**Lab: Macro-DNA Fingerprinting Name Pd**

**Purpose:** Students will use a model of a restriction enzyme, ECO R1, to cut their DNA strand into restriction fragment length polymorphisms (RFLP's). Students will use these RFLP's to produce DNA fingerprints to determine the parents of a child.

**Background:** It's hard not to turn on the evening news or open a newspaper without one story or article making a reference to DNA "fingerprinting." 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 your DNA into restriction fragment length polymorphs (RFLP's). We will then separate the pieces we obtain on a simulated gel electrophoresis, which will allow you to distinguish your DNA fingerprint pattern from the others in the class. But that's not all. It appears that a baby has been found, and the parents are known to be in this class and we will use DNA fingerprints to figure out who they are!

**Materials (for each person):**

one DNA sequence (male #1-9 OR female #10-18)

tape

one ECO R1 restriction enzyme model

scissors

**Pre-Lab Questions:**

1. What is the purpose of Gel Electrophoresis?
2. How are restriction enzymes important in Gel Electrophoresis?

**Procedure:**

1. Obtain one DNA sequence. On the gel electrophoresis data table, put a \* on the sequence that you have. The number is found on the top left of each strip. (In the example from #3 below, the sequence is #1).
2. ***Prepare your DNA****:* The sequence is actually made up of three pieces that you will need to cut and then tape together. The three strips are labeled at the top left end with a number and either X, Y, or Z.) Cut around the solid lines of the rectangle and then along the dashed lines. Then tape the ends of the paper together in increasing alphabetical order, as shown by the example below. Make sure to overlap the ends located in the middle so there isn’t a break in the sequence of bases. (You should not see the y or the z once it is taped!)

1

z

1

y

1

x

**notch**

1

x

1. Now examine your ECO R1 Restriction Enzyme model. Look at the upper line of code on the model and note the G-A-A-T-T-C sequence there. The ECO R1 enzyme cuts between the G and the A of that specific sequence and if you look at your model, there is a small notch located at that place. See the image to the right.
2. Your next step is to cut the DNA sequence into RFLP's. Take your ECO R1 enzyme and place it at the left end of the top strand of DNA. Slide down the strand, and at each point where there is a G-A-A-T-T-C sequence appears in the holes, use the notch in your enzyme to make a mark on the DNA strip. Continue this until you have identified all of the sites on the strand that the restriction enzyme will cut.
3. At each spot marked on your DNA strip, make a straight cut of the DNA sequence between the G and A.
4. You will end up with several RFLP's of different lengths. Arrange the strips in order, with the longest at the top of your desk and the shortest at the bottom of the desk.

AATTCGCAAG

AATTCAAG

AATTCG

TCG

**= 3**

**= 6**

**= 8**

**= 10**

1. Count the number of bases on each of the RFLP's, and shade in the appropriate boxes on the Gel Electrophoresis Sheet. This is your DNA Fingerprint! Note that you may end up with more than one fragment that has the same number of bases… that is okay – you can just stack them on top of each other! In an actual electrophoresis gel plate, the largest DNA fragments move the slowest through the gel, and the smallest DNA fragments move the fastest (and thus the furthest). Thus, they appear as separate lines or bands on the gel electrophoresis sheet but there may be more than one strand of DNA in that line.

**Gel Electrophoresis Data Table:**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| # of bases | Male #1 | Male #2 | Male #3 | Male #4 | Male #5 | Male #6 | Male #7 | Male #8 | Male #9 | Baby’s Fingerprint | Female #10 | Female #11 | Female #12 | Female #13 | Female #14 | Female #15 | Female #16 | Female #17 | Female #18 | # of bases |
| 18 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 18 |
| 17 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 17 |
| 16 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 16 |
| 15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 15 |
| 14 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 14 |
| 13 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 13 |
| 12 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 12 |
| 11 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 11 |
| 10 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 10 |
| 9 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 9 |
| 8 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 8 |
| 7 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 7 |
| 6 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 6 |
| 5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 5 |
| 4 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 4 |
| 3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 3 |
| 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 2 |
| 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |

**Lab Analysis (If it asks you to explain, please answer in complete sentences):**

1. How much DNA does a child get from each of its parents?
2. Should a child have exactly the same RFLP pattern as its parents? Explain why or why not.
3. Based on your results, do you think you are the parent of the child? Why or why not?
4. Compare your DNA sequences to those of your classmates. What differences do you see? What similarities do you see?
5. Based on the results from the entire class, what conclusions can you make about who are the parents of the baby? Are your results conclusive or do you need to do something further?
6. What effect would using a different restriction enzyme have (one that cuts between, say, a T-T sequence)? Explain.
7. What other uses are there for using DNA fingerprinting? List at least two!
8. Explain the steps of gel electrophoresis in your own words.

**Gel Electrophoresis Data Table: KEY**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| # of bases | Male #1 | Male #2 | Male #3 | Male #4 | Male #5 | Male #6 | Male #7 | Male #8 | Male #9 | Baby’s Fingerprint | Female #10 | Female #11 | Female #12 | Female #13 | Female #14 | Female #15 | Female #16 | Female #17 | Female #18 | # of bases |
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| 17 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 17 |
| 16 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 16 |
| 15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 15 |
| 14 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 14 |
| 13 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 13 |
| 12 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 12 |
| 11 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 11 |
| 10 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 10 |
| 9 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 9 |
| 8 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 8 |
| 7 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 7 |
| 6 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 6 |
| 5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 5 |
| 4 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 4 |
| 3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 3 |
| 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 2 |
| 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |

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