



## DNA Fingerprinting A Simulation

### Background

Many of the revolutionary changes that have occurred in biology over the past fifteen years can be attributed directly to the ability to manipulate DNA in defined ways. The principal tools for this recombinant DNA technology are enzymes that can cut, mend, wind, unwind, transcribe, repress, and replicate DNA. Restriction enzymes are the “chemical scissors” of the molecular biologist; these enzymes cut DNA at specific nucleotide sequences. A sample of someone’s DNA, incubated with restriction enzymes, is reduced to millions of DNA fragments of varying sizes. A DNA sample from a different person would have a different nucleotide sequence and would thus be enzymatically “chopped up” into a very different collection of fragments. The DNA fragments are separated by electricity – agarose gel electrophoresis – and tagged or stained in some fashion so that they can be visualized and studied. The resulting pattern of restriction fragments resembles the pricing bar code used on supermarket products – small bars lined up in a column, the largest fragments closest to the beginning of the gel, the smallest at the end of the gel.

Methods of DNA identification have been applied to many branches of science and technology, including medicine (prenatal tests, genetic screening), conservation biology (guiding captive breeding programs for endangered species), and forensic science. In the latter discipline, analysis of the pattern of DNA fragments in a restriction digest loosely called a *DNA fingerprint*, enables us to discriminate between suspects accused of a crime or potential fathers in a paternity suit.

In DNA fingerprinting, radioactive pieces of DNA called probes are added to the separated DNA fragments. The probes are designed to bind 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 variation in DNA from one person to the next is so great that the probability of two people sharing the same DNA fingerprint is essentially zero. And unlike conventional fingerprints, which are often difficult to gather at a crime scene, a DNA fingerprint can be made from a very small sample of blood, skin or semen – or even a single hair!

## PURPOSE

In this laboratory, to prepare and analyze a simulated DNA fingerprint, we will:

- cut a DNA sample – the evidence! – by incubating it with a restriction enzyme
- load an agarose gel with the restriction digest
- conduct gel electrophoresis to spread out the mixture of DNA fragments in the digest
- photograph the gel to visualize the DNA
- analyze the resulting banding pattern or “DNA fingerprint”, and use it to “solve” an imaginary crime

## Materials: per team

- gel electrophoresis box
- agarose,[0.8%], with ethidium bromide 1ug/ml added, liquid held @ 65°C (≈ 20 ml)
- power supply
- casting tray and comb
- DNA samples, on ice
- P-20 micropipette and tips
- electrophoresis buffer, ≈ 300 mL (1XTEA)
- 6x loading dye

**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 Receive DNA Sample (“crime scene” or “suspect”).**

1. You will be assigned to one of six forensic groups. Each group will receive DNA from suspects 1,2,3, and from crime scene (CS)and a control (C) DNA which will contain NO restriction enzyme. Do not share reagents or equipments with another forensic group. Keep these tubes on ice while we start Part II.

## **Part II Cast an agarose gel**

1. Prepare your casting tray by carefully placing a glass plate into bottom of tray.
2. Obtain a test tube with 20 ml of hot liquid agarose (**USE CAUTION – HOT!**) The agarose solution contains ethidium bromide, a carcinogen, so wear gloves when pouring the solution. When the test tube is just cool enough to hold, pour the agarose evenly into the floor of the casting tray, making sure that it is completely in place and even.
3. DO NOT JAR or MOVE the casting tray as the gel solidifies. This ensures a smooth, even gel. As the agarose hardens (about 10 min), it changes from clear to slightly opaque.
4. Fill the plastic electrophoresis box with about 300 ml of 1X TEA— electrophoresis running buffer. TEA is a salt solution, made of Tris, pH8.0, which keeps the pH constant, EDTA, which pulls out low levels of extraneous divalent ions and sodium acetate, a salt.
5. When the gel has solidified, remove the comb in a straight up motion and transfer the glass plate and gel from the casting tray to the gel box and submerge the gel and plate onto its platform in the gel box. You'll notice that removing the comb left behind six little empty 'slots' or *wells* in the gel. The wells should be located at the cathode end (black lead; (-)). Remember- black-top; red-bottom. The level of the buffer should be only slightly covering the surface of the gel.

## **Part IV Load the gel**

1. After the appropriate incubation period, your DNA digests will be ready to be electrophoresed on the agarose gel
2. To each of your five tubes, add 2  $\mu$ l loading dye. Then give them all a quick spin in the microcentrifuge to mix in the dye. Temporarily set them aside.
3. Is your gel ready to load? It should be in the gel box, under buffer solution; the comb should have been removed and the six empty wells in the gel should be at the cathode (negative) end of the box.

4. Load 10 µl of each sample into a separate well in the gel.

-Lower the pipet tip under the surface of the buffer, but don't puncture the bottom of the gel

-Gently depress pipet plunger and slowly expel a sample into a well. Keep plunger depressed until the pipet is out of the gel box.

-Change tips between samples.

### **Part V Gel Electrophoresis**

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 of the piece; small pieces experience less resistance and move faster (farther) than the larger pieces.

### **CAUTIONS**

- Remember, it is good practice to 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**
1. WITH THE POWER SUPPLY **OFF**, secure the lid of the gel box and connect the leads to the same channel of the power supply (red-red, black-black)
  2. Set the power supply at about 100 Volts and 40 milliAmps current  
80 mAmps will automatically result if a second box is connected to one power supply.
  3. Turn the power supply ON. Notice there is a switch to direct the LED display to read in either volts or milliamps. Use it to verify that current is to read in either volts or milliamps. Use it to verify that current **is**

flowing through the gel.

4. Shortly after the current is applied, you should notice something happening at each electrode... what is it? You may also notice that the loading dye “behaves” in an unexpected way. Why?
5. Continue to electrophorese until the fastest-moving dye front has advanced at least  $\frac{3}{4}$  of the way across the gel (about 45 minutes).
6. Then, turn the power supply OFF and disconnect the leads.
7. Remove the casting tray from the gel box. **Wear gloves, remember there is ethidium bromide in the gel and also in the buffer.** CAREFULLY slide your gel off the casting tray and into its labeled plastic tray or “boat”.

### **Part VI Photograph the gel**

There are many stains and tagged probes that allow us to visualize DNA. In our lab, we will stain the DNA with a fluorescent dye called ethidium bromide (EtBr). EtBr sticks to the surface of the DNA double helix. When excited by ultraviolet (UV) light, the EtBr absorbs some of the energy and emits orange (visible light).

### **CAUTIONS**

- Do not allow you skin, eyes, mouth, etc. to come in contact with ethidium bromide solution. Always wear goggles and gloves if working with this chemical.
- If you accidentally spill ethidium bromide staining solution, notify the instructor. S/he will deactivate it using powdered activated charcoal.
- UV light can damage unprotected eyes and skin. Never look directly into an unshielded UV light source. Our transilluminators are safe, since a plastic safety shield is lowered over the gel.

## DNA FINGERPRINTING SIMULATION

### Crime Scene Scenario

**Sharfmans in Worcester was robbed last night! The perpetrator appeared to cut him/herself on the broken store window; blood was found on glass at the scene of the crime. The blood-spattered shards of glass were taken to a forensics lab to analyze the DNA.**

<b>SUSPECTS</b>	<b>Woman, in 40's</b>	<b>Man, ex-con in 30's</b>	<b>Young man in 20's</b>
<b>ACTIVITY AT TIME OF ROBBERY (probable cause)</b>	Owner of jewelry store. Had worked in the store that day. Was observed meeting with jewelry appraiser in "bad" part of town earlier	Notorious jewel thief, paroled from prison. Crime statistics show that a large fraction of ex-cons return to criminal behavior. Police had observed the man in the area of the jewelry store.	Busboy at restaurant near jewelry store was seen running down the street near the store late at night (after midnight).
<b>MOTIVE</b>	Would collect on theft insurance; if she had "arranged" for the robbery, she'd also still have the jewels.	Had returned to theft / fencing of jewelry for money.	Was in need of money to support his family; his hours at the restaurant had been cut way back.
<b>ALIBI</b>	Had gone home from the store not feeling well. Spent evening alone at home. As far as the appraiser – he was a friend of a friend.	Had gone to a crowded movie by himself. Ticket taker could neither confirm nor deny that the man had indeed gone to that theater.	Had worked the late shift and was running down the street to catch the bus.