Name: _____ Date:_____

"Whose DNA?" Gel Electrophoresis (Edvotek)

Product Link

I. Background and Introduction

DNA fingerprinting allows for the identification of the source of a DNA sample, a procedure often used in forensic cases to identify suspects or victimes. Several steps are involved in DNA fingerprinting:

- Obtain a sample (hair, body fluids, skin)
- Digestion of DNA using **restriction enzymes**
- Polymerase chain reaction (PCR) amplifies DNA
- Gel electrophoresis separates DNA fragments by size

The bands seen on the gel represent different sizes of DNA. Small bands travel farther than larger bands. When the bands match, that indicates the sample contains the same DNA.

DNA markers are DNA molecules of different lengths that are used to compare other DNA samples. They are measured in kb (kilobase), which equals 1000 base pairs.

Paternity determination is another application of DNA fingerprinting. In this technique, samples are obtained from the mother, the child, and possible fathers. A child's DNA is a composite of its parents. The bands created will match both the father and the mother.

Pre-Lab Questions

1. Examine the crime scene evidence. Highlight the suspect bands that match the evidence. Which suspect is a match to the crime?

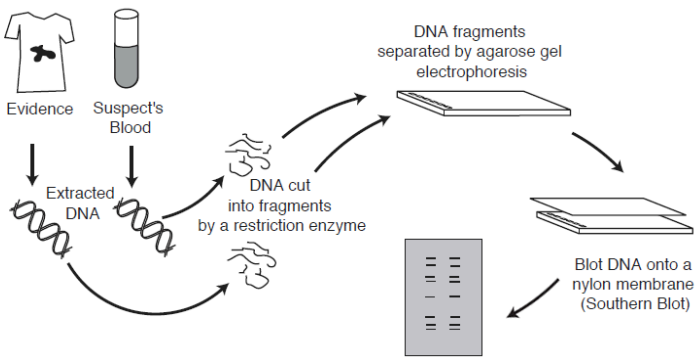
2. The second fingerprint shows a paternity test. Highlight the mother's bands in pink, and the father's bands in blue.

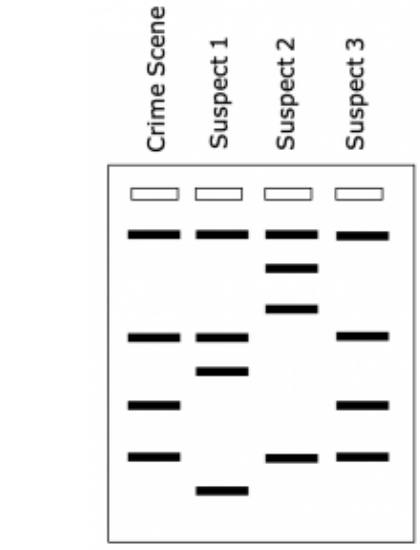
3. Use the DNA markers to determine the length of the mother's fragment. How large is the fragment?

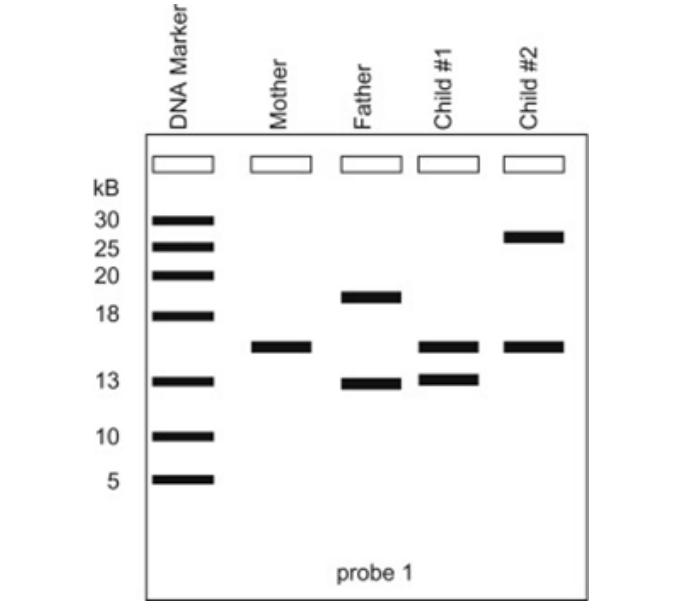
4. What is the role of restriction enzymes in this lab?

5. How is PCR used in DNA analysis?

6. What is the role of gel electrophoresis?







II. Day 1: Prepare agarose gels

1. Agarose is melted in DI water and heated until it is clear. DO NOT OVERHEAT! (Remove as soon as the solution is clear and starts to bubble.)

Heat on hot plate or use microwave

2. Place a comb in the casting tray and pour enough agarose to submerge the casting tray without going over the rubber end caps.

3. Cover the casting tray with plastic wrap and store in the refrigerator.

4. When the gel solidifies, the comb will create wells for loading the DNA sample.

Day 2: Loading DNA into wells

*Pay close attention to which Lane you are loading the samples into

1. Unwrap the agarose molds and remove the endcaps

2. Place them into an electrophoresis chamber. The wells should be positioned toward the black leads.

3. Carefully pour water (with buffer) into the chamber to cover the molds

3. Use a micropipette to load samples into the wells.

Lane 1 (A) Crime Scene DNA 1
Lane 2 (B) Crime Scene DNA 2
Lane 3 (C) Suspect 1 DNA Digest 1
Lane 4 (D) Suspect 1 DNA Digest 2
Lane 5 (E) Suspect 2 DNA Digest 1
Lane 6 (F) Suspect 2 DNA Digest 2

4. Connect the leads to the power source (match red to red and black to black)

5. Turn on the power source. You may notice bubbles in the water.

6. The samples will begin to move!

While you wait for the results, work on the synthesis questions

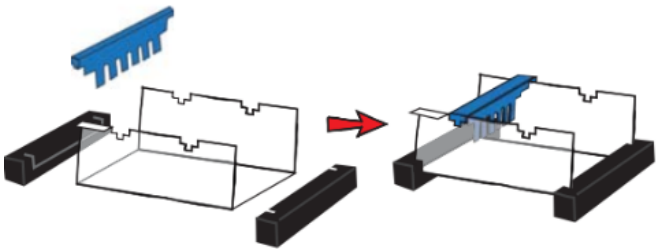
Gel Mixture

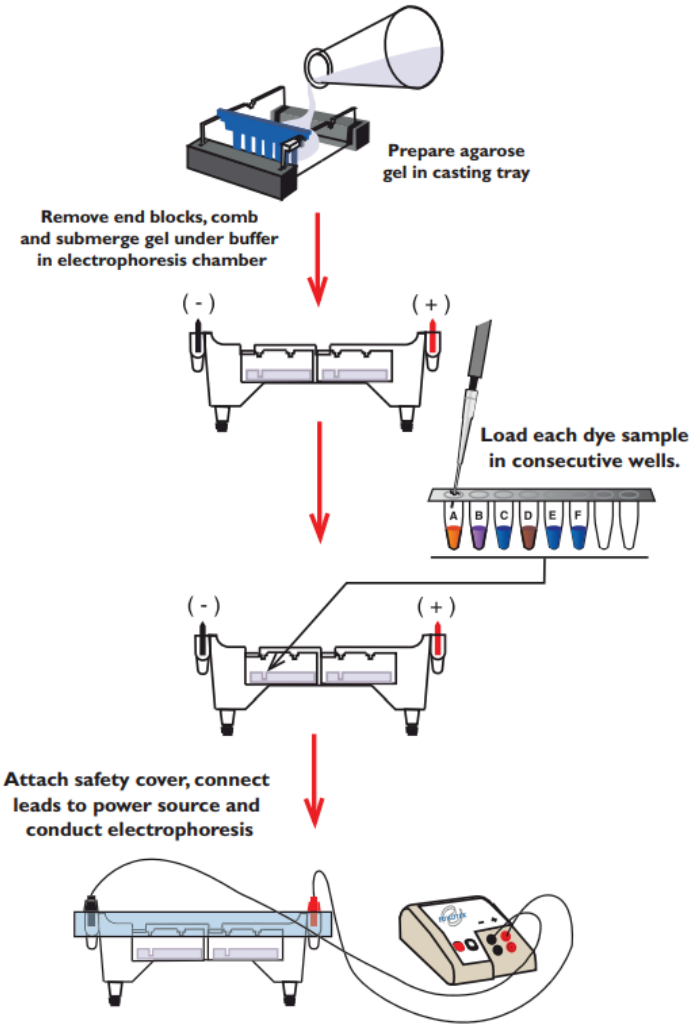

Wear gloves and safety goggles

For 7x14 cm Gel Casting Tray
(or two 7x7)

1.2 ml Concentrated buffer
58.8 ml Distilled Water
0.46 g Agarose *total volume 60ml

Buffer Solution: 6 ml 50x Conc. Buffer + 294 ml DI water
(Class): 20 ml 50x Conc Buffer + 980 ml DI water



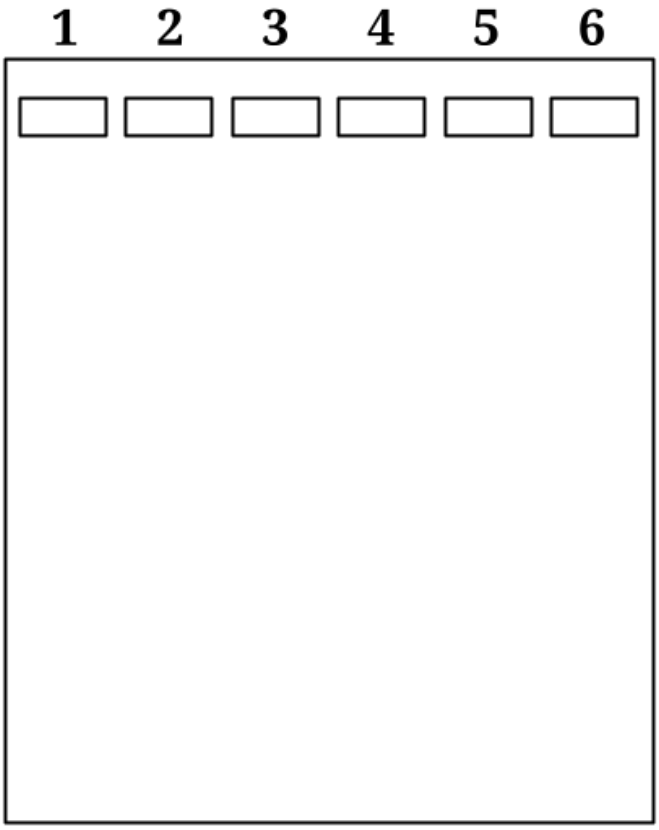


Case Background

A house was burglarized and the criminal left behind a water bottle. Police turned the water bottle over to the evidence lab where DNA was extracted from the saliva on the bottle. The DNA was digested using two restriction enzymes. Each enzyme digest will create an individual's unique fingerprint. Your goal is to analyze the DNA fingerprint patterns and decide if suspect 1 or suspect 2 was at the crime scene.

III. Interpreting Results

1. Sketch the location of the bands after your gel has run. The top boxes are where the samples were loaded.



• Lanes 1 and 2 (set one) represent the crime scene DNA digested by two different restriction enzymes, which yield distinctly different DNA banding patterns.

• Lanes 3 and 4 (set two) represent DNA from Suspect 1. The suspect's DNA has been digested with the same two restriction enzymes as in Lanes 1 and 2.

• Lanes 5 and 6 (set three) represent DNA from Suspect 2. It also has been digested with the same two enzymes as the crime scene DNA (Lanes 1 and 2) and DNA from Suspect 1 (Lanes 3 and 4).

Wells *(Identify the samples, see previous page)*

1) _____

2) _____

3) _____

4) _____

5) _____

6) _____

7. DNA Digest 1 (Lane 1) matches which suspect?

8. DNA Digest 2 (Lane 2) matches which suspect?

9. Which suspect matches both crime scene digests?

10. Why is it important that forensic scientists use more than one digest?

DNA Lab Synthesis

1) What are some reasons that scientists would want to isolate human DNA?

2) What is the purpose of the DNA markers?

3) What is the purpose of submerging your gel in a buffer solution while doing gel electrophoresis?

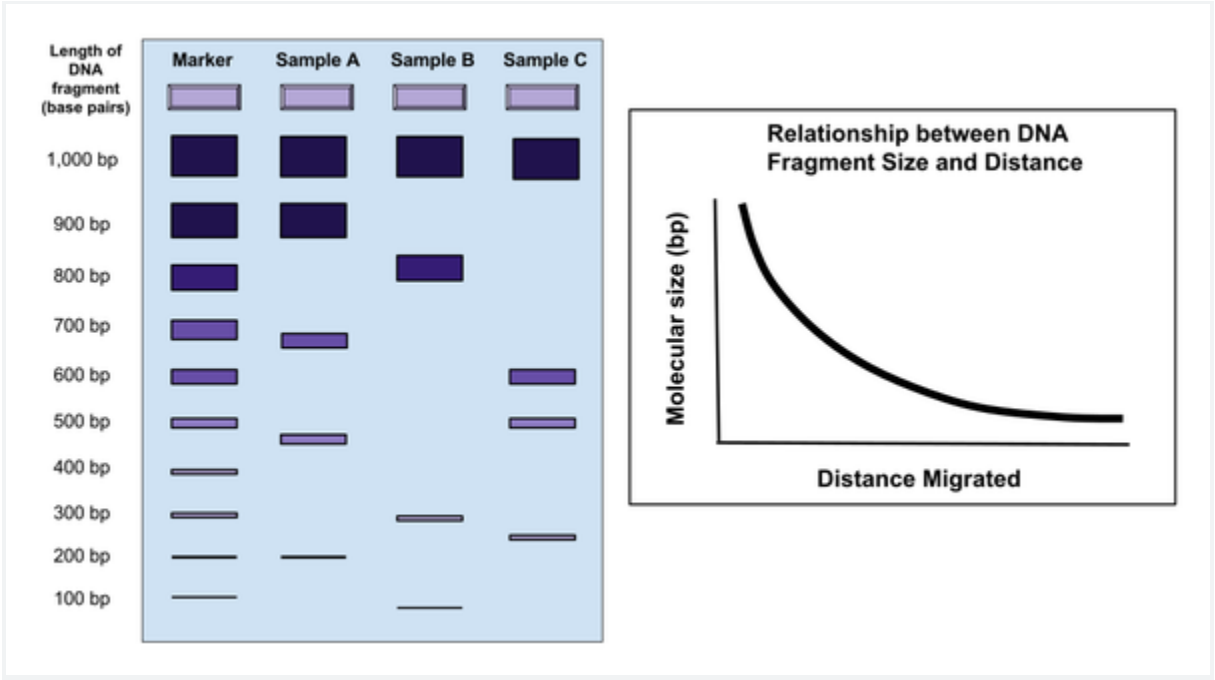
4) Circle one choice from each pair of words in parentheses:

When we separate PCR products on an agarose gel, the (positively / negative) charged DNA migrates toward the (positive / negative) electrode or anode. (smaller / larger) fragments move through the gel faster and end up the farthest away from the starting position.

Gel electrophoresis separates fragments based on their (charge / length)

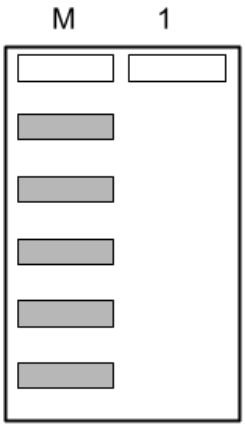
The tiny air bubbles coming from the electrodes in the box indicate that the current (is / is not) running.

5) Markers are used to estimate the length of a sample. A known sequence is used to compare samples to determine the size of the fragments. Use the marker to estimate the size of the fragments in **sample C**.



Fragment 1 _____ Fragment 2 _____ Fragment 3 _____ Fragment 4: _____

6. The fragments in the marker lane (M) in the gel shown below range in size from 100 to 500 base pairs in increments of 100.

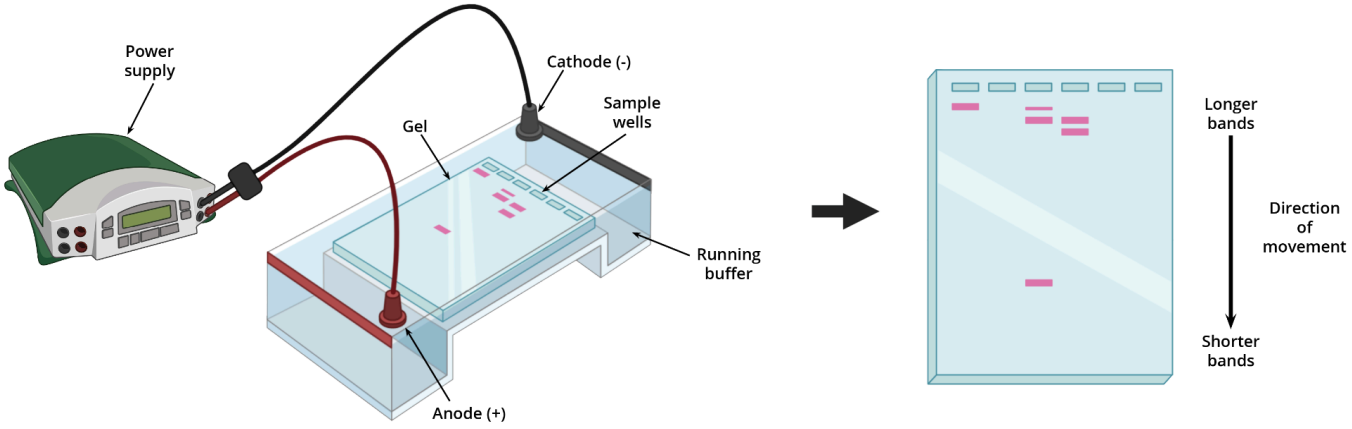


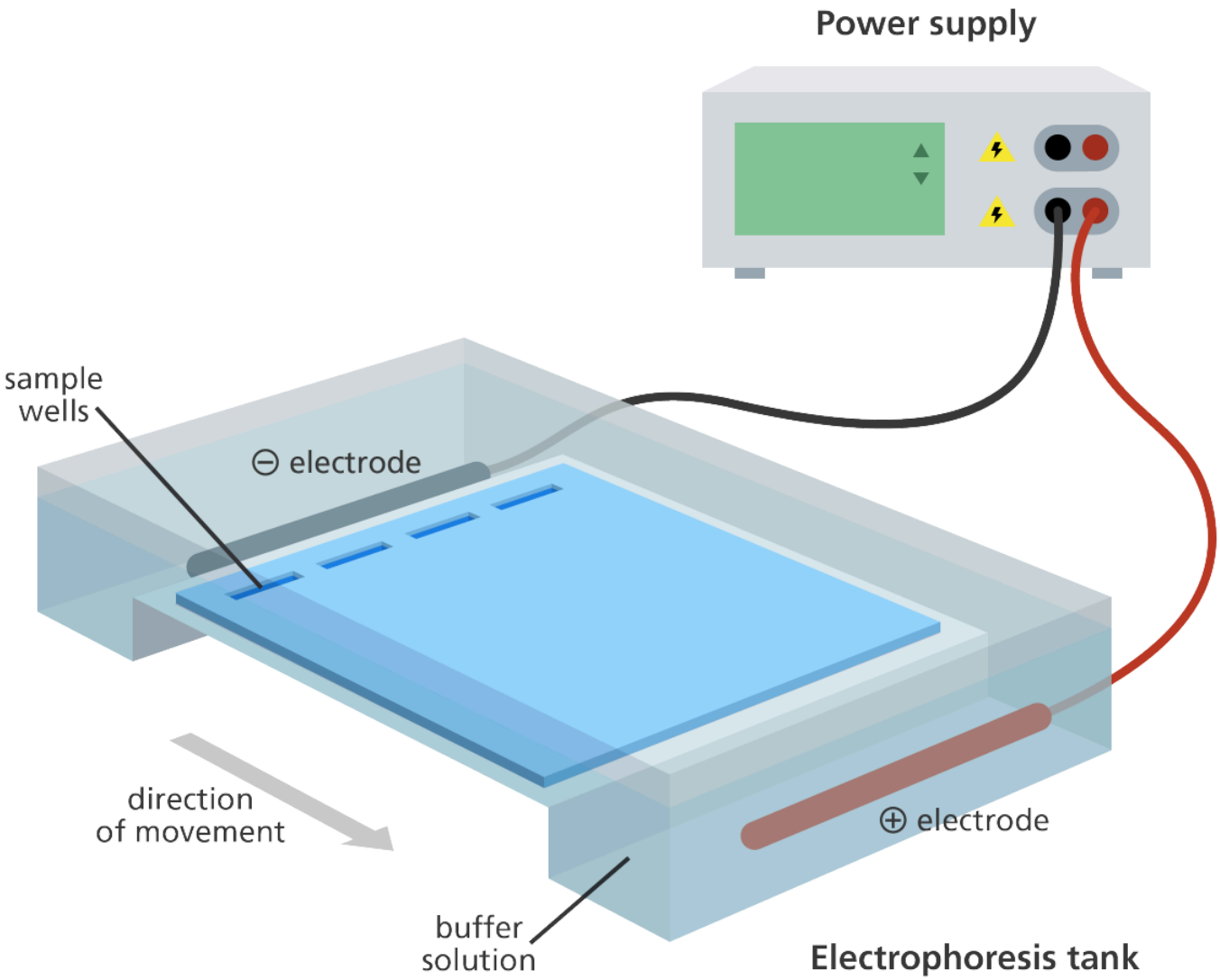
a) Label the positive and negative electrodes and draw an arrow showing the direction the DNA migrated when the gel was running.

b) Label the marker band sizes on the gel and draw a band in lane 1 showing where a 425 base pair fragment would run.

7. A common error beginning biology students make is placing the leads on wrong (red to black instead of red to red). Consider what would happen if you had made this mistake. How would this affect the outcome of your experiment?

8. In gel electrophoresis, the buffer provides ions that carry a current through the gel, and to maintain a constant pH. This is the liquid you poured into the chamber. What would happen if you just poured water into the chamber?




Power supply
sample wells
⊖ electrode
direction of movement
⊕ electrode
buffer solution
Electrophoresis tank