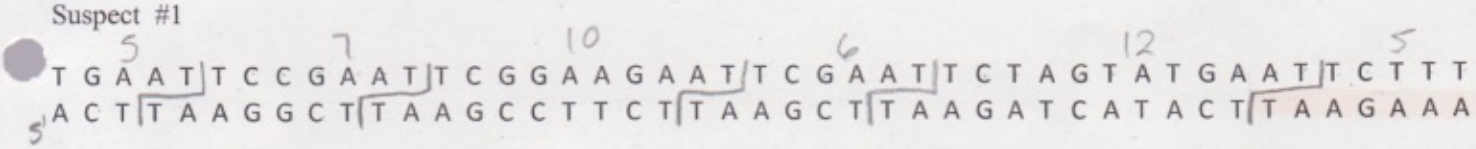
Evidence # 1 A



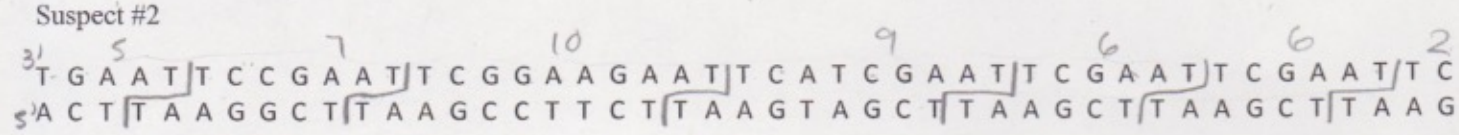
Evidence #2B



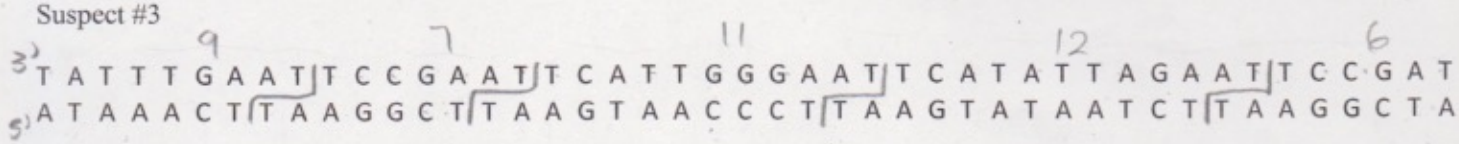
Suspect #1



Suspect #2



Suspect #3



RFLP Length	Evidence #1A blue	Evidence #2B green	Suspect 1 yellow	Suspect 2 red	Suspect 3 orange
12					
11					
10					
9					
8					
7					
6					
5					
4					
3					
2					
1					