COC Biology Outreach Program

Check out this awesome video by Hortensia Jimenza Diaz and Cinematic Sweden!!!!

https://www.youtube.com/watch?v=Mehz7tCxjSE&ab_channel=TED-Ed

DNA FINGERPRINTING: VERSION B

**Watch the video above to answer the questions below, including the background questions *before* class.

1.If the mother pea's genotype is RRYY and the father's is RRyy, could they have a wrinkled pea baby? Why or why not?

2. From the video's Punnet square, what percentage of DNA does the RRYY mother and RRyy father each contribute to a single offspring?

3.How do you think this percentage is useful in a paternity test? How might you prove someone is NOT the father of a child?

***DID YOU KNOW THAT DNA EXONERATES MORE PEOPLE THAN IT CONVICTS? THAT BY 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: Patrick Allen & Jim Wolf, College of the Canyons Professor 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.

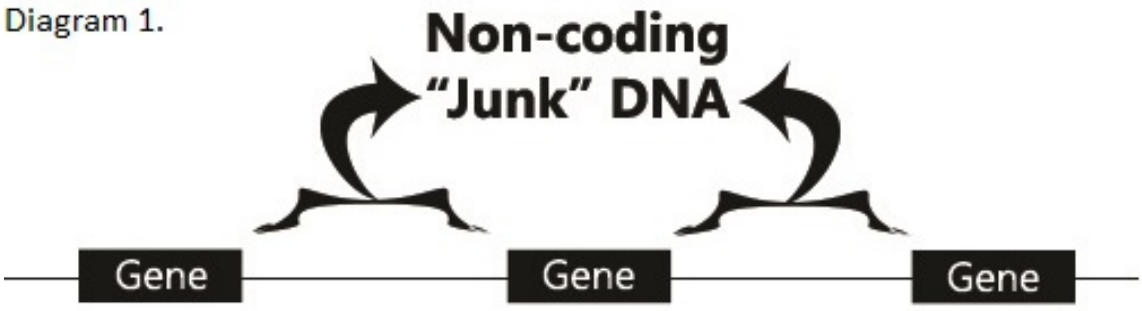
2. To introduce the many practical uses of DNA fingerprinting.

3. Refine micropippetting skills.

DNA Fingerprinting Background

DNA Fingerprinting has long been the backbone of many wide-reaching disciplines; from Forensics, Epidemiology, even diagnosis of disease within an urgent care room. If you or a loved one have ever been diagnosed with pneumonia, strep throat, or any other bacterial disease then chances are, your bacteria's DNA has gone through a DNA Fingerprinting experiment similar to this one. This technique is also used to keep people from being unfairly sent to prison. Many people would assume that biologists examine the gene's of people to judge who is guilty, or who is innocent within a criminal case. The real origin of DNA fingerprints are much more involved, and much more interesting.

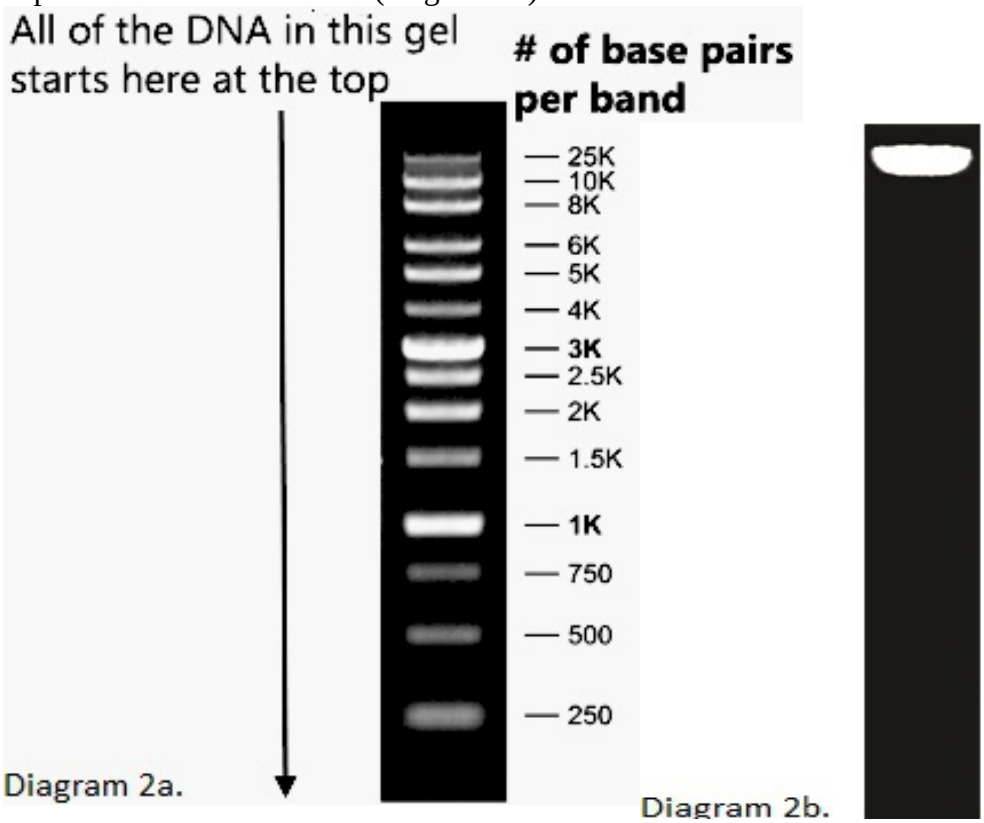
Diagram 1.



What is actually looked at are the *gaps* between coding genes (diagram 1). Our genomes aren't just gene after gene after gene. There are gaps between these coding regions filled with non-coding DNA, sequences that will *never* make any proteins for your body. Many scientists, and even textbooks that you may read have called these non-coding regions "junk DNA"**[2]**. Junk they say!? That's just rude behavior from those who didn't appreciate how they are equally as important as coding regions. In fact 98% of Human DNA is so-called "junk".

These non-coding regions can contain what are called "Microsatellites" **[4]**. These are small repeating sequences (tandem repeats) of DNA such as "**AGAGAG AGAGAG**" between genes. It is this DNA that is truly unique for each person and it is this DNA that makes your own DNA fingerprint. This contrasts with genes, which because they hold important information (like telling your heart to beat) they are much less likely to vary. You and a friend might share the repeating sequence **"ATAT ATAT ATAT"** together, but yours may repeat 3 times and your friend's might repeat 6 times. Biologists examine many microsatellites at once to create a unique DNA fingerprint and distinguish between different people. If you were to compare 10 microsatellites, how would you prove two people are related, such as a father and daughter? Would they share all of the same repetitions? How many should they theoretically share?

These small microsatellites make it easier than comparing whole genes, especially when it comes to Gel Electrophoresis. This is how DNA Fingerprints are made distinguishable, by using an agarose gel (a jello-like sugar that comes from algae) placed on a glass slide to filter and separate DNA based on size (diagram 2a).



If the gel separates DNA based on size, then what kind of DNA travels faster within the gel? What kind of DNA travels slower?

The Hemoglobin *gene* in diagram 2b didn't move at all within the gel. It is actually at least 5,225,466 bp long **[3]**. Why didn't the DNA in that well move, and what might that say about using whole genes to create DNA Fingerprints?

So how does the DNA move through the gel? Electrophoresis from the technique's name means “to carry with an electrical current”. The agarose gel and some salt buffer are placed in a “gel box” to serve as an electrical bridge between two electrodes. The electrical current will travel from one electrode to the second because the agarose gel and salt buffer connects them (making a stream of electrons). This will cause the DNA to move through the gel because the current pushes on the negatively charged phosphate groups of DNA (diagram 3).

These phosphates groups that form the backbone of the double helix give DNA an overall negative charge. What separates the DNA is the solid agarose gel, which acts like an obstacle course. The larger, heavier DNA moves slower because it has a harder time moving through the obstacles. The smaller, lighter DNA moves quicker because they aren't hindered as much by the agarose gel. They are like little children who are more agile and athletic than an average adult (larger DNA).

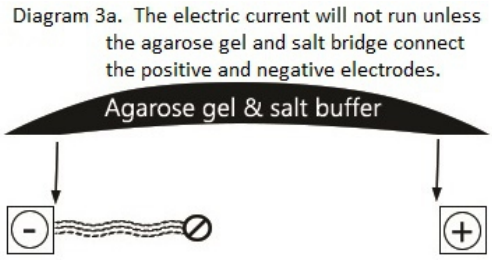
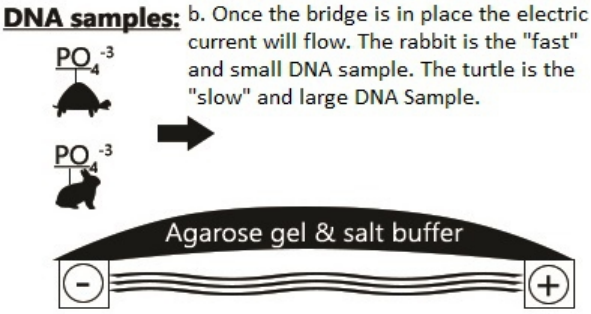
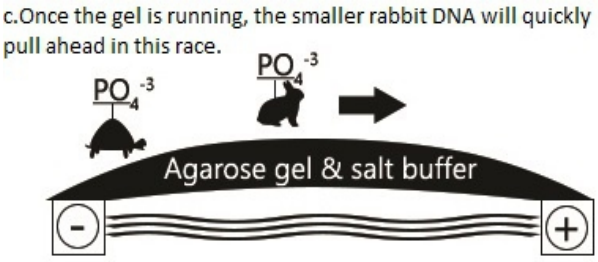


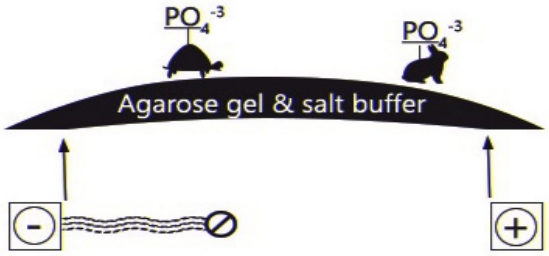
Diagram 3a. The electric current will not run unless the agarose gel and salt bridge connect the positive and negative electrodes.



b. Once the bridge is in place the electric current will flow. The rabbit is the "fast" and small DNA sample. The turtle is the "slow" and large DNA Sample.



Electric current pushes the negatic phosphate groups



The agarose gel is removed from the chamber and the salt buffer is recycled. When the electric current stops moving,so do the DNA samples.

After electrophoresis has finished separating the DNA, a staining liquid called methylene blue will bind to the to DNA allowing it to become visible. This is how DNA fingerprints are seen, whether they're from a crime scene, a paternity test, or even an infected person. These Microsatellites are cut from each person's or bacteria's genome by special bacterial enzymes called Restriction Enzymes. They will specifically recognize and cut out these repeating sequences of DNA. One restriction enzyme, called PvuII, was used in a 1989-1992 study to analyze Tuberculosis (TB) patients in New York City **[1]**. The team of scientists found that at least 37.5% of infected people shared TB strains with at least one other person. That out of 104 patients, there were only 77 unique TB DNA fingerprints. This technique is so powerful that it doesn't just show what disease someone has, but it can show how that disease behaves and show patterns of the infection. The technology may have been invented back in 1985 **[4]**, but even older technologies are still relevant and used by modern labs today!

If the study had found 104 unique TB fingerprints out of 104 patients, what might that say about Tuberculosis behavior? Would all of the infections be very recent, or are just the symptoms very recent? What might the ratio between unique TB fingerprints and patients look like if there was almost no time between infection and symptoms?

Sources (Always cite your sources!!)-

[1] Alland, David, Gary E. Kalkut, Andrew R. Moss, Ruth A. Mcadam, Judith A. Hahn, William Bosworth, Ernest Drucker, and Barry R. Bloom. "Transmission of Tuberculosis in New York City -- An Analysis by DNA Fingerprinting and Conventional Epidemiologic Methods." New England Journal of Medicine N Engl J Med 330.24 (1994): 1710-716. Web.

[2] Doolittle, W. F. "Is Junk DNA Bunk? A Critique of ENCODE." Proceedings of the National Academy of Sciences 110.14 (2013): 5294-300. Web.

[3] "HBB." Genetics Home Reference. U.S. National Library of Medicine, 1 July 2015. Web. 9 Nov. 2015. <http%3A%2F%2Fghr.nlm.nih.gov%2Fgene%2FHBB%23location>.

[4] Jeffreys, A. J., V. Wilson, and S. L. Thein. "Individual-specific ‘fingerprints’ of Human DNA." Nature 316.6023 (1985): 76-79. Web.

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 liquid 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 gels 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

Lab supplies:

- ☐ 1.5ml test tube containing loading dye "L" (provided by teacher)
  1.5ml test tubes with DNA unknowns (also provided by teacher)
  Two 1.5ml test tubes
  Test tube rack

- ☐ 1 micropipettor
  10ml graduated pipette
  1 box of micropipettor tips
  Pipet-aid

- ☐ Bottle for "used methylene blue stain"
  Small cup for used tips

- ☐ 60-70°C water bath with flasks of melted agarose gel
  3" X 2" glass slide
  Plastic "comb"

- ☐ Electrophoresis device ("gel box" shared by two stations)
  Power supply for gel boxes (shared by four stations)
  Bottle of "electrophoresis solution" (shared by the class)

- ☐ Bottle of methylene blue stain (shared by the class)
  Plastic staining tray
  Deionized water
  Gel viewers (white light box)

- ☐ Marking pen (Please use regular pen or pencil on paper.)
  Transparent plastic sheets for tracing staining pattern (cut from plastic baggies)
  Known DNA pattern sheet
  Transparent plastic ruler

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

Preparing the gel

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

2. Fit the pipet-aid to the 10ml pipette and go to the flask of melted agarose gel; obtain 9ml of melted agarose solution; return to your station and promptly spread 8ml of the warm solution on the glass slide; position the comb as has been demonstrated to you.

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

Meanwhile...

Preparing the samples for electrophoresis

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

2. 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.

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

Loading the gel

1. By now the gel should be solidified. It will appear cloudy. Gently press down on the gel and gently lift the comb. Return comb to your lab station kit.
2. Lift the slide with the gel and place onto the platform of the gel box with wells near the black (negative electrode).
3. 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.

4. Decide which lab partner will load the gel first. Set the micropipette for 12 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).

1. Close the lid of the gel box without disturbing the solution or sloshing the DNA samples.

2. 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.

***note: it is OK if it is not exactly 200 volts. 197 or 203 is just fine.*

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

While you wait: Housekeeping and practice analysis

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

2. Observe the patterns for samples of DNA that you have been given on page 10. Write a few sentences describing the distance and pattern of the bands for each DNA. Use the answer page (pg. 9).

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. Overall, six bands are visible.

3. 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.)

4. Remove the lid of the power supply. Carefully slide the glass out from underneath and then place the gel into the staining tray. Return the glass slide to your kit.

VI. Staining the DNA in the gel.

1. Add methylene blue dye until the gel is barely submerged. Note the time and begin rocking the tray. You will stain for 5 EXACTLY minutes (no longer).

2. 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 (out of direct light).

3. 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. If time permits, repeated destaining and changing of water will help make DNA strands more visible. 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.

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

5. Discard or save the gel as instructed.

VI. Observations and conclusions.

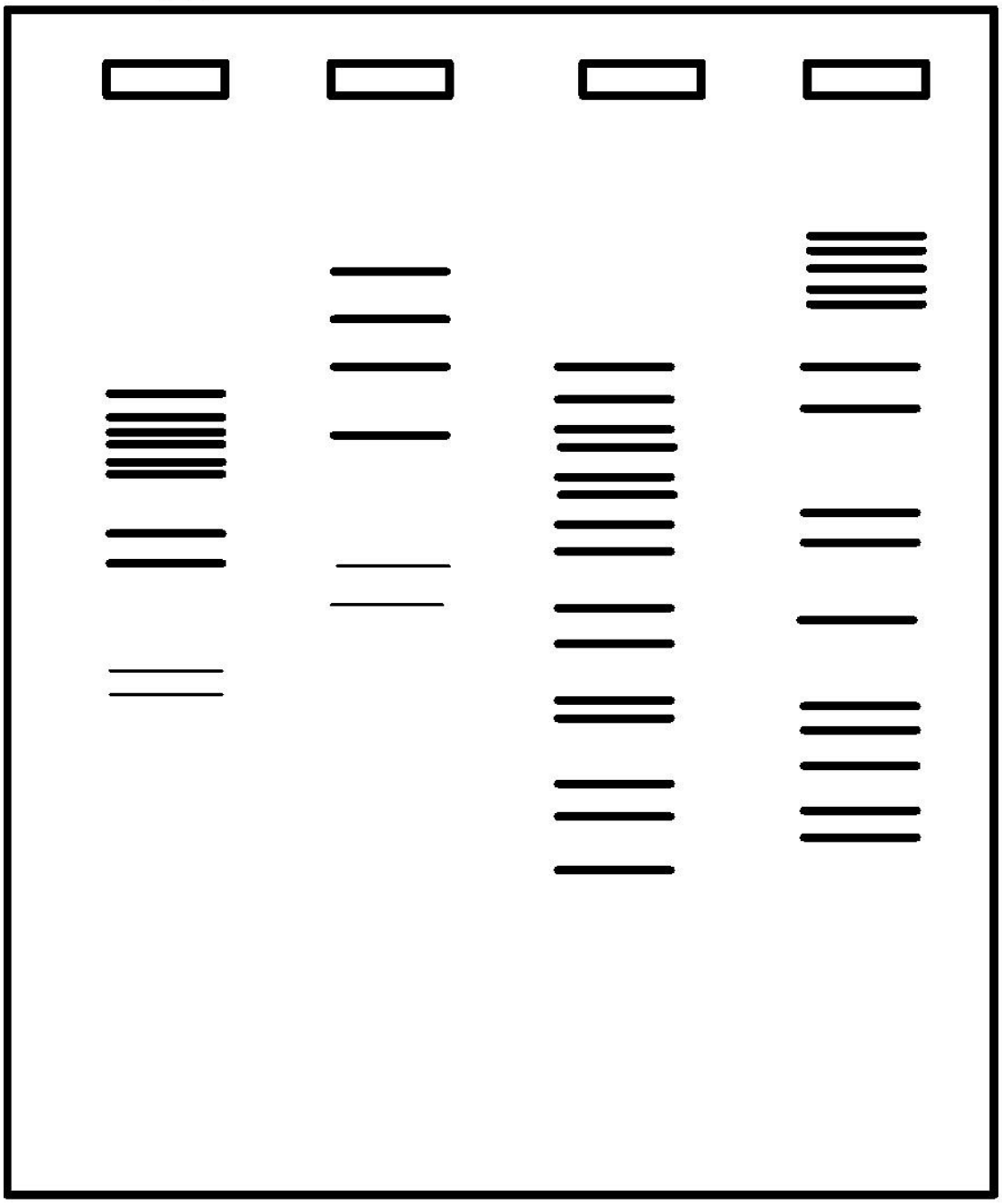
1. Measure the distance that your stained DNA bands migrated in the gel and record on below.

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

3. Compare your sample to the patterns on page 9 (or the comparison sheet provided to you by your instructor) 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

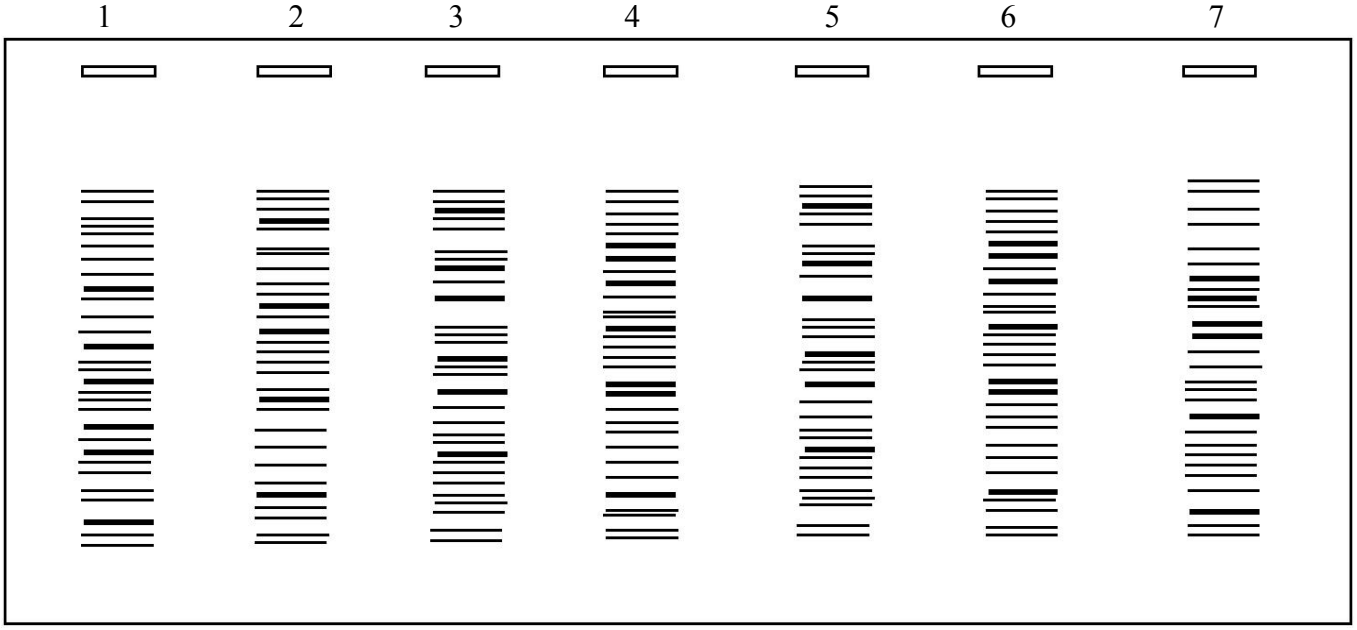
PART A: FORENSIC SCIENCE - RAPE CASES

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

3. You ran your gel for 15 minutes at 200 volts. It is true that when you increase the voltage all DNA within the gel will move faster and the inside of the gel box will *heat up* from the stronger current. Why didn't you turn up the voltage to have a faster experiment? (Remember how you prepared your agarose gel and the different states of matter it was in.)

4. The agarose gel used in the experiment was made with 0.8-1% agar. You can change the percentage of the gel to alter how fast DNA moves. Sometimes very small linear DNA will move too quickly and will leave the gel. Sometimes very large linear DNA will not separate based on the percentage of agar within the gel. What percentage of agar do you think could be used to separate very small DNA (say 3-5bp)? Very large DNA (say 1 million bp)? Why? (use agar percentages from 0.5% to 4% in your answer)

5. Based on your answer above, how would you design a Gel Electrophoresis experiment to test your answer from question 4? You are given a DNA sample with 5 different sizes; 25,000 bp, 12,500 bp, 6,000 bp, 3,000 bp, and 300 bp. You will run gels at 200 volts for 15 minutes. (First think how many different gels at least you need to run and how to interpret each of the results.)