Lab 4: Biotechnology
Pre-Lab Test

True or False

1. DNA molecules vary from person to person however all DNA molecules weigh the same.

2. The presence of a suspects DNA at a crime scene proves that the suspect had some part in the crime.

3. Every human has two identical copies of an allele, one from their mother and one from their father.

Define:

4. Allele

5. Locus:

THE USE OF DNA IN FORENSIC ANALYSIS

Today we'll try out one of the workhorse tools of criminal investigation—agarose gel electrophoresis. This is an essential technique of DNA analysis, widely used in many fields.

How does Gel electrophoresis work?

- Gel electrophoresis separates charged molecules according to their size (molecular weight) by pulling them through a porous gel. All the molecules are pulled by the electrical current applied across the gel, but smaller molecules slip more easily through the pores of the gel and thus move further in a given amount of time. The gel is submerged in a liquid buffer that conducts the current.

When the current is turned off, the molecules stay as visible bands at positions in the gel based on their relative sizes. These bands are made visible by various techniques. A common technique is staining the DNA with ethidium bromide, which must be handled very carefully as it is carcinogenic. To minimize this risk in our lab we will be comparing standard dyes rather than actual DNA in our test, but the procedure and relevance of the technique are just the same.

Because the odds of known DNA segments from two or more people being identical are infinitesimally small, where the DNA ends up in the gel following electrophoresis is a valuable bit of info! If a suspect's crime-scene electrophoresis result matches his/her actual DNA sample (which may be taken from blood, semen, hair roots, skin cells, saliva etc.), then odds are very high that suspect is a perpetrator. But the tests aren't infallible.

As we run our experiment today, we'll also take a look at a film documentary of one of the most notorious crimes in our country's history--the Sheppard murder of the 1950's. As you watch the film, note how much the techniques of forensics have changed since this crime was committed, as well as why the new techniques are still limited!

AGAROSE GEL ELECTROPHORESIS

The instructor will demonstrate the use of the gel apparatus and molding trays. Each lab group will then make an experimental gel and run the "DNA" samples from a hypothetical crime scene (not the crime depicted in the film).

Please pay close attention to the instructions given and outlined below. The two major steps take time, and we need to be sure everyone completes each step at the same time.

Gel pouring procedure:

1. Prepare the mold for the gel by taping the open ends of the mold securely.

2. Place the white comb in the slots in the center of the mold, along the red line. This will produce wells in the gel into which you'll load the samples.

3. Combine the pre-measured amount of agarose (a polysaccharide derived from seaweed) and 100 ml buffer (in large carboy) in a small flask and heat in the microwave until the mixture boils. (This step may have already been done for you.)

4. Place the taped mold in a spot on your desk where it'll be safe from bumping or falling. Pour the hot, boiled agarose into the center of the mold, all at once. Do not move the mold until the gel has set (about 30 minutes—we'll watch the start of the film during this time). Rinse out the agarose flask under running water immediately. Invert flask on a paper towel on the side counter.

5. When the gel has cooled and turned somewhat whitish (after about 30 minutes), remove the tape from the mold carefully, gently pull out the comb, and place the mold in the gel tray of the electrophoresis chamber with the red line closest to the red pole. Do not tilt the mold while you're doing this, or you might end up with your gel on the floor!

Loading the wells: Using the following procedure, load 10.0 microliters of each sample into a separate well. Each person in your group should have a chance to load a sample. Be sure to keep track of what sample is in what well! Skip a lane between each sample.

1. Push the pipetman firmly into a disposable micropipet tip.

2. Check that the pipetman is set to measure 10.0 microliters.

3. Depress the plunger to the first stop and hold. Dip the disposable tip into the sample tube and slowly release the plunger to draw up the fluid. Slide the tip along the wall of the tube to release excess fluid clinging to the outside of the tip.

4. Hold the pipetman above the gel. Gently lower the tip with sample into the appropriate well—do not push too far or you'll puncture the gel.

5. Slowly depress the plunger of the pipetman to the first stop, wait a moment, and press to the second stop to fully release the sample into the well. Hold the pipet plunger at the second stop position as you slowly withdraw the pipet tip from the gel.

6. Discard the disposable tip by pushing the ejector button, replace with a new tip, and proceed loading the other samples in their own wells. You must use a new, sterile tip for each sample! Used tips may be discarded in the trash.

Make a note or drawing here of the order in which your wells were loaded:

Pouring the buffer and starting the run:

1. Place your electrophoresis chamber in a spot where it will not be bumped or disturbed. We will be using one power supply for every two chambers, so you may need to move yours to another desk. Pour 300 ml of running buffer into the chamber. The gel itself should be covered with approximately 2 mm of the buffer.

2. Carefully slide the chamber lid onto the chamber. Do not tilt or force the lid or you will
break the connection between the chamber and the patch cords!

3. The next step is to apply current to the gel and samples. Wipe dry the safety lid of the chamber and any spills on the desktop. Make sure that the power supply is unplugged and that all wires and patch cords are dry and free of signs of damage or wear.

4. Connect the red terminal of the power supply to the red terminal (+) of the electrophoresis chamber with a red patch cord. Connect the black terminal of the power supply to the black terminal (-) of the electrophoresis chamber with a black patch cord.

Have your set-up checked by the instructor before plugging in the power supply

5. After your set-up is checked, set the power output to 100 volts and plug in the power supply to begin the electrophoresis run. A light should go on and a voltage readout appear; if that exceeds or falls below 100 you may adjust the knob so that the readout is as close to 100 as possible (it'll fluctuate some). You should see tiny bubbles rising from the fine platinum wires at each end of the electrophoresis chamber.

6. The run should take about 30 minutes to complete. Check the progress of the migration of the dyes in the electrical field periodically, as we watch the rest of the film. The apparatus is set up so that both positively and negatively charged components of the dyes will be separated (the positively charged go towards the negative pole, and vice versa). The run should be stopped when any band nears the end of the gel (turn off the power supply).

7. After the run is complete, turn off the power and unplug the power supply. Wait 10 seconds. Disconnect the patch cords from the power supply first, then from the electrophoresis chamber. Do not allow the cords to touch each other or get in the buffer. Slide off the chamber lid carefully—do not tilt or force it!

8. Remove the gel tray from the chamber and place it on a piece of white paper. Immediately rinse the electrophoresis chamber with tap and then distilled water and turn it upside down on paper toweling on the side counter. Be sure not to touch the fine platinum wires at each end of the chamber. Loop the patch cords together in a loose knot.

9. Examine the gel results. For each sample, measure the distance (in cm) that each band has traveled from its well, noting also whether each move toward the + or – electrode. Also note the color of each sample. For the crime scene sample, there should be two bands; record info for both. See the table below and fill it in for your group's results.

Victim	color	+ or -	Distance
Suspect #1			
Suspect #2			
Crime Scene			

Can you tell whether or not one of the suspects left DNA at the crime scene? If so, which one.

Discuss your results with your lab partners, and then answer the questions on the practice quiz on your own.

Post Lab 4 Test

Electrophoresis questions:

1. Did all the samples move toward the same end of the gel? Why/why not?

2. Which two DNA samples were found in the crime scene sample?

3. If this were a real crime scene investigation, who if anyone would you question, and maybe arrest? Why?

4. What physical characteristic of the DNA sample causes these bands of different lengths to appear in the gel?

Video questions:

5. What are the difficulties in using DNA in the analysis of a 45 year old crime?

6. We learned that an individual carries two alleles for every locus (gene "address" on a chromosome). Why is only allele 4.1 found in Eberling's blood?

7. Does finding the 4.1 allele in the blood stains on the porch and closet door prove that Richard Eberling left blood in the Sheppard house? Why/why not?

9. After seeing the video, are you convinced that Dr. Sheppard is innocent? Why/why not?

10. Are you convinced that Eberling is the murderer? Why/why not?