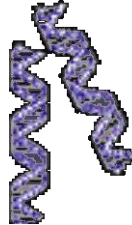


Biotechnology CLUES* Program



DNA FINGERPRINTING

Version A

DNA FINGERPRINTING ALLOWS SCIENTISTS TO COMPARE DNA FROM VARIOUS ORGANISMS AND IDENTIFY A PARTICULAR INDIVIDUAL.

DNA IS EXTRACTED AND CUT INTO FRAGMENTS. THE FRAGMENTS FORM A PATTERN ON AN ELECTROPHORESIS GEL. THAT PATTERN PROVIDES AN IDENTITY PROFILE.

IN THIS EXPERIMENT YOU WILL BE GIVEN A MYSTERY SAMPLE OF DNA. YOU WILL SEPARATE THE PIECES, PRODUCING A PATTERN ON A GEL, AND IDENTIFY YOUR DNA.

USES FOR DNA FINGERPRINTING:

- **CRIME WORK: RAPE AND MURDER***
- **PATERNITY SUITS**
- **MISSING PERSONS AND UNIDENTIFIED BODIES**
- **IMMIGRATION DISPUTES**
- **ANIMAL WORK**
 1. **BREEDING COMMERCIAL SPECIES (HORSES, CATTLE, DOGS, CATS ETC.)**
 2. **BREEDING ZOO ANIMALS**
 3. **BREEDING ENDANGERED SPECIES**

***DNA FINGER PRINTING IS PARTICULARLY IMPORTANT TO SOCIETY IN CRIMINAL CASES BECAUSE IT PROTECTS THE INNOCENT AND IMPROVES THE CONVICTION RATE OF THE GUILTY.**

For more information on College of the Canyons Biotechnology program, contact Professor of Biology Jim Wolf at (661) 362-3092 or email: jim.wolf@canyons.edu

***CLUES is an acronym for California Lutheran University Enriched Science Program, after which the College of the Canyons program is structured. College of the Canyons gratefully acknowledges the help of CLU in establishing this program.**

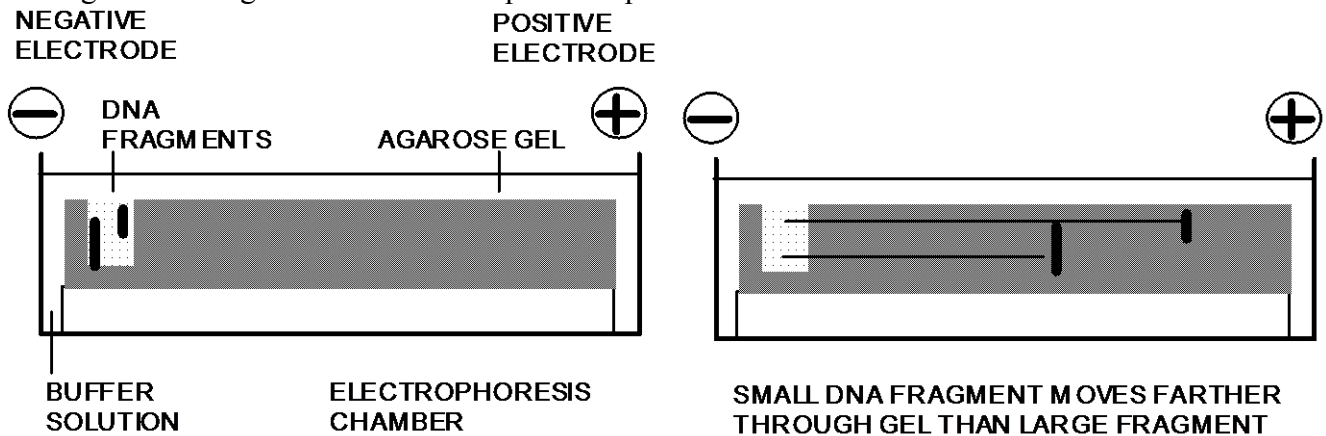
LAB UNIT 4: GEL ELECTROPHORESIS
Version A

OBJECTIVES:

1. To introduce students to agarose gel electrophoresis as a tool for separating DNA fragments
2. To allow students to work with restriction enzymes and understand their mechanism of action
3. To introduce students to the concept of DNA fingerprinting

I. BACKGROUND

One of the most basic and frequently used tools of the molecular biologist is electrophoresis. In this experiment, you will be using agarose gel electrophoresis to separate DNA fragments of different sizes. Electrophoresis means "to carry with an electrical current". What you will observe is that different sized pieces of DNA will be carried different distances by an electric current as the pieces migrate through agarose, a Jell-O like substance derived from seaweed, which you will apply in its melted state to a glass slide. The gel coated slide will be placed in an electrophoresis chamber filled with a buffer solution which contains the ions needed to conduct electricity. You will place your DNA samples into small wells cast at one end of the gel. Current supplied by electrodes at either end of the chamber creates an electric field across the gel. The DNA will migrate through the pores in the agarose toward the other end of gel. The diagrams below should prove helpful.



It is possible to do gel electrophoresis with DNA because the DNA molecule has an overall negative charge. The negative charge is due to the phosphate groups which alternate with the sugar, deoxyribose, to form the "rails" of the "twisted ladder" which makes up the double helix. When the current is turned on, the negatively charged DNA migrates along with other anions away from the electron- rich negative electrode toward the electron- poor positive electrode. The different DNA fragments separate according to their size because the smaller fragments migrate farther than the larger ones which experience more friction from the agarose network of the gel. After separating the fragments, you will turn off the current, remove the gel and stain it with methylene blue stain which binds to the DNA pieces and allows them to become visible.

While electrophoresis has many applications in state-of-the-art molecular biology laboratories, one of its most interesting and important uses is in the courtroom with a technique called DNA fingerprinting. DNA fingerprinting can reveal a DNA pattern which is unique to an individual and hence link a suspect to a particular piece of evidence with an extremely high degree of certainty, and since its first use in a 1987 Oklahoma murder trial, it has been used in several hundred criminal cases and in several thousand disputed paternity cases. For example, a hair sample found at the scene of a crime is taken to a forensic laboratory. There the DNA in the cells in the root of the hair is extracted and then is cut into pieces by special bacterial enzymes called restriction enzymes. Restriction enzymes do not cut the DNA randomly but cut at specific base pair sequences called recognition sequences. There are several hundred restriction enzymes available, and each has a specific recognition sequence which is usually composed of four to twelve base pairs. For example, the double stranded recognition site for the enzyme EcoRI is



The lengths of the fragments generated by a restriction enzyme digest depend upon the number of cuts made in a given piece of DNA and the location of each recognition sequence. An individual's DNA is as distinctive as a fingerprint, since restriction enzymes cut each person's DNA into different sized pieces. The different sized pieces result from cutting non-coding regions of the chromosomes called simple sequence DNA which probably serves to maintain the structural integrity of the chromosomes. Each person's simple sequence DNA cuts uniquely with restriction enzymes. When the cut DNA is separated into its pieces by electrophoresis, the visible end result looks something like the bar code on a super market package. The DNA from the root of the hair can then be compared with the DNA obtained from one or more suspects. A sample of the victim's DNA is also run as a control.

Use the practice problems (practice page 1 and 2 at end of module) to see examples of DNA fingerprinting.

The practice examples are vastly over-simplified. In actual criminal cases, several restriction enzymes may be used resulting in complex patterns consisting of dozens of DNA bands. The probability of any two individuals having identical band patterns is then computed. Usually the probability of two individual having the same bands is approximately 1 in 10^{19} which is many times the world's population. It is important to note that this method will also free an innocent suspect.

OVERVIEW OF THE EXPERIMENT:

You and your partner will be given three identical samples of a single "unknown DNA" which you are to identify through DNA fingerprinting. The experiment will take two periods.

1. On the first day you will save one of your DNA samples as a control, and you will digest the other two samples with two different restriction enzymes. One sample will be digested with BamH I, and the other will be digested with Hind III. BamH I is from the bacterium *Bacillus amyloliquefaciens*, and Hind III is isolated from *Haemophilus influenzae*. The digestions will be left overnight.
2. Following the protocol directions, the next day you and your partner will pour an agarose gel with four wells for DNA samples.
3. After the gel has hardened you will place the gel in the electrophoresis chamber which contains an electrolytic buffer.
4. Loading dye will be added to your DNA samples; they will be loaded into the wells and the current will be switched on.
5. After 15 minutes, you will turn off the current, remove your gel and stain the DNA samples with methylene blue stain.
6. You will finally identify the source of your DNA by comparing it with the gel patterns provided.

GEL ELECTROPHORESIS AND DNA FINGERPRINTING LAB Version A

Supplies for each lab station:

<u>Day 1</u>	<u>Day 2</u>	
<input type="checkbox"/>	<input type="checkbox"/>	Test tube rack
<input type="checkbox"/>	<input type="checkbox"/>	Three 1.5ml test tubes (day 1); one 1.5ml test tube (day 2)
<input type="checkbox"/>	<input type="checkbox"/>	1.5ml test tube containing distilled water "W"
	<input type="checkbox"/>	1.5ml test tube containing loading dye "L"
<input type="checkbox"/>		Restriction buffer (in 1.5ml tube) "R"
<input type="checkbox"/>	<input type="checkbox"/>	Micropipettes and tips
	<input type="checkbox"/>	10ml graduated pipette
	<input type="checkbox"/>	Pipet-aid
	<input type="checkbox"/>	3" X 2" glass slide
	<input type="checkbox"/>	Plastic comb
	<input type="checkbox"/>	Plastic staining tray
	<input type="checkbox"/>	Transparent plastic ruler
<input type="checkbox"/>	<input type="checkbox"/>	Marking pen (Please use regular pen or pencil for paper.)
	<input type="checkbox"/>	Electrophoresis device ("gel box")
<input type="checkbox"/>	<input type="checkbox"/>	Known DNA pattern sheet
<input type="checkbox"/>	<input type="checkbox"/>	Cup for used tips

Two lab stations will share:

- Power supply for two gel boxes

Supplies at a central location for use by the whole class:

- DNA unknowns (in 1.5ml tubes)
- Restriction enzymes (in 1.5ml tubes)
 - BamH I
 - Hind III
- Micropipettor tips
- 60-70° water bath with flasks of melted Agarose gel
- Bottle of "electrophoresis buffer"
- Bottle of methylene blue stain

Day 2

- Bottle for "used methylene blue stain"
- Transparent plastic sheets for tracing staining pattern and "gel viewers"
- Clock
- Deionized water
- 1 Kb ("one kilobase") ladder DNA Standard

Procedure for the Electrophoresis/DNA fingerprint lab
Check the list of materials against supplies at your station.

I. Cutting the DNA into fragments with restriction enzymes.

A. Label three 1.5 ml tubes with group initials and either "C", "B", or "H" ("C" is the control tube and will receive no enzyme. "B" will receive the restriction enzyme BamH I. "H" will receive restriction enzyme HinD III.)

B. Use the matrix below as a checklist. Read down each column, adding the same reagent to the appropriate tubes. Use a fresh tip for each reagent. If the micropipet stops turning before 1 μ l, do not force further. Use the smallest volume it will reach without forcing.

Record the letter of the unknown DNA you used on your answer page.

TUBE	DEIONIZED WATER	DNA	"R" (BUFFER)	Enzyme BamH I	Enzyme Hind III
C	8 μ l	2 μ l	0	0	0
B	5 μ l	2 μ l	2 μ l	1 μ l	0
H	5 μ l	2 μ l	2 μ l	0	1 μ l

C. Close the tubes, and place them in the portion cup where they will be kept at room temperature overnight.

Question. What part or location on a restriction enzyme enables it to recognize a certain nucleotide sequence? (Answer on answer page.)

- II. Pouring the agarose gel. (Next day or same day if gels can be kept submerged in buffer until they are run).
- A. Set glass slide on a smooth level section of lab bench where comb can be positioned over it later.
 - B. Fit the 10ml pipette into the pipet-aid and go to the flask of melted agarose gel; obtain about 8ml of the solution, return to your station and promptly spread 8ml of the warm solution on the glass slide and put the comb into position as has been demonstrated.
 - C. Allow the agarose solution to cool and solidify for at least 3-4 minutes.

Question. Compare the average kinetic energy of a water molecule in a sol (like liquid jello) to the average kinetic energy of a water molecule in a gel (like gelled jello). (Use answer page.)

III. Preparing for the electrophoresis

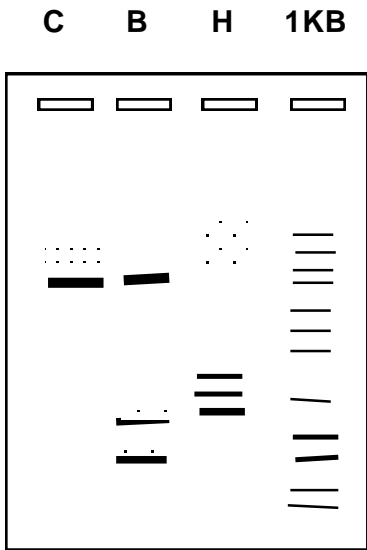
Record the letter of your unknown here _____

After reaction time for restriction enzymes:

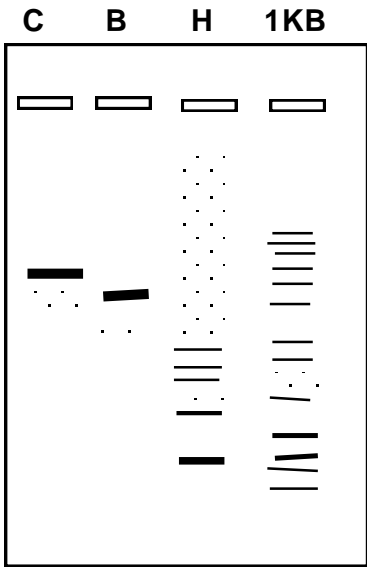
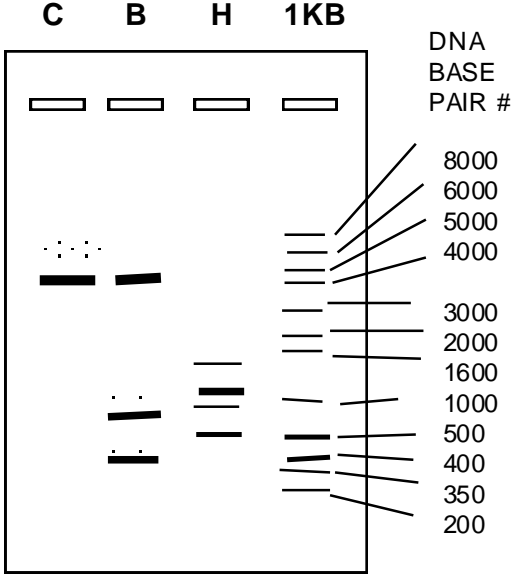
- A. Set up a fourth tube marked "1 Kb" for a DNA Standard. Add 9 μ l of water and 1 μ l of 1 Kb Ladder DNA. If micropipet will not go to 1 μ l, use the smallest volume it will reach without forcing.
- B. Add 2 μ l of loading dye to each of the four tubes with micropipette using a new pipette tip for each.
- C. By now the gel should be solidified. It will appear cloudy. Press down on the gel and gently lift the comb. Return comb to your lab station kit.
- D. Lift the slide with the gel and place onto the platform of the gel box with the wells toward the negative electrode (your left). Place the spacer block next to the slide on the platform.
- E. If necessary, add electrophoresis buffer to the chambers at the ends of the gel box until the buffer covers the surface of the gel, filling any dents at the location of the wells to give a smooth surface.
- F. Set the micropipette to 10 μ l and use it to transfer the contents of the reaction tubes into the wells in your gel. Change tips between samples! Lane 1 (closest to you): C, Lane 2 :B, Lane 3: H, Lane 4: 1 KB standard.

- IV. Running the gel (two groups share a power supply)
- A. Close the lid of the gel box without disturbing the buffer. Be sure that there is no spilled liquid on the table under the gel box, wires, or power supply.
 - B. Turn on the power supply. Adjust the power supply to be just under 200 volts. Note the time. Look to see if bubbles are being produced at the electrodes in the gel box and that the colored dyes in the loading dye are migrating. In exactly 15 minutes you will stop the electrophoresis run.
Meanwhile...
 - C. Label the top edge of the staining tray with your names or initials
 - D. Turn off the power supply after 15 minutes. The bluish dyes in the loading dye will have migrated about 1/2 and 3/4 of the distance to the end of the gel. These dyes are not DNA, but they help you to tell at a glance if the electrophoresis has taken place.
 - E. Remove the lid of the gel box.
- V. Staining the DNA in the gel.
- A. Transfer the gel to the staining tray. Return the glass slide to your kit.
 - B. Add Methylene Blue dye until the gel is barely submerged. Note the time and begin rocking the tray. You will stain for 5 minutes.
 - C. After 5 minutes pour the stain back into the "used stain" bottle and rinse the gel once in deionized water (NOT TAP WATER!). Then add enough deionized water to submerge the gel. If you will not "read the gel" until later, place the labeled tray in the designated location in your lab.
 - D. After the gel has destained (at least 5 minutes, or the next day) rinse the gel once more (deionized water) and pour off the water. Place the transparency on top of the gel and trace the locations of the wells and the bands of stained DNA onto the transparency. Each lab partner should trace his or her own transparency. Ignore the loading dye locations as you trace (loading dye is usually not "band-like").
 - E. Use transparent tape to attach the transparency to your lab report sheet.
 - F. Discard or save the gel as instructed.
- VI. Observations and conclusions.
- A. Complete questions for victim's DNA patterns on "Victim's DNA Pattern". (Victim's pattern page.)
 - B. Describe in a few sentences the pattern of the bands in your sample. Use the answer page.
 - C. Compare your samples to the patterns obtained from "known DNA" and 1 Kb ladder. Give your conclusions about the identity of the "unknown" DNA. Use the answer page.
 - D. Explain your results. If some uncertainty exists, describe and suggest explanations for it. Base your explanation on the overall pattern you observe as well as the lengths of the fragments on your gel. Use the answer page.

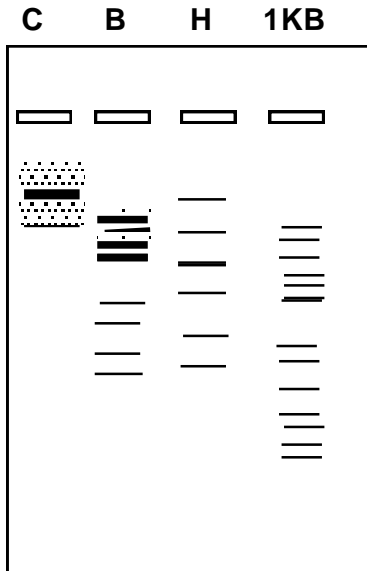
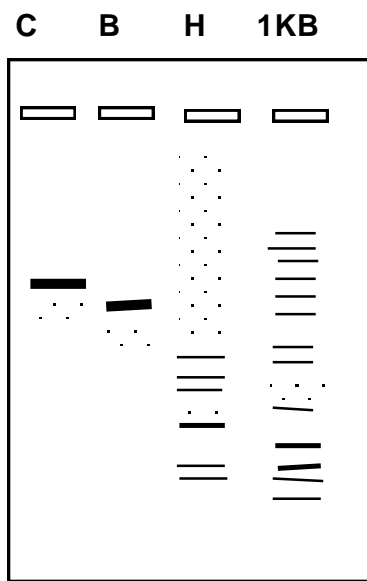
KNOWN DNA PATTERNS PAGE (NOTE: IMPERFECTIONS IN DIAGRAMS INTENTIONAL AS TO REFLECT TYPICAL GEL IMPERFECTIONS).



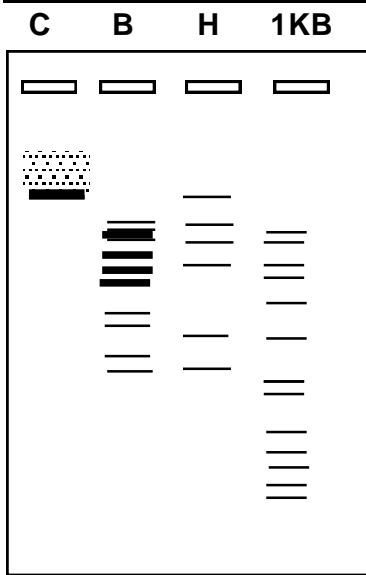
SUSPECT A
(BOTH GELS)



SUSPECT B
(BOTH GELS)



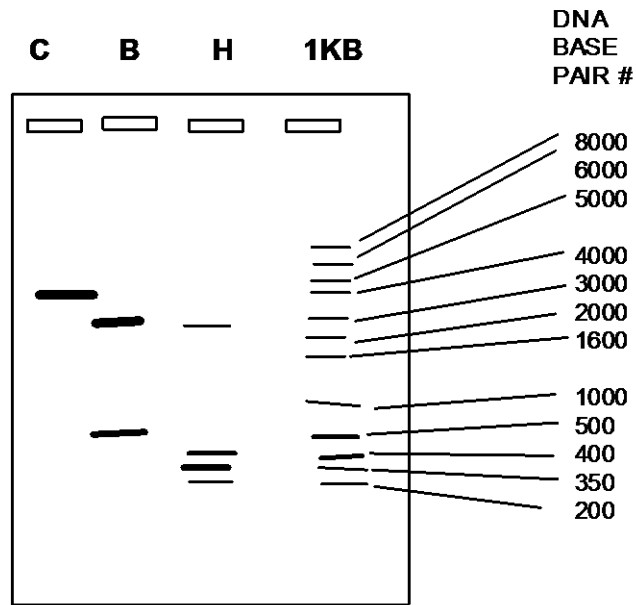
SUSPECT C
(BOTH GELS)



VICTIM'S DNA PATTERN PAGE

VICTIM'S DNA PATTERN

NAME _____



1. Approximately how long is the uncut control piece of the victim's DNA?
2. How long are the pieces of DNA that were cut with BamH I?
3. How long are the pieces of DNA that were cut with HinD III?
4. Describe the general patterns for the lanes C, B, and H starting from the gel wells.

Name _____

Record the letter of the unknown DNA you are using in your tubes: _____

Question. What part or location of a restriction enzyme enables it to recognize a certain nucleotide sequence?

Question. Compare the average kinetic energy of a water molecule in a sol (like liquid Jello) to the average kinetic energy of a water molecule in a gel (like gelled Jello).

YOUR RESULTS:

Describe in a few sentences the pattern of bands in your sample.

Compare your samples from the "known DNA" and 1 KB ladder. Give your conclusions about the identity of the unknown DNA.

Explain your results. If some uncertainty exists, describe and suggest explanations for it. Base your explanation on the overall patterns you observe as well as the lengths of the fragments on your gel.

Name _____

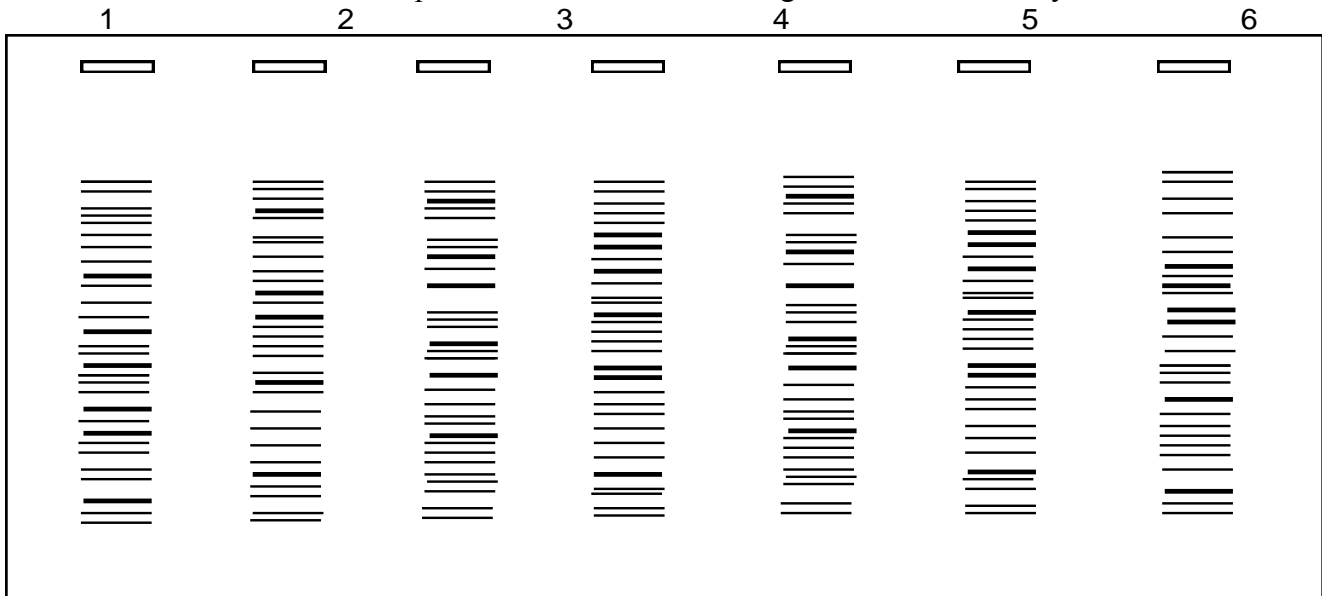
PART A: FORENSIC SCIENCE - RAPE CASES

Assume that you are a molecular biologist involved in forensic medicine. Two women have been raped within a span of 2 weeks and you have been given the following evidence relating to the crime: Blood samples from both women, semen collected on each victim, and blood samples from three possible suspects.

You purify the DNA from each sample, cut the DNA's with restriction enzyme and then perform agarose gel electrophoresis on the DNA as follows:

- Well 1 -- Blood from victim A
- Well 2 -- Blood from victim B
- Well 3 -- Semen collected on victim A
- Well 4 -- Semen collected on victim B
- Well 5 -- Blood from suspect X
- Well 6 -- Blood from suspect Y
- Well 7 -- Blood from suspect Z

Results from the electrophoresis of DNA after cutting with restriction enzyme:



Questions:

1. A. Were both women assaulted by the same man? _____
B. Explain your reasoning.

2. A. Which, if any, suspect or suspects, is/are involved?
B. Explain your reasoning.

Name _____

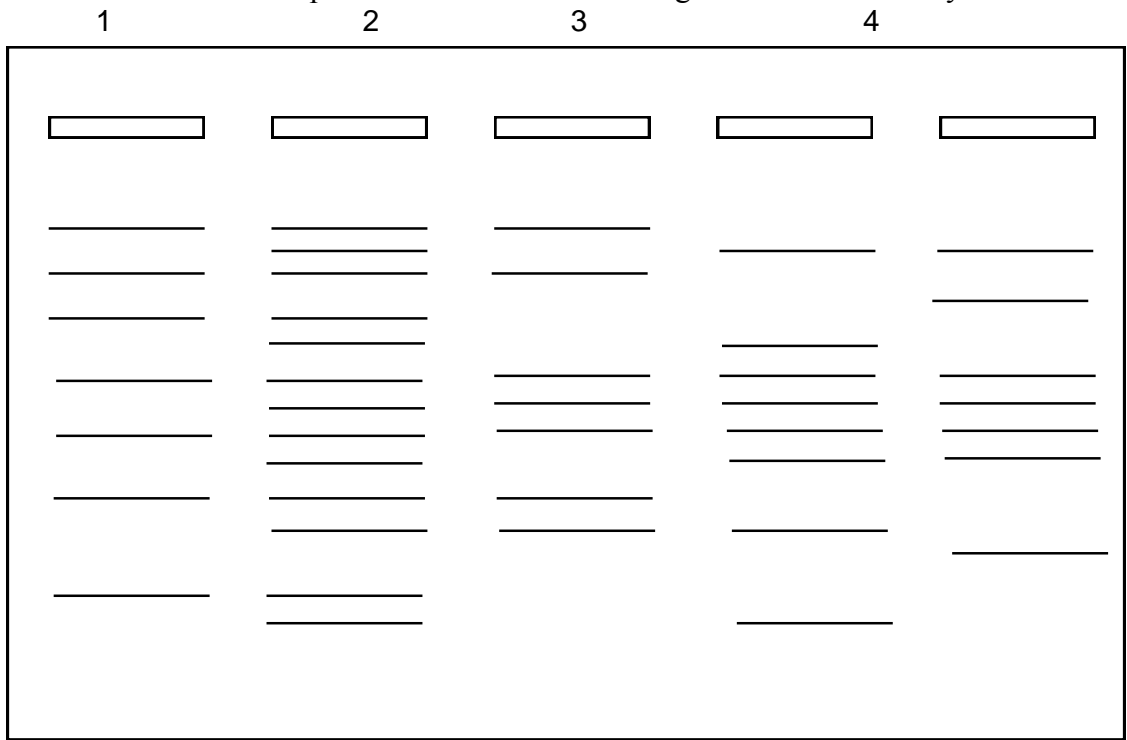
PRACTICE page 2

PRACTICE PART B: CONSERVATION SCIENCE - A PATERNITY CASE

Assume that you are a molecular biologist at a zoo. You are asked to help identify the biological father of a baby chimpanzee. After adding an appropriate restriction enzyme to each of the DNA samples, you perform agarose gel electrophoresis on the DNA.

- Well 1: Sample from mother's blood
- Well 2: Sample from baby's blood
- Well 3: Sample from blood of possible father E
- Well 4: Sample from blood of possible father F
- Well 5: Sample from blood of possible father G

Results from the electrophoresis of DNA after cutting with restriction enzyme:



Determine which DNA sample came from the blood from the child's actual father, and explain your findings.