Lab RE1

DNA Fingerprinting – A Simulation

Background

Many of the revolutionary changes that have occurred in biology over the past fifteen years can be attributed to the ability to manipulate DNA. In this lab we will be using ***restriction enzymes*** as a kind of "chemical scissors". ***Restriction enzymes*** look for specific base sequences, and when they have a match, they cut. Because everybody's DNA is unique, where the 'scissors' cut is slightly different for each person. When the restriction enzymes are done cutting the DNA, you end up with a bunch of different sized DNA chunks. The number of big, small and medium size chunks is unique for each person, because our DNA is unique.

The DNA fragments can be separated electrically by **agarose gel electrophoresis.** They can be tagged or stained so that they are visible can be studied. The resulting pattern of fragments resembles the pricing bar code used at supermarkets. We call this bar code pattern a DNA fingerprint.

In forensic science, DNA fingerprints are used to find a match between a suspect and DNA found at the crime scene. This investigative technique is called DNA fingerprinting because each pattern of DNA fragments is unique to each individual, just like a fingerprint. Unlike conventional fingerprints that are often difficult to gather at a crime scene, DNA fingerprints can be made from a very small sample of blood, skin, semen-- or even a single hair!

In DNA fingerprinting the DNA is labeled or "tagged" with a radioactive probe. The probes are designed to bind to specific sequences on the DNA, thus marking some of the fragments. X-ray film placed on top of the gel will become exposed by the radioactivity, and black bands will become visible on the film.

The term *'electrophoresis'* literally means "to carry with electricity." It is a technique for separating and analyzing mixtures of charged molecules. Due to its sugar-phosphate backbones, DNA is a negatively-charged molecule. When placed in an electric field, it will migrate toward the anode (+). The speed of migration of DNA in an agarose gel depends on the size, shape, and strength of the charge associated with the DNA fragment.

Purpose

In this laboratory, to prepare and analyze a simulated DNA fingerprint, *the student will:*

- **cut** a DNA sample -- the evidence! -- by incubating it with restriction enzymes,
- **prepare and load an agarose gel** with the restriction enzyme,
- conduct **gel electrophoresis** to spread out the mixture of DNA fragments in the digest,
- **stain and photograph** the gel to visualize the DNA,
- **analyze** the resulting banding pattern or "DNA fingerprint," and use it to "solve" an imaginary mystery.

Materials

gel electrophoresis box
power supply
casting tray and comb
P-20 micropipet and tips
racks for 0.5 mL reaction tubes
0.5 mL reaction tubes (5)
plastic trays for staining
Ziploc™ baggie
400 mL beaker for buffer
beaker or graduate cylinder for agarose
agarose, [0.8%], liquid held @ 65°C (≈ 25-30 mL)
restriction enzymes, on ice
DNA samples, on ice
electrophoresis buffer, ≈ 300 mL (1X TBE or 1X TAE)
restriction buffer [2X]
loading dye
sterile water
marking pen
cotainer for waste
mini ice bucket

Class use: water bath, crushed ice containers, microcentrifuges, documentation stations (camera, film, filter, hood, UV transilluminator) ethidium bromide staining solution [1 μg/mL].

Part I. Restricting DNA Samples (Day 1).

1. Each team should obtain 5 reaction tubes. Label the caps with a sharpie.
   1 U (DNA from crimes scene uncut control)
   2 CS (DNA from crime scene, cut)
   3 V (DNA from Vincent)
   4 I (DNA from Irene)
   5 D (DNA from the new Director)

2. Obtain DNA, enzyme and necessary reagents as directed by your teacher. Keep the DNA, buffer, and enzyme ON ICE. Add the appropriate solutions as indicated below and answer the questions:

Test Tube	**Buffer**	**Enzyme**	**Uncut DNA "U"**	**Crime Scene DNA**	**Vincent's DNA**	**Irene DNA**	**New Director DNA**	**Sterile H2O**
1U	6 μL		2 μL					4 μL
2CS	6 μL	4 μL		2 μL				
3V	6 μL	4 μL			2 μL			
4I	6 μL	4 μL				2 μL		
5D	6 μL	4 μL					2 μL	

3. Place the tubes in a 37°C water bath for at least 30 minutes. They will be stored in the refrigerator overnight.
4. After all three solutions have been added to each tube, close the caps tightly and mix by giving the tubes a quick 2-3 second spin in a microcentrifuge.

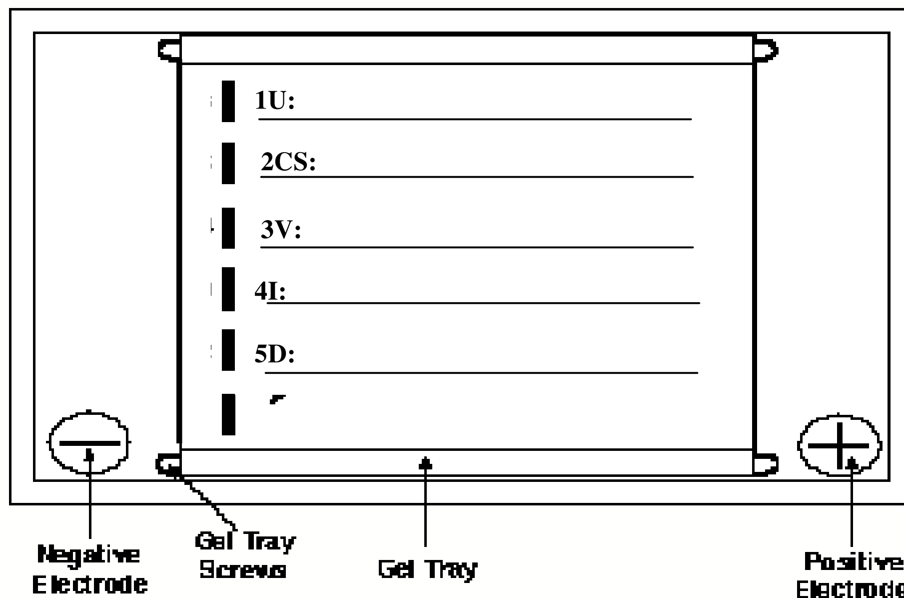
CAUTION : **BE SURE THE REACTION TUBES ARE BALANCED IN THE ROTOR -- YOU'LL NEED A SIXTH TUBE, EITHER SOMEONE ELSE'S OR ONE WITH 12 μL OF WATER AS A BALANCE TUBE.**

Part II. Load an agarose gel (Day 2).

1. To each of your five tubes, add 2 μL loading dye. Then give them all a quick spin in the microcentrifuge to mix in the dye. (Be sure to BALANCE the tubes in the rotor!)
2. Follow your instructor's directions for preparing your gel plate. If you will be pouring your own gel, be sure that…
    - you use 30 mls of agarose gel
    - comb is in the correct position for pouring
    - both gates are up, screws are barely tight
    - pour slowly and steadily
    - do not bump the tray while the gel is setting
    - wait at least 3 minutes before putting the gel tray into the box

In the diagram below write down what DNA samples belong in each well.

BACK



FRONT

Orientation of gel for loading

Part III. Run your agarose gel (Day 2 & 3).

1. After loading your DNA sample you must run your gel.
2. Fill the plastic electrophoresis box with about 300 mL of 1X TAE --electrophoresis buffer.
3. Lower the "gates" on the casting tray and submerge the casting tray onto its platform in the gel box. The comb should be located at the left side by the negative black lead (cathode).
4. Carefully remove the comb from the gel (pull it straight out). You'll notice that this left behind six little empty "slots" or **wells** in the gel.

TO LOAD YOUR DNA INTO THE GEL…

Load 12 μL of each sample into a separate well in the gel. Be sure to change tips between samples!

a. Fill your pipette tip correctly to 12 μL.

b. Position your arm and pipette over the gel box. Steady that arm with your other arm and place both elbows on the table

c. Lower the pipet tip under the surface of the buffer to the top edge of a well.

d. **Check:** BE VERY CAREFUL NOT TO PUNCTURE THE BOTTOM OF THE GEL.

e. Gently depress the pipette plunger and slowly expel the sample into a well. Keep the plunger fully depressed while removing the pipette tip out of the buffer.

f. Make sure to label the above diagram with the DNA sample you put into each well. This will help you with your results.

CAUTIONS: Remember, turn the power supply OFF before touching or opening a gel box.

If two teams are connecting their gel boxes to one power supply, be sure to communicate with each other whenever the power supply is turned ON or OFF.

5. WITH THE POWER SUPPLY **OFF**, slide the lid onto the gel box and connect electrical leads anode to anode (red to red) and cathode to cathode (black to black).
6. Turn the voltage knob down to ZERO. Then turn the power supply ON. Set the power supply to about 100 Volts
7. **Check:** Notice there is a switch to direct the LED display to read in either volts or milliamps. Use it to verify how much current *is* flowing through the gel. (It should read about 40 milliamps with one gel box hooked up or 80 milliamps with two boxes hooked up with 1XTAE buffer.)
8. Allow electrophoresis to proceed until the dye, and DNA, are out of the wells and safely into the agarose gel.
9. Turn the power supply OFF and disconnect the leads.
10. Remove the casting tray from the gel box. CAREFULLY slide your gel off the casting tray and into an empty plastic tray or "boat." Label the rim of the tray with your initials. Place the gel and "boat" into a Ziploc™ baggie and seal. The gels will be stored in the refrigerator overnight.

1. The operator, wearing safety glasses and gloves, will transfer your gel to a staining dish containing a dilute EBr solution.
2. The gel will be left in the EBr stain for 5-10 minutes, and then rinsed in distilled water for 5-10 minutes to increase contrast and make the gels safer to handle.
4. An operator will then transfer the gel to a UV photographic station. The ultraviolet light makes EBr-coated DNA fragments glow. A UV photograph can then be taken for analysis.

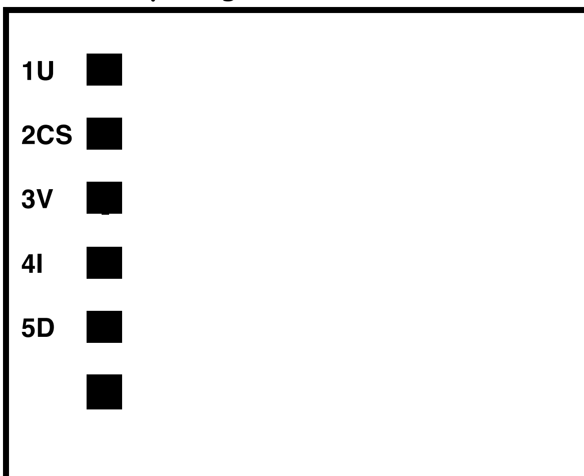
Part IV: Stain and photograph the gel. (Day 4)

The DNA will be stained with a fluorescent dye called ethidium bromide (EBr). EBr slides between the rungs of the DNA double helix. When excited by ultraviolet (UV) light, the EBr absorbs some of the energy and emits light.

CAUTION: Ethidium Bromide is extremely toxic. Therefore operator will conduct the actual photographic procedure. Students will see the results and obtain a printout for analysis.

Part V: Data and Data Analysis (Day 4).

Examine your gel and sketch the results in the diagram below.



Check: Due to its sugar-phosphate backbones, DNA is a negatively-charged molecule. When placed in an electric field, it will migrate toward the anode (+). The speed of migration of DNA in an agarose gel depends on the **size**, **shape**, and strength of the **charge** associated with the DNA fragment.

Analysis Questions:

1. Which lane has the control sample? Which lane has DNA from the crime scene?
2. Which lane contains the DNA that most closely matches the DNA found in the spit of the murdered director's eye (crime scene DNA)?
3. Based on this data, who would ou proclaim the murderer? Why?
4. Describe two ways you could end up with a false match between the suspect's DNA and the crime scene DNA?
5. What was the role of restriction enzyme in the process of DNA fingerprinting?
6. In the movie GATTACA, how did genetic engineering lead to prejudice and discrimination?
7. Does your gel show any signs of improper laboratory technique? (i.e. blurry or smudged bands, crooked band fronts, multiple matching banding patterns in several lanes, multiple bands in the "1U" lane, etc.) If yes, describe the kind of technique error you may have made. More than likely your instructor will be helpful during the UV photography session.