EXERCISE 1

Biotechnology

INTRODUCTION

You have undoubtedly heard on the news or read in the newspaper numerous instances of biotechnology being used in various ways in today's society (DNA fingerprinting in criminal cases, DNA analysis used in paternity suits, genetic engineering, human genome project, etc.). In today's lab, you will participate in different experiments that address some of the main techniques currently used in molecular biology and DNA analysis. DNA is a remarkable molecule which consists of a different sequence of base pairs (adenine, guanine, cytosine, thymine) in every person (except for identical twins).

The ability to visualize and identify sequence differences and alter base sequences in DNA have provided researchers with powerful tools with many different applications.

Activities: DNA Fingerprinting

Materials

Equipment	Supplies
Waterbath set at 60° C	Agarose
Gel electrophoresis apparatus	Running buffer
Power Pack	Pre-digested DNA samples
Micropipettor and tips	Ethidium bromide
	Carolina blue

Part 1- Casting a gel (each group will perform this part)

Agarose is a polysaccharide that is purified from algae. Agarose is purchased as a dried powder like jello. The agarose is mixed with water and boiled to dissolve the agarose. As the boiled solution cools the large strands of the macromolecules of agarose polymerize, join or crosslink forming a molecular mesh. Although the agarose appears to be a solid, it really is made of strands of agarose surrounded by the liquid buffer. If you were of molecular size, moving through the agarose gel would be like navigating through a dense forest or trying to move through mesh-like spider webs. The smaller you are the easier it would be to move around the trees and webs. The larger you are the harder it would be to move through the gel and the longer it would take. Agarose can be used to separate pieces of DNA based on size. Gel electrophoresis involves running an electrical current through an agarose gel which causes DNA to migrate toward the positive electrode. Larger pieces of DNA will travel slower than smaller ones.

Part 2- Gel Electrophoresis and DNA Fingerprinting

Electrophoresis is a powerful technique that can be used to separate pieces of DNA as well as proteins and other charged molecules.

Read the appropriate sections in your online reference and answer the following questions.

Although you can't actually see DNA as it is traveling through a gel, what is one method used to see the bands of DNA once the run is finished?

Why does DNA move toward the positive electrode and not the negative electrode (hint: What is the charge of DNA fragments?)?

Which would travel faster, a 2000 base pair piece of DNA (2 kilo base, or 2 kb) or a 9 kb fragment?

This section describes the basic concepts of DNA fingerprinting. The samples you will run today represent DNA from body fluids (semen) found on a victim of a rape as well as DNA from two different suspects. You will perform an RFLP (restriction fragment length polymorphism) analysis. Basically, each DNA sample was treated with two different enzymes that cut DNA at very specific regions (restriction sites). The resulting fragments will be separated through electrophoresis in hopes of determining from which suspect the body fluid found on the victim came.

A generalized electrophoresis apparatus is shown to the right. The electrodes carrying current from the

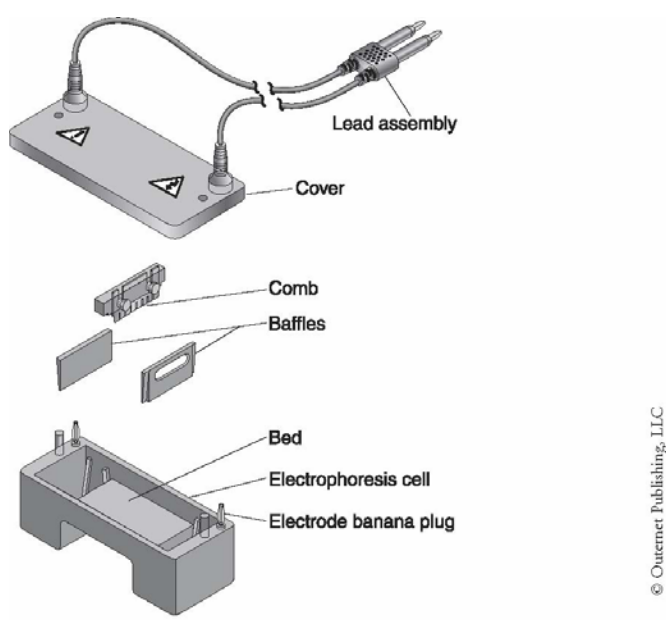

Figure 5.1 - Electrophoresis apparatus.

power pack are attached to the cover. **DO NOT** pull on the electrodes to remove the cover! Remove the cover by grasping it from the sides and lifting. Inside the apparatus on the raised platform is a removable gel bed. The upright sides of gel bed are notched to allow the placement of the comb. There is a center notch and an end notch. The end notch should be located at the negative electrode (black) terminal. Aluminum baffles should be located on either side of the gel bed. These are used only when pouring the gel so that the agarose doesn't leak into the buffer chamber. A Teflon comb should be with the apparatus.

Before class, your instructor will prepare the agarose gel by adding the appropriate amount of agarose to boiling water. Agarose will solidify when it cools. So, it is very important to keep the agarose in the hot waterbath until needed. Your instructor will demonstrate how to pour your gel. When you are ready to pour your gel, make sure the baffles are snuggly in place at the ends of the gel bed. Remove the container of agarose from the waterbath, add 3 drops of the Carolina blue solution to the gel and then pour the agarose into the gel bed apparatus. Add the "comb" to the end notch. The "teeth" of the comb will form small wells in the gel as the gel solidifies. You will eventually add DNA samples to each of the wells. Each sample will contain many different pieces of DNA and these will be separated through electrophoresis. Allow the gel to solidify for 5 minutes. DO NOT move the apparatus during this time.

Once you have cast your gel and it has solidified, **gently** remove the comb by rocking it back and forth. Remove the baffles. Ask your instructor for help if needed.

You will be provided with 6 different DNA samples: 2 samples of body fluid found on the victim, 2 samples of suspect 1, and 2 samples of suspect 2. The 2 samples of each represent DNA treated with a different restriction enzyme. A loading dye (blue color) has been added to the samples. The loading dye has two important functions. It makes the sample denser than the buffer so the samples will sink to the bottom of the well. The dye is a small molecule and will move very quickly through the gel along the electrical front. The color provides a visual indication of the progress of the electrophoresis.
Using a micropipette, you will add 25 μl of sample to each well. Be very careful when adding the sample to the gel, try not to puncture the bottom of the gel as this will cause the sample to diffuse out of the well. ***Use a new pipette tip for each sample.*** Discard used tips appropriately. In the space below, record which sample you added to each well.

lane 1 ___________________________________

lane 2 ___________________________________

lane 3 ___________________________________

lane 4 ___________________________________

lane 5 ___________________________________

lane 6 ___________________________________

Once you have added your DNA samples, you will need to add the running buffer. The running buffer maintains the correct pH and provides ions to permit the conduction of electrical current through the gel. Gently pour buffer into one of the buffer chambers until the buffer is level with the gel. Then add buffer to the other chamber and continue to pour the buffer until a thin layer of buffer covers the entire gel and fills the sample wells. Replace the cover and attach leads to the power supply (remember black leads are plugged into the black receptacles on the power supply).

Set the power supply to 120 volts. Turn on the power supply and let it run for roughly 40 minutes or until the dye front is 1 cm from the end of the gel. While you are waiting for the electrophoresis to be completed continue with **Activities: DNA Isolation (below).**

You will not be able to see the DNA bands, even after the run is complete. Your gel (along with the other groups) will be stained according to the following:

After 40 minutes, turn off the power supply and disconnect the electrodes from the power supply. Remove the gel bed and gently slide the gel into the staining tray. Your instructor will continue the staining and destaining process. The stain, ethidium bromide binds to DNA and is a mild mutagen and must be handled carefully. Your instructor therefore will perform the staining and destaining procedures.

Once destaining is complete, the gels will be placed on a UV viewing chamber. After you have viewed the gel your instructor will provide you with a photograph, a print out, or a digital image of the gel for you to analyze further.
Staple your image to your lab report.

Which sample contained the smallest fragment of DNA? ______________________

Which sample contained the largest fragment of DNA? ______________________

Which sample contained the most fragments of DNA? ______________________

Based on your results, which suspect is likely the guilty one? Why?

Lets say you repeated your experiment, but this time added a third restriction enzyme. The bands from the third enzyme were identical in the two suspects as well as the evidence found on the victim. Would your conclusions be any different?

Is it possible for two different (genetically different) individuals to have fragments of identical-length DNA produced when treated with the same restriction enzyme? If so, how might this cause a problem in court cases?

If you were on a jury and were presented with an RFLP analysis similar to the one you performed, would you be willing to convict the suspect based only on the results of just two restriction enzymes? How many tests would it take for you to be convinced that the body fluid evidence did in fact come from the suspect you feel is guilty?

Online report (Please include your name and the names of your partner/s when posting information):

1. Import you gel image into a windows journal document. Label the wells and the fragments. Review the example given in the pre-lab activities.
2. Upload the digital representation of your DNA fingerprint gel to the discussion board. Include a 'key' that describes which sample is included in each well and indicates the number of fragments seen in each well.
3. On the discussion board, post your conclusions regarding which suspect is guilty of this crime. Discuss how you came to this conclusion.
4. Review the postings of the other groups in your section. Comment on their conclusions.

ACTIVITIES : DNA Isolation

Materials

Equipment	Supplies
Mortar and pestle	Liver
Cheesecloth	Sodium chloride (NaCl)
Ice bath	SDS (a detergent)
250 mL beaker	95% Ethanol (ice cold) in dropper bottles
	Glass rod
	Test tube

Part I- DNA Isolation (work in groups of 2)

In this experiment, you will isolate DNA (along with its associated proteins) from liver just to see what DNA looks like at the macroscopic level and to gain an appreciation of how much DNA is present in cells.

Obtain a small piece of liver.

Grind the liver, along with 10 ml of NaCl (saltwater) and 3 ml of SDS (a detergent, used to disrupt membranes), with a mortar and pestle for 3 minutes.

Then, strain your mixture through a piece of 4-ply cheesecloth (to remove the larger cellular debris) into a 250 ml beaker. Pour the mixture into a clean test tube.

Gently, drop cold ethanol down the side of the test tube (try not to stir things up too much). You should see a cloudy precipitate at the interface between the liver extract and ethanol. Gently stir with a glass rod if you don't see the precipitate.

Using either your PDA or the digital camera take a picture of your spooled DNA; you will need to upload this image to the course website.

Briefly describe your observations.

The DNA becomes visible and precipitates out of solution due to the interaction of long strands of DNA with the proteins found in the cell. If we treated the liver with harsh chemicals that denatured the DNA and cut it into individual bases, would you expect to see a precipitate?

Why do you think we chose animal cells instead of plant cells to isolate DNA (what structure is located exterior to the cell membrane)?

When finished, dump the extract down the sink, wash and rinse the test-tube and mortar and pestle.

Online report (Please include your name and the names of your partner/s when posting information):

1. Take a picture of your spooled DNA. Upload your group's image of the spooled DNA to the discussion board for your section. Provide a description of the image.