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Gen Bio 1 Lab #9: CSI and Strawberry DNA Analysis

Pre-Lab Reading: Page 234-244 in the 8th edition or Page 331-335 and in the 9th edition.

Pre-Lab Vocabulary:

1. Agarose –
2. Electrophoresis –
3. Restriction enzymes –
4. DNA enzyme recognition site–
5. Vector –
6. Plasmids –
7. Complementary DNA –
8. Nucleotides –
9. Purine –
10. Pyrimidine –
11. Semiconservative replication –

Who Done It?

DNA fingerprinting allows for the identification of the source of a DNA sample, which is important in many forensic cases. DNA fingerprinting can provide positive identification with great accuracy by matching DNA obtained from a crime scene to individual suspects.

Several steps are involved in DNA fingerprinting. First, a suitable sample must be obtained. DNA is then isolated from the evidence, such as blood or hair samples. Once the DNA is isolated, it is either digested with special enzymes called restriction endonucleases (restriction enzymes), or checked for specific genomic markers (particular repeats of sequence in the DNA of humans) using the polymerase chain reaction.

Restriction endonucleases are enzymes that cleave the sugar-phosphate backbone of DNA according to specific base-pairings. In most practical settings, a given enzyme cuts both strands of duplex DNA within a stretch of 4-10 base pairs. The site at which a restriction enzyme will cleave the DNA is called a **recognition site**. Most recognition sites are palindromes, they read the same forward (5' to 3' on the top strand) and backward (5' to 3' on the bottom strand).

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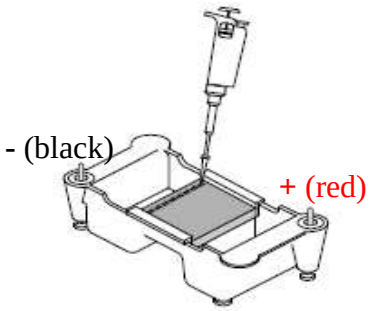
Materials
Gel casting tray with dams and a comb
Electrophoresis chamber
Running buffer (1X concentration)
0.8% agarose
DNA samples from the crime scene and 2 suspects, each cut with 2 different restriction enzymes.

Procedure 1:

1. Prepare gel casting tray and add 30 ml of 0.8% agarose after melting in the microwave. Allow gel to solidify in the refrigerator.
   NOTE: When separating small fragments the concentration of agarose is increased (to 1-3%) to slow them down. When separating large fragments, the concentration of agarose is decreased (0.8%) to speed them up.
2. Carefully remove the comb from the casting tray and then carefully remove the dams.
3. Place the tray with the gel into the electrophoresis chamber with the wells closest to the **negative electrode** (first examine how the lid fits onto the chamber; **see diagram to right**). Next add running buffer until the top of the gel is covered.
4. Add 35 microliters of each sample to a separate well (hole) produced when you removed the comb. **NOTE: make sure you know the order in which you added the samples.**



Remember: "run to red"

Label	Sample	
A	Crime scene DNA 1	NOTE: DNA 1 denotes cut with restriction endonuclease #1 and DNA 2 denotes cut with restriction endonuclease #2.
B	Crime scene DNA 2	
C	Suspect 1 DNA 1	
D	Suspect 1 DNA 2	
E	Suspect 2 DNA 1	
F	Suspect 2 DNA 2	

5. Place lid on the electrophoresis chamber plug cord into the power supply, adjust the voltage to 75V and turn the power supply ON.
6. Allow samples to run until the "fastest" piece (dye) has moved along about 2/3 of the gel.
7. Compare the bands in the crime scene samples to the bands from the 2 suspects and determine which suspect is the perp.

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Questions:

1. **Which suspect is the perpetrator? Explain how you know.**

2. **What is the variable in this experiment?**

3. **Did you use a positive control? If not, what would make a good control?**

4. **What kind of evidence would you look for at a crime scene to obtain DNA?**

5. **Why was the suspects' DNA cut with 2 different restriction endonucleases?**

6. **Why did you place the wells near the negative electrode of the electrophoresis chamber?**

7. **Why does each person have a unique pattern in their DNA?**

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Strawberry DNA Extraction Lab

Every cell in a strawberry contains eight copies of each of its chromosomes. As a result, strawberries contain large amounts of DNA. Strawberry DNA is easy to extract because strawberries are easy to mash, and ripe strawberries produce enzymes that contribute to the breakdown of cell walls.

Materials

Strawberries
Detergent + salt solution
Ice-cold ethanol
Zip-lock baggie
Graduated cylinder (10-mL)
Funnel
Cheese cloth
Test tube
Wire loop

Procedure 1:

1. Obtain one strawberry and place it inside a self-sealing plastic bag. Press the air out of the bag and seal it carefully. Mash the bagged strawberry with your fist for two minutes.
2. Add 10 mL of detergent to the bag. Press the air out carefully and reseal the bag. Mash the bagged strawberry for one minute.
3. Set up the filtration apparatus as shown in front of the class, using the test tube rack.
4. Pour the liquid extract into the filtration apparatus, and let it drip directly into the test tube.
5. When the test tube is approximately 1/8 full, remove the funnel. Discard any extra mashed strawberry pulp with the cheesecloth.
6. Slowly drizzle cold ethanol (your instructor will get this for you when you are ready for this step) along the side of the test tube, until the test tube is about half full of liquid. The ethanol should form a separate layer on top of the filtered extract.
7. Dip the loop into the test tube to where the ethanol and extract layers meet. Gently twirl the loop. Keep the tube at eye level so that you can see what is happening. Observe the characteristics of the DNA as it precipitates (clumps together) out of the solution.

Questions:

1. **Match the following lab steps with the effects on the strawberry cells:**

A) Mash the fruit to slush.	**_____ breaks open the cells**
B) Filter the strawberry extract.	**_____ dissolves plasma membrane**
C) Add detergent solution.	**_____ clumps DNA together**
D) Layer cold ethanol over	**_____ separates organelles and cell debris, such as fragments of cell walls and membranes, from DNA and small dissolved molecules such as proteins and sugars.**

2. **A person cannot see a single strand of cotton from 30 meters away, but if thousands of threads are wound together into a rope, the rope can be seen at some distance. How is this statement an analogy to the DNA extraction you just completed? Describe the DNA you extracted. Was it what you expected to observe?**

3. **DNA dissolves in water, but not in ethanol. Explain what happened when the ethanol came in contact with the strawberry extract during the DNA extraction.**

4. **If DNA is invisible under a light microscope, how will you be able to see the strawberry DNA you extract?**

5. **Why do you think clumps of precipitated DNA molecules have a ropelike shape?**

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 **Questions to e x p a n d your mind.** 

1. Can you think of a case when 2 people may have identical DNA patterns?

2. List 3 things that can affect how material (DNA, dyes, etc.) moves through an agarose gel.

3. In order to study human genes, scientists must first extract the DNA from human tissues. Would you expect the method of DNA extraction for human DNA to be the same as the method you used to extract DNA from strawberries? Why or why not?

4. If you were a strawberry farmer what questions would you pose to a scientist about strawberry DNA to increase your bottom line?