COC Biotechnology Program

DNA FINGERPRINTING: VERSION C

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
GOT SCIENCE? GET AHEAD!

OVERVIEW OF THE EXPERIMENT:

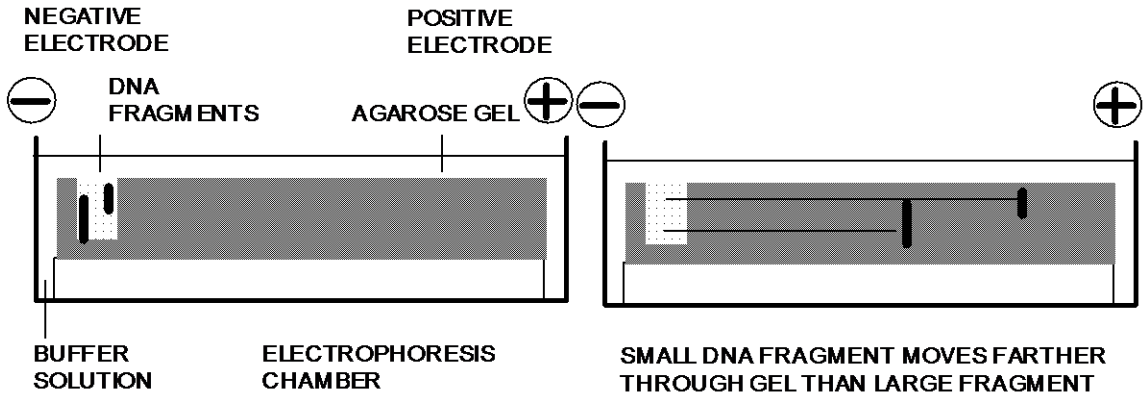
1. You will work in pairs. You will receive samples of four simulated DNA unknowns.
2. You and your partner will pour an agarose gel. The gel will have wells for loading the samples.
3. While your gel is hardening, you will add glycerol to the "unknown DNA" samples. Glycerol makes the samples dense enough to sink into the wells.
4. After the gel has hardened, it will be placed in the solution in the gel box. The "DNA" samples will be placed in the wells on the gel, and you will turn on the electrical current.
5. While the gel is running you will carefully compare the patterns of the known DNA's.
6. After 8 minutes, you will turn off the current, remove your gel and observe the patterns produced by your unknowns.
7. Finally you will compare the patterns on your gel to the known DNA patterns provided and draw conclusions about the identity of each unknown.

I. BACKGROUND

DNA "fingerprinting," like ordinary fingerprinting, makes use of a characteristic pattern that can be compared between individuals. What makes DNA "fingerprinting" special is that no fingers are required to do it, so an animal without fingers, a plant, or any other kind of living thing can be identified by its DNA fingerprint.

DNA is the code molecule that is found in every living cell and that tells cells what to do. Since every organism is a little bit different from every other (except for identical twins), the DNA instructions in one organism are different from those of others. DNA is found in cells, so it is found throughout the body. DNA can be collected painlessly from blood, skin, or even hair. In the lab the DNA is cut at specific code sequences using special enzymes made by bacteria. The resulting pieces from different organisms vary in length just as the overall DNA's vary. Finally, the DNA pieces are made to "line-up" according to their length by a process called electrophoresis. The resulting pattern of "lines" lets scientists identify all organisms by their DNA, just as people are identifiable by the patterns on their fingertips.

The name electrophoresis means "to carry with an electric current." Electrophoresis is a process of moving molecules by attracting them to an electric charge that is opposite to their own charge. DNA has a negative charge, so it moves toward the positively charged electrode and away from the negatively charged electrode. The molecules are forced to travel through a Jell-O like substance placed between the oppositely charged electrodes. Larger pieces of DNA move more slowly than smaller pieces because larger pieces get tangled up more often in the web-like structure of the gel. The gel is made of agarose, a product made from seaweed.


NEGATIVE ELECTRODE
POSITIVE ELECTRODE
DNA FRAGMENTS
AGAROSE GEL
BUFFER SOLUTION
ELECTROPHORESIS CHAMBER
SMALL DNA FRAGMENT MOVES FARTHER THROUGH GEL THAN LARGE FRAGMENT

Name _____ PRACTICE page 1

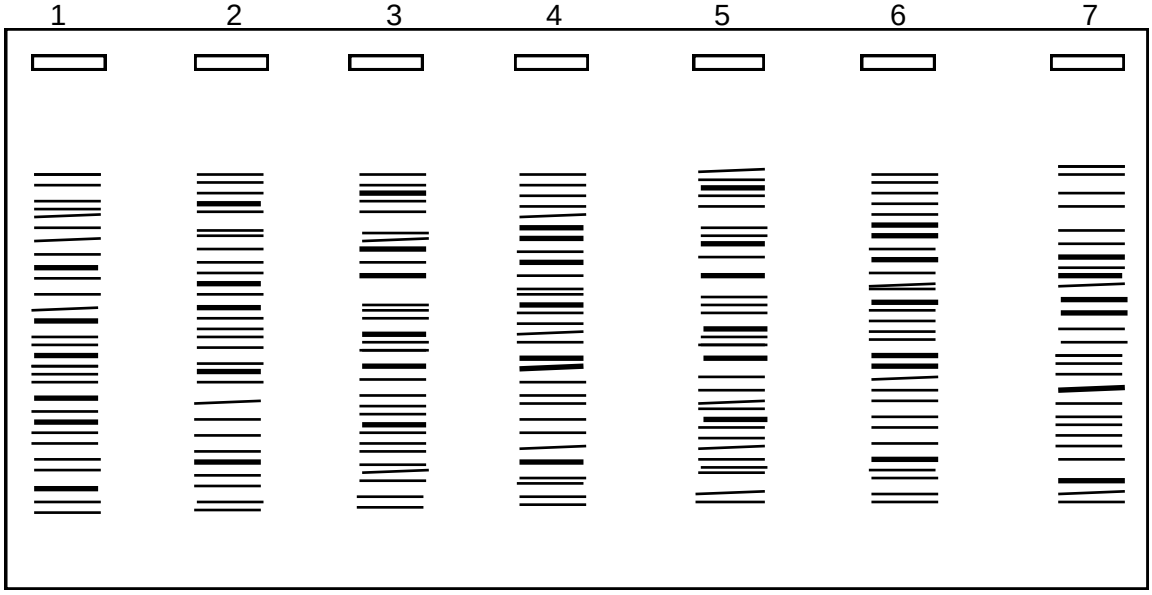
PART A: FORENSIC SCIENCE - RAPE CASES

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? _____

Explain your reasoning.

2. A. Which, if any, suspect or suspects, is/are involved?

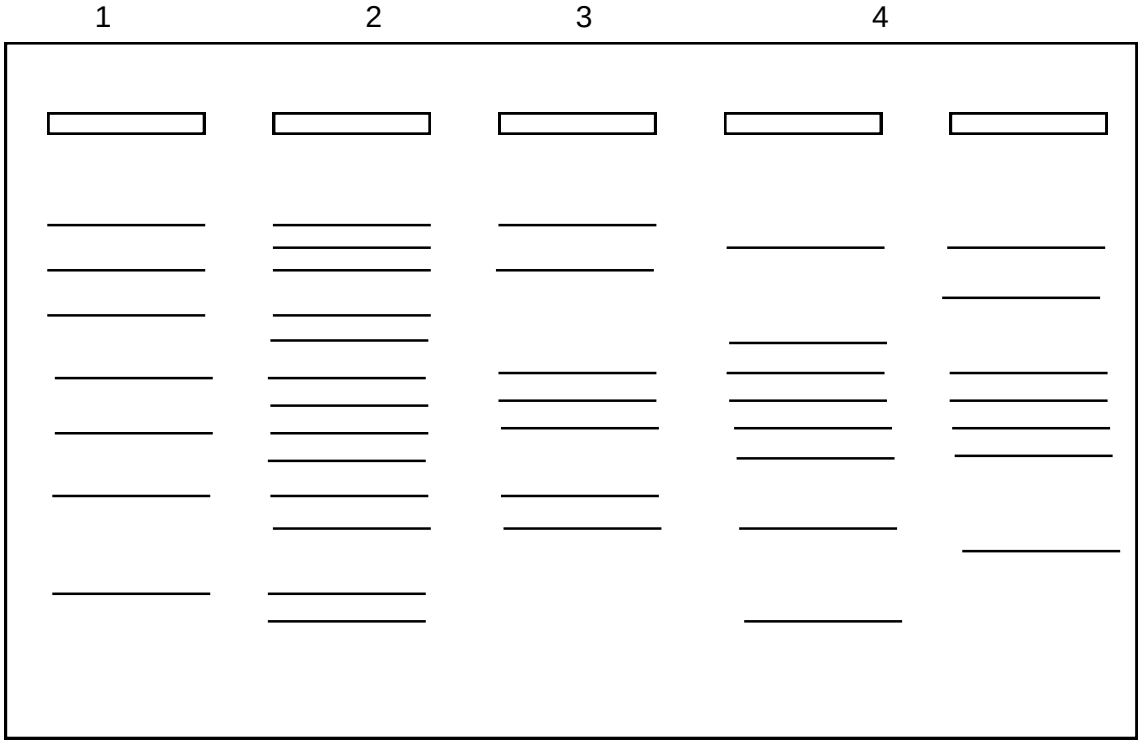
B. Explain your reasoning.

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: Samples 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.

GEL ELECTROPHORESIS AND DNA FINGERPRINTING LAB:
Version C

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

- ☐ 65°C water bath with flasks of melted agarose gel
- ☐ Bottle of electrophoresis solution

Check off the checklist of materials for the supplies at your station. A pair of students should work at each lab station.

- ☐ Test tube rack
- ☐ Four empty 1.5 ml test tubes
- ☐ Four 1.5 ml test tubes containing "DNA unknowns" "11", "12", "13", "14"
- ☐ 1.5 ml test tube containing glycerol "G"
- ☐ Micropipette and tips
- ☐ 10 ml graduated pipette
- ☐ Pro-pipette
- ☐ 3" X 2" glass slide
- ☐ Plastic "comb"
- ☐ Plastic sandwich bag
- ☐ Marking pen (Please use regular pen or pencil on paper.)
- ☐ Small cup for used tips
- ☐ Electrophoresis device ("gel box")
- ☐ Transparent plastic sheets for tracing staining pattern
- ☐ Power supply for gel boxed (Two lab stations will share one power supply.)

Procedure (Wait until your teacher tells you to start.)

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 pro-pipette to the 10 ml pipette and go to the flask of melted agarose gel; obtain 8 ml of melted agarose solution; **return** to your station and promptly empty the warm solution in the center of the glass slide; **place** the comb one centimeter from one end of the slide. ☐

C. **Allow** the agarose solution to cool and solidify for at least 3-4 minutes. While you wait for the solution to cool, **go on** to the next instruction. ☐

II. Preparing the samples for electrophoresis

A. **Label** the empty 1.5 ml test tubes with the numbers 11, 12, 13 and 14. ☐

B. Add samples of unknown DNA to your tubes as follows:

1. Set micropipette to 10 µl. ☐
2. Put a clean tip on your micropipette. ☐
3. Check to see that the micropipette is set for 10 µl. ☐
4. Add 10 µl of "DNA unknown #11" to your tube labeled 11. ☐
5. Discard your pipette tip and get a new tip. Go to step 2 and repeat the process for samples 12, 13, and 14. Use a new tip each time.
   12 ☐ 13 ☐ 14 ☐

C. **Add** glycerol to each tube as follows:

1. **Set** micropipette to 2 (l. **Get** a new tip. **Add** 2 (l of glycerol to the tube containing your DNA 11. Mix the contents well and **discard** the tip. ☐
2. Repeat step 1 for tubes 12, 13, and 14. Use a new tip each time. ☐ ☐ ☐

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 glass slide with the gel and **place** slide and gel onto the platform of the gel box with the wells at the left end, near the black electrical terminal. ☐

C. **Check** to see that the electrophoresis solution covers the gel with no dents at the wells. Only if dents are present should you add more solution. ☐

D. **Be sure** the gel box is placed near the power supply because the gel box cannot be moved after the samples are loaded. ☐

E. Decide which lab partner will load the gel first. **Set** the micropipette for 10 µl. **Get** a new tip. Transfer 10 µl of your sample #11 into well #1. **Get** a new micropipette tip and transfer Sample #12 into well #2. Now the other member of the team will **load** samples #13 into well #3 and #14 into well #4. **Use** a new pipette tip every time. ☐

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

A**.** **Taking care** not to move the gel box, close the lid. Avoid moving the gel box to avoid sloshing the DNA samples out of their wells. ☐

B. **Check** to see that the wires connect the gel box to the power supply. Turn on the power supply. Adjust the voltage to be just under 200 volts. Record the time. You will stop the electrophoresis in 8 minutes. **Look** to see if bubbles are being produced at the electrodes in the gel box and that the samples are migrating. **Ask** for help if no bubbles are visible. ☐

V. A. While you wait, **label** the edge of the plastic sandwich bag with your names. ☐

B. **Observe** the patterns for the "known DNA fingerprints" and write a short description of each pattern on your Observations and Conclusions Page. ☐

C. After 8 minutes turn off the power supply. ☐

D. Remove the lid of the gel box. Pick up the glass slide with the gel on the top (be careful because the gel tends to slip off the glass) then **push** the gel off the glass slide into the sandwich bag. Return the glass slide to your kit. ☐

VI. Tracing your gel

A. **Place** the transparency on top of the plastic bag containing the gel. On the transparency, **draw** a line around the edges of the gel, **then** trace the locations of the wells. Finally, **draw** outlines around the patterns on your gel. Label the patterns with their colors and label the lanes 11, 12, 13, 14. Then your partner will use another transparency to make a tracing for his or her lab report sheet. ☐

B. Use transparent tape to attach the transparency to your lab report sheet. ☐

VII. Compare your gel to the patterns obtained from “Known DNA” and give your conclusions about the identity of each person on your gel whose “DNA” was “unknown.” Put answer on the Observations and Conclusions Page.

Name _____**Observations and Conclusions Page**

IV. B **Observe** the patterns for the "known DNA fingerprints" That you have been given. **Write** a sentence describing the pattern for each "known DNA fingerprint." Example description: Starting from the well, the pattern for "Suspect M" is a space, then a circular area of red, then a space equal to the first space, then a circular area of blue

Suspect R:

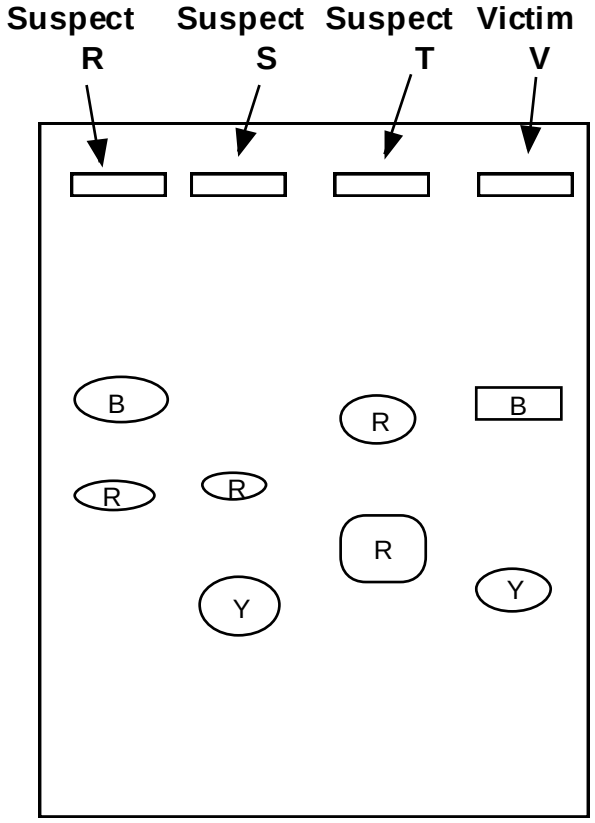
Suspect S:

Suspect T:

Victim V:

KNOWN DNA FINGERPRINTS

VI.B: Attach your transparency here.



B= BLUE R= RED Y = YELLOW

VII. Compare your gel to the patterns obtained from "known DNA" and give your conclusions about the identity of each person on your gel whose "DNA" was "unknown."

Your Sample	Identity of Known Person	Explain your reasoning:
11.		
12.		
13.		
14.		

Name _____**Observations and Conclusions Page**

B **Observe** the patterns for the "known DNA fingerprints" that you have been give**n.**
Write a sentence describing the pattern for each "known DNA fingerprint." Example description: Starting from the well, the pattern for "Suspect M" is a space, then Small oval-shaped stained area, then a space equal to the first space, then a larger oval-shaped stained area. (Include color of each stained area if available.)

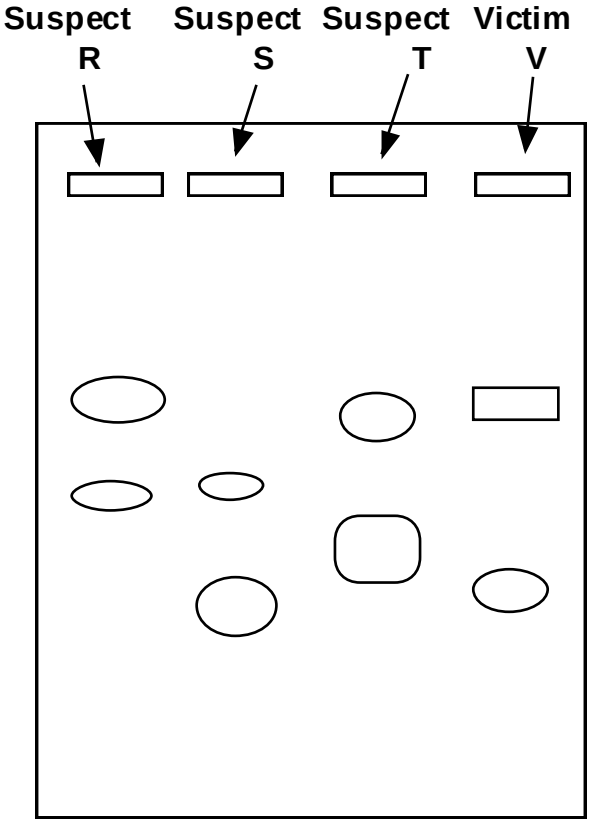
Suspect R:

Suspect S:

Suspect T:

Victim V:

KNOWN DNA FINGERPRINTS VI. B: Attach your transparency here.



Compare your gel to the patterns obtained from "known DNA" and give your conclusions about the identity of each person on your gel whose "DNA" was "unknown."

Your Sample	Identity of Known Person	Explain your reasoning:
11	_____	_____
12	_____	_____
13	_____	_____
14	_____	_____