COC Biotechnology Program

DNA FINGERPRINTING: VERSION B

In the time it takes you to complete this lab, your DNA could be extracted, amplified, analyzed and compared. Everything from a criminal past to family history and even the top ten most likely ways a disease will kill you (and when) could be assessed. This is a brave new world, and with it comes awesome responsibility. As members of today's scientific society, you should be aware of some of the technologies of DNA… here is lab on DNA Fingerprinting

- ✓ **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 AN UNKNOWN SAMPLE OF DNA. YOU WILL SEPARATE THE PIECES, PRODUCING A PATTERN ON A GEL, AND IDENTIFY A SUSPECT BASED ON DNA ANALYSIS (SIMILAR TO WHAT YOU WOULD SEE IN A COURT OF LAW OR ON CSI).**
- ✓ **USES FOR DNA FINGERPRINTING INCLUDE: CRIME WORK, PATERNITY SUITES, MISSING AND UNIDENTIFIED BODIES, IMMIGRATION DISPUTES AND ANIMAL WORK.**

***DID YOU KNOW THAT DNA EXONERATES MORE PEOPLE THAN IT CONVICTS? THAT B Y 2016, 20% OF ALL THE MONEY SPENT IN THE US WILL BE SPENT ON SCIENCE.**

Did you know that rigorous science training would make you more competitive for ANY type of job? Employers know that students who can tackle hard science will do well with almost ANY challenges presented them. For information on biotechnology and other robust science courses, contact: Jim Wolf, College of the Canyons Biotechnology Director at (661)362-3092 or email: jim.wolf@canyons.edu

LAB UNIT 4: GEL ELECTROPHORESIS
Version B

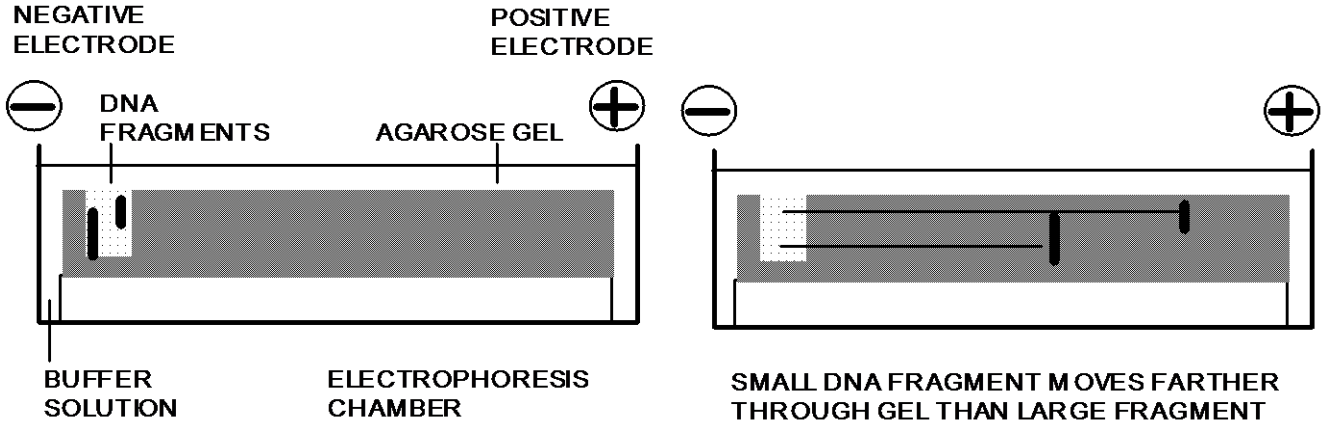
OBJECTIVES:

1. To introduce students to agarose gel electrophoresis as a tool for separating DNA fragments

2. 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 pieces 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 to a glass slide. The gel coated slide will be placed in a "gel box" where the gel serves as an "electrical bridge" between two electrodes submerged in a conducting solution. 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 and other negatively charged ions will migrate through the pores in the agars toward the positive 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 comes from the phosphate groups which alternate with the sugar, deoxyribose, to form the "rails" the "twisted ladder" of the double helix. The negatively charged DNA is attracted to the positively charged electrode when the current is turned on. The different sized DNA pieces separate because the smaller fragments will migrate farther than the larger. After the fragments are separated, you will turn off the current, remove the gel and stain it with methylene blue stain which will bind to the DNA pieces and allow 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 using a technique called DNA fingerprinting. DNA fingerprinting can reveal a DNA pattern which is unique to an individual and hence can link a suspect to a particular piece of evidence with an extremely high degree of certainty. For example, a hair found at the scene of a crime and is taken to a forensic laboratory. There the DNA in cells in the root of the hair is extracted and then is cut into pieces by special bacterial enzymes called restriction enzymes. An individual's DNA is as distinctive as a fingerprint since restriction enzymes cut each person's DNA into different sized pieces. 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 hair root cells can then be compared with the DNA obtained from one or more suspects.

The PRACTICE PAGE (at end of module) exercises should help you understand 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 individuals having the 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:

1. You will work in pairs; however, each of you will run a fingerprint on an "unknown" piece of DNA.

2. Following the directions on the next page, you and your partner will pour one agarose gel. The gel will have several wells so that each of you has a lane for your "unknown" DNA.

3. While your gel is hardening, you will each select one of the "unknown" DNAs from your teacher, and following the directions, you will add loading dye to your DNA.

4. After the gel has hardened, it will be placed in the solution in the gel box. The DNA samples of you and your partner will be placed in the wells on the gel and, and you will turn on the current as directed.

5. While the gel is running you will carefully examine the patterns of the possible "unknown" DNA's.

6. After fifteen minutes, you will turn off the current, remove your gel and stain the DNAs with methylene blue stain.

7. Finally you will figure out which "suspect" your DNA came from by comparing your gel pattern with the patterns provided.

GEL ELECTROPHORESIS AND DNA FINGERPRINTING LAB:

Version B

Supplies for each lab station:

- ☐ Test tube rack
- ☐ Two 1.5ml test tubes
- ☐ 1.5ml test tube containing loading dye "L"
- ☐ 1 micropipettor and tips
- ☐ 10ml graduated pipette
- ☐ Pipet-aid
- ☐ 3" X 2" glass slide
- ☐ Plastic "comb"
- ☐ Plastic staining tray
- ☐ Transparent plastic ruler
- ☐ Marking pen (Please use regular pen or pencil on paper.)
- ☐ Known DNA pattern sheet
- ☐ Small cup for used tips

Two lab stations will share:
Electrophoresis device ("gel box")

Four lab stations will share:
Power supply for gel boxes

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

- ☐ 1.5ml test tubes with DNA unknowns
- ☐ Micropipettor tips
- ☐ 60-70°C water bath with flasks of melted agarose gel
- ☐ Bottle of "electrophoresis solution"
- ☐ Bottle of methylene blue stain
- ☐ Bottle for "used methylene blue stain"
- ☐ Transparent plastic sheets for tracing staining pattern
- ☐ Gel viewers
- ☐ Clock
- ☐ Deionized water

Procedure for the Electrophoresis/DNA fingerprint lab

A pair of students should work at each lab station

Check the list of materials against supplies at your station

I. Preparing the gel

A. Set glass slide on a smooth level section of lab bench where comb can be positioned over it later.

B. Fit the pipet-aid to the 10ml pipette and go to the flask of melted agarose gel; obtain 8ml of melted agarose solution; return to your station and promptly spread 7ml of the warm solution on the glass slide; position the comb as has been demonstrated to you.

C. Allow the agarose solution to cool and solidify for at least 3-4 minutes.

Meanwhile...

II. Preparing the samples for electrophoresis

A. Each lab partner should label one of the 1.5ml test tubes with his or her initials.

B. Each member of the lab partner team will now get his or her sample of unknown DNA. Decide which of you is to go first. Set micropipette to 10µl and put on a tip. Take your micropipette and your tube to the location where your teacher is giving out the unknown DNA solutions.

1. Put a clean tip on your micropipette.
2. Check to see that the micropipette is set for 10µl.
3. Add10µl of DNA unknown solution to your tube.
4. Discard your pipette tip and get a new tip. Set your micropipette to 2µl.
5. Add 2µl of loading dye to the tube containing your DNA solution. Mix the contents well and discard the tip.
6. Give the micropipette to your partner.
7. Record the number of your DNA unknown solution on the answer sheet.

C. The second member of the team will now get his or her unknown DNA following Steps 1-5 and 7 in Part B.

III. Loading the gel

A. 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.

B. Lift the slide with the gel and place onto the platform of the gel box with wells at the left end.

C. Check to see that the solution gives a smooth surface over the gel with no dents at the wells. Only if dents are present should you add more electrophoresis solution.

D. Decide which lab partner will load the gel first. Set the micropipette for 10 µl. Get a new tip. Transfer the contents of your DNA sample into one of the wells in the gel. The other member of the team will get a new micropipette tip and transfer his or her DNA into the other well. Record which well contains your sample and which contains your lab partner's.

IV. Running the gel (two groups share a power supply).

A. Close the lid of the gel box without disturbing the solution and sloshing the DNA samples.

B. Turn on the power supply. Adjust the power supply to be just under 200 volts. Record the time. You will stop the electrophoresis run in 15 minutes. 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. Ask for help if no bubbles are visible.

Go on to next instructions while you wait for the gel to run.

V. While you wait: Housekeeping and practice analysis

A. Label the top edge of the staining tray with your names.

B. Observe the patterns for samples of DNA that you have been given on page 9. Write a few sentences describing the distance and pattern of the bands for each DNA. Use the answer page (pg. 8).

Sample description:

The overall pattern, starting from the well, is of a space, then two bands close together, then a space equal to the first space, then two more bands close together.

C. After 15 minutes turn off the power supply. The bluish dyes in the loading dye will have migrated about 1/2 to 3/4 of the distance to the end of the gel. (These dyes are not DNA, but they help you tell at a glance if the electrophoresis has worked.)

D. Remove the lid of the power supply. Pick up the slide with the gel (be careful because the gel tends to slip off the slide) and then place the gel into the staining tray. Return the glass slide to your kit.

VI. Staining the DNA in the gel.

A. Add methylene blue dye until the gel is barely submerged. Note the time and begin rocking the tray. You will stain for 5 minutes.

B. 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.

C. 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. Ignore the loading dye locations as you trace (loading dye is usually not "band-like"). Each lab partner should make his or her own transparency.

D. Use transparent tape to attach the transparency to your lab report sheet (pg. 8).

E. Discard or save the gel as instructed.

VI. Observations and conclusions.

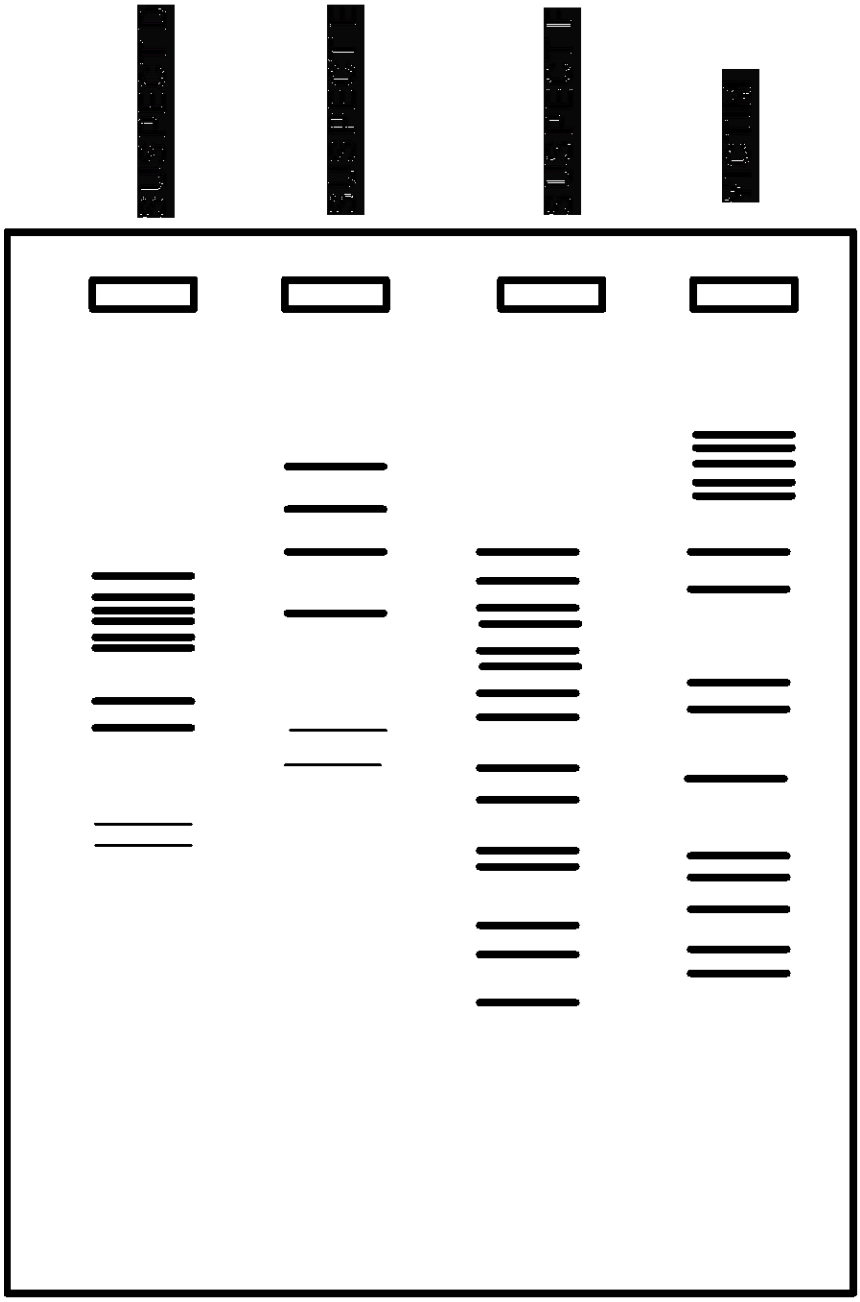
A. Measure the distance that your stained DNA bands migrated in the gel and record on below.

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

C. Compare your sample to the patterns on page 9 and give your conclusions about the identity of the person whose DNA was your "unknown." Explain how you made the determination.

KNOWN DNA FINGERPRINTS
VERSION B

(NOTE: ACTUAL DNA BANDS MAY NOT BE AS DISTINCT OR NUMEROUS)



Name_____ **PRACTICE page 1**

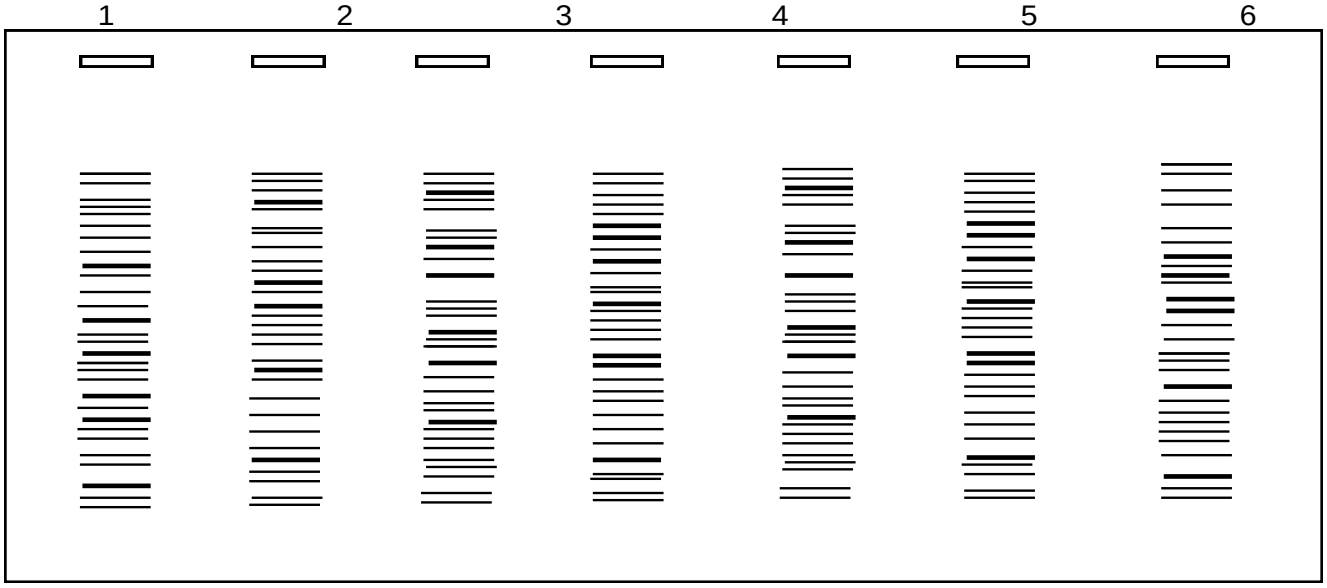
PART A: FORENSIC SCIENCE - RAPE CASES

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.

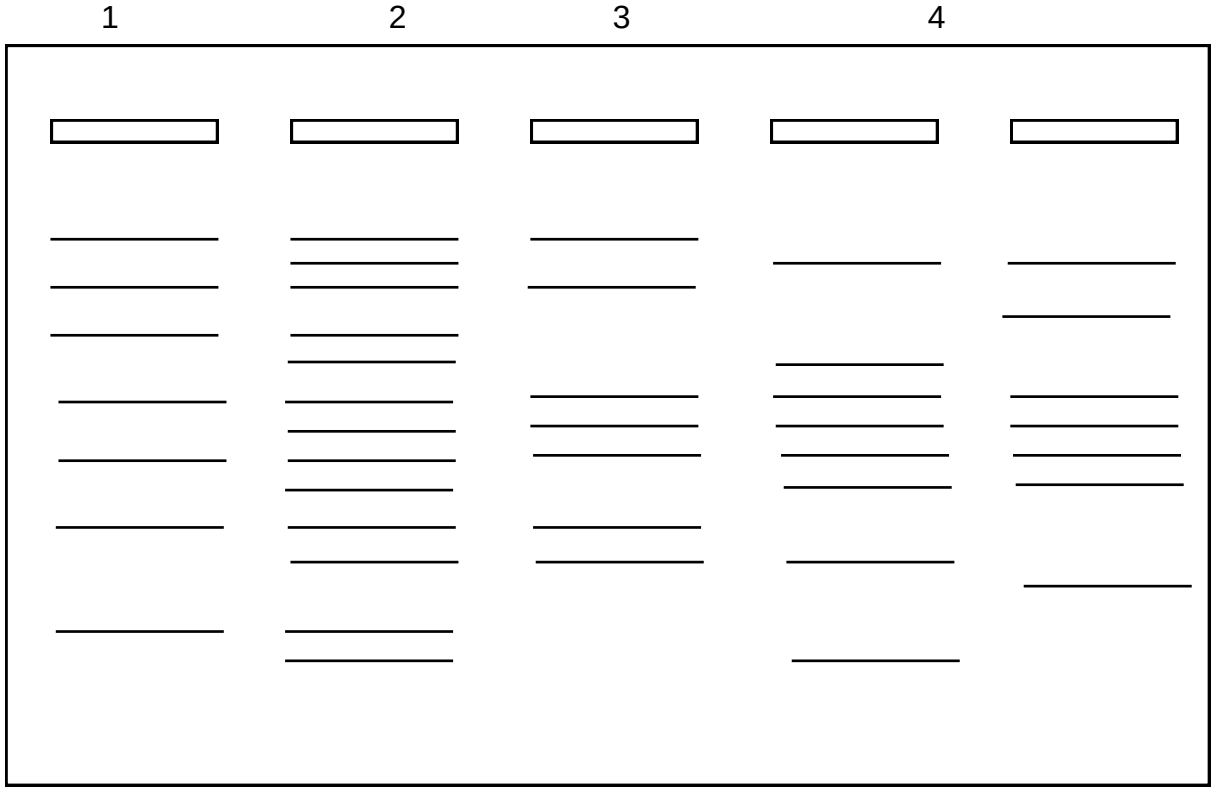
Name_____ **PRACTICE page 2**

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.