

BIOLOGY – LABORATORY EXERCISE		
	Name:	
	Lab Day: M T W Th F	
	Table / Group Number:	
BIOTECHNOLOGY		

Objectives:

At the end of this laboratory, you should be able to:

1. Understand the process of DNA fingerprinting.
2. Identify the steps used in the process of recombinant DNA technology.
3. Understand how the process of gel-electrophoresis separates molecules.
4. Explain and support your ethical decision regarding recombinant technology.

Introduction:

Previously we focused on how the DNA in genes controls the formation of proteins. Today we will see how proteins affect phenotypes. We will also learn how to separate proteins by their size and charge using a process called electrophoresis. Currently electrophoresis is used to separate DNA samples and is one step in the process of DNA fingerprinting. Then we will transfer a gene for making a protein from a human to a bacteria. Finally we will view some of the ways gene transfers are being used to alter plants; to make human proteins as well as the ethical questions raised by these new techniques.

Activity 1: DNA Fingerprinting:

DNA fingerprinting takes advantage of the normal variation that occurs within DNA from person to person. These differences can then be used as a means of comparison that may help police convict a criminal suspect or determine the paternity of a child.

To do this, scientists analyze fragments of DNA called RFLP's (restriction fragment length polymorphisms) and look for similarities/differences between RFLP's of two or more people. RFLP's are obtained by cutting DNA with restriction enzymes that cut DNA at specific base pattern locations. Since everyone's DNA is unique (with the exception of identical twins) the number of cuts and their specific locations within the DNA varies from person to person producing fragments of different sizes. (See Figure 1 below on left)

The DNA from each subject is then separated out by electrophoresis producing a specific banding pattern. (See Figure 1 below on right)

Figure 1: Restriction enzymes and fragment analysis



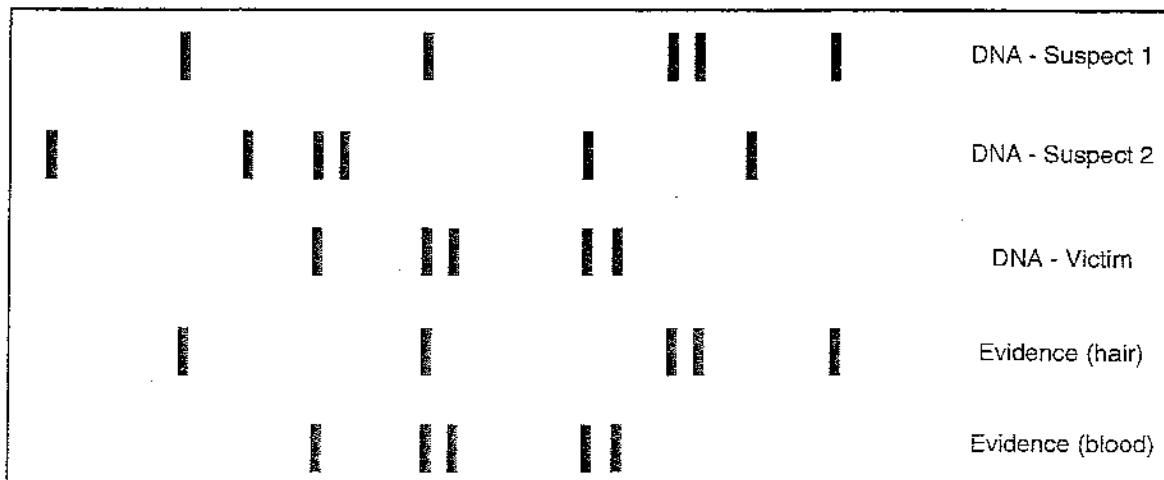
By comparing the banding patterns, scientists have an incredible diagnostic tool that can reach into many areas of science.

Activity - DNA Analysis

In a criminal investigation, DNA from biological material left at the crime scene (e.g. blood, hair, tissue, or semen) is compared to that of the victim and any suspects in the case. In the following activity, you will be analyzing DNA fingerprints and answering the questions below.

A 23 year old male was found murdered in the hallway of his apartment building. Investigators collected many samples of evidence including blood found on a knife near the body and hair from the victim's shirt. Police had two suspects due to eye - witness accounts. Both suspects, the victim and the evidence were analyzed using DNA fingerprinting.

Figure 2: DNA fingerprinting in a criminal case



a. Who does the blood evidence most likely belong to?

b. Who does the hair evidence most likely belong to?

c. What can you say about suspect 1 and suspect 2 as related to this criminal case?

d. Could you say you know who committed the crime without a doubt?

Why or why not? Give reasons for your answers

Activity - Simulating DNA Fingerprinting

Procedure:

1. Prepare Materials Needed for the Simulation

a. Construct the Human Cell DNA.

Obtain a copy of each CELL DNA page from the teachers desk. Being careful to keep the pieces from each page separate, cut the CELL DNA into 5 strips and assemble them in order (tape strip 1 to strip 2 to strip 3, etc.) to form one long strip for each CELL DNA molecule. When completed you should have 3 paper strips each representing a CELL DNA molecule from a different individual. You may wish to mark each strip to distinguish one from the other. Throughout this procedure it is critical that you are able to keep each CELL DNA molecule separate from all others.

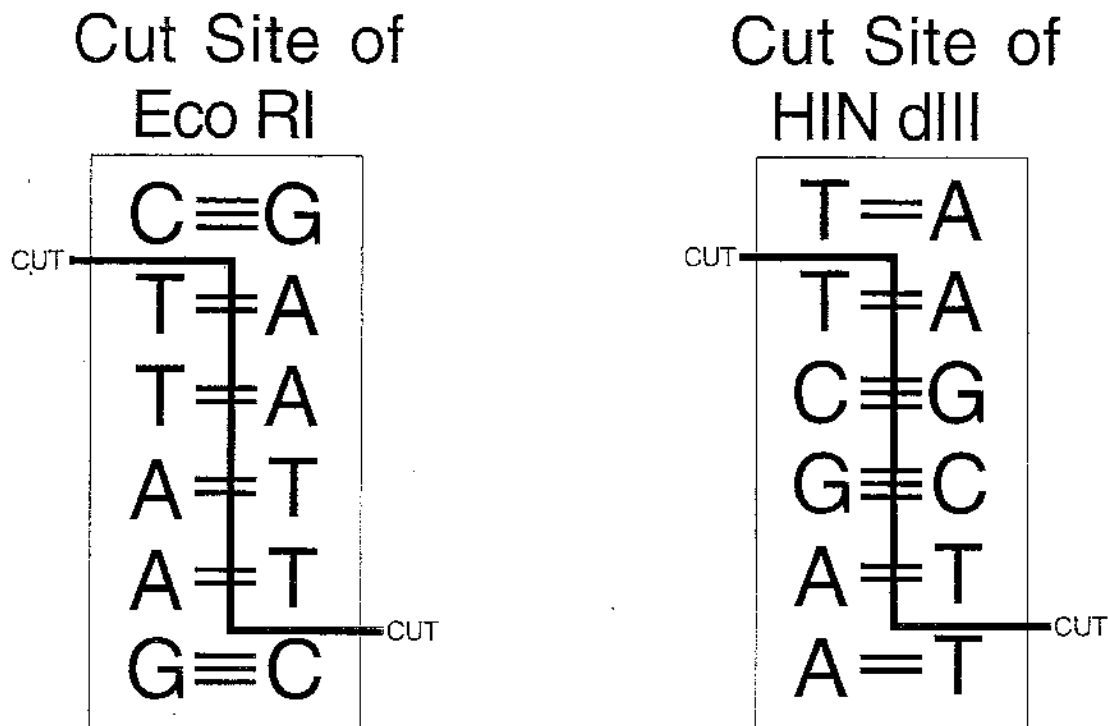
2. Use of Restriction Enzymes.

Use the Eco RI and Hin DIII RESTRICTION ENZYMES illustrated (fig. 3) to identify ALL possible cut sites on each CELL DNA molecule. Notice that each restriction enzyme can only cut DNA at very specific sites determined by the sequence of nucleotides and produces a staggered cut forming "sticky ends." Use a highlighter to mark each restriction site (use different colors to indicate restriction sites of the different restriction enzymes). There are several sites where the DNA can be cleaved or cut.

3. Cut the CELL DNA.

Use scissors to simulate the action of the selected restriction enzymes and cut the CELL DNA at all of the previously labeled restriction sites (be sure your cuts are properly staggered). Keep the resulting fragments separate, do not allow fragments from different CELL DNA molecules to become mixed.

Figure 3: Eco RI and HIN DIII Restriction Enzymes



4. Simulate the Separation of the DNA Fragments by Electrophoresis

Electrophoresis is a process that is used to separate molecules based on their size, shape and charge. The molecules are placed in a support of agarose gel and subjected to an electrical field. The molecules then move through the gel dependent upon their inherent properties. In this simulation we will assume that all CELL DNA fragments will move a distance inversely proportional to their length (shorter fragments travel farther than longer fragments). Determine the length of each fragment by counting the number of nucleotides (single or paired) from one end of the fragment to the other. Shade in the appropriate box in figure 4 to represent the position that the DNA fragment will migrate to and thus produce a visible band. Repeat for all DNA fragments.

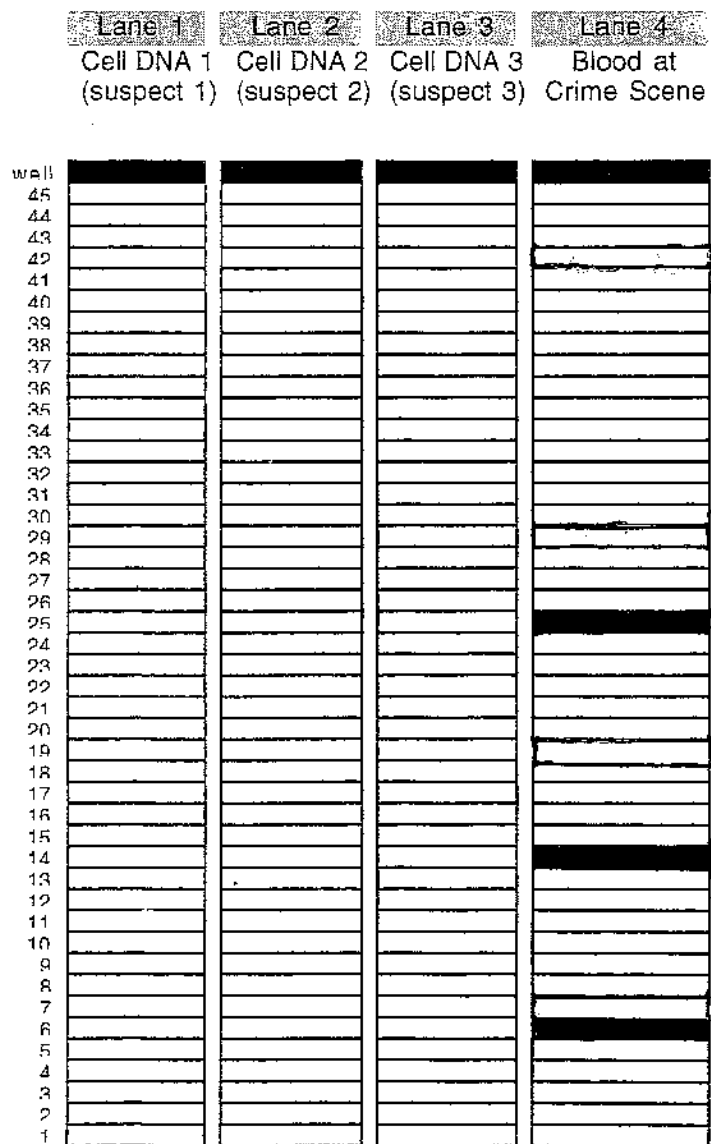
5. Compare the Banding Patterns Produced

a. Why was it imperative that the CELL DNA molecules and their fragments be kept separate?

b. Who does the blood evidence most likely belong to?

c. What can you conclude about each suspect as related to this criminal case?

Figure 4: Simulated DNA Gel Electrophoresis



Activity 2: Recombinant DNA Biotechnology

These days recombinant DNA is a common topic in papers and TV. A genetically engineered tomato recently was added to the foods we eat, bacteria have been used to make insulin for many years, and the cure for cystic fibrosis appears to be just around the corner. What do all these things have in common? They all fit under the

general heading of **genetic engineering** or the use of genes to make products mankind desires. In this activity we will examine the first step in this process called **recombinant DNA**.

Essentially, recombinant DNA means taking a gene from one organism and transferring it into another host organism. For example, the gene for human insulin has been removed and inserted into the DNA of a bacterium. Then, the recombinant bacteria are grown in huge vats and the human insulin they produce (following the instructions on the inserted human gene) is collected for use by persons with diabetes. The steps used in this process are:

1. Identify the gene that codes for the desired protein.

Scientists do this by identifying the sequence of amino acids used to make a protein and then identifying the nucleotide sequence (codons) of which the gene is composed.

2. Isolate the gene from the DNA.

This can be done with the use of **restriction enzymes** (taken from bacteria) to cut DNA at a specific sequence; These enzymes can be used to cut the DNA on either side of the gene. Some enzymes make a staggered cut of the DNA yielding "**sticky ends**" or single strands of nucleotide bases that can bind with complementary "sticky ends." For example, the restriction enzyme called Eco RI will cut the sequence $\dots\overset{\text{G}}{\text{GAATTC}}\dots$ at the junction between the G and A.

The result is the DNA fragments $\dots\overset{\text{G}}{\text{CTTAA}}\dots$ and $\overset{\text{A}}{\text{AATTC}}\dots$. These asymmetrical ends are made of complementary nucleotides. Thus, ensuring the DNA from two different organisms will "stick" together.

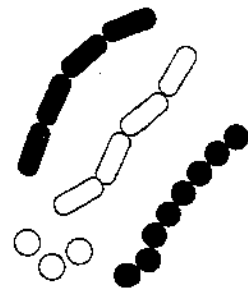
3. Transfer the gene to the host cell.

A common vector for this purpose is a small ring of DNA found in bacteria called a plasmid. The plasmid is first removed from the bacteria. Then the same restriction enzyme that was used to cut the gene is used to cut the plasmid. This produces "sticky ends" that will match those on the cut gene.

Because plasmids also contain a **replication origin** they will be duplicated when the bacterial cell that they have been inserted in divides. Thus the replication origin region must also be included with the gene.

Then, the cleaved plasmid and the cleaved gene are mixed together. Hopefully, the "sticky ends" will come together forming a new plasmid containing the replication origin, the gene of interest and any marker gene selected. A marker gene, such as a gene that provides for antibiotic resistance, will help identify the bacteria that incorporate the recombinant DNA plasmid.

Finally, the recombinant DNA plasmid is mixed with the host bacteria. To check that the plasmid has been taken up, it is tested for some characteristic such as drug resistance associated with the marker gene.



Procedure:

1. Prepare Materials Needed for the Simulation

a. Construct a bacterial plasmid.

Obtain a PLASMID page from the teacher's desk. Cut the plasmid into 5 strips of nucleotides. Tape the strips together end to end to form a circle. This can be done in any order as long as your plasmid contains the replication origin the gene for antibiotic resistance.

b. Construct the Human Cell DNA.

Obtain a CELL DNA page from the teachers desk. Cut the cell DNA into 5 strips and assemble them in order (tape strip 1 to strip 2 to strip 3, etc.) to form one long strip. This has already been completed in Activity 1.

2. Use of Restriction Enzymes.

Use the Eco RI and Hin DIII RESTRICTION ENZYMES illustrated (fig. 3) to identify ALL possible cut sites on the PLASMID DNA molecule. Notice that each restriction enzyme can only cut DNA at very specific sites determined by the sequence of nucleotides and produces a staggered cut forming "sticky ends." Use a highlighter to mark each restriction site (use different colors to indicate restriction sites of the different restriction enzymes). There are several sites where the plasmid can be cleaved or cut. The restriction enzymes must be the *same as those selected on the CELL DNA* to ensure that there will be "sticky ends" on the human CELL DNA and bacterial PLASMID DNA fragments that will match.

3. Mark the Important Sites on the Plasmid and Cell DNA.

Label the replication origin site and the antibiotic resistance gene on the plasmid, and the gene of interest on the cell DNA.

4. Cut the Plasmid and Human Cell DNA.

Use scissors to simulate the action of the selected restriction enzymes and cut the plasmid and cell DNA at all of the previously labeled restriction sites (be sure your cuts are properly staggered).

5. Insert the Cell Gene into the Plasmid DNA.

Use tape to simulate the action of ligase enzymes and fit matching sticky ends back together. If done correctly you should have a single circle of DNA, part bacterial (plasmid) and part human (cell DNA) containing the cell gene, the replication origin and the antibiotic resistance gene. Several pieces of DNA may be left over

Questions

a. Explain why it is possible to insert a gene from a human cell into the DNA from a bacterium?

b. What is a plasmid?

c. Describe the basic "tools" used in recombinant DNA. What are they and how do they serve as "tools"?

d. What are "sticky ends" and why are they important?

e. Explain what is meant by "recombinant" DNA.

f. What restriction enzymes did you choose to cut the cellular DNA and the plasmid DNA?

g. Explain how you could tell if the plasmid you designed was taken up by the bacteria into which you tried to insert it.

Activity 3: Separating proteins by electrophoresis:

Background: Electrophoresis is a process that is used to separate molecules based on their size and charge. The molecules are placed in a support of agarose gel and subjected to an electrical field. The molecules then move through the gel dependent upon their inherit properties.

In the following activity three known proteins (and one unknown protein mixture) will move within the gel and separate at a rate dependent upon their charge and mass.