BIO 1101
LAB 5: BIOTECHNOLOGY & GENETICS
Gel Electrophoresis and Forensics - Human Genetics and Blood Typing

READING: Please read pages 153 - 161 and 220 - 237 in your text.

INTRODUCTION: This is a time-consuming lab, so you will need to work efficiently (and carefully!) to ensure your lab is completed by the end of the period. Let's get started! While your gel is "running" (it will take 45-60 minutes) you can read the background information on gel electrophoresis and work on Part 2 of today's laboratory, Human Genetics and Blood Typing.

(A) Gel Electrophoresis and Forensics

Criminology. While the use of molecular biology in crime labs is not biotechnology *per se*, it is certainly an area where genetic engineering tools increasingly are being used. DNA "fingerprinting" is a widely used technique that is based on the fact that every human (and other sexually reproducing organisms) has a unique combination of DNA base pairs, thanks to crossing over during meiosis. When a sample of DNA is cut up into small pieces using a restriction enzyme, the resulting pattern of pieces forms a unique "fingerprint" for that individual. DNA is becoming a powerful piece of evidence both for convicting and for exonerating criminal suspects.

In order to manipulate DNA and identify genes to work with, geneticists first conduct a **restriction digest**. A restriction digest uses special enzymes called **restriction enzymes** that cut DNA at specific points along the molecule identified by the order of the nucleotide bases. For example, the restriction enzyme called **BamH1** cuts DNA at the base pair sequence "GGATCC" wherever it appears in the genome. It cuts both DNA strands between the two G nucleotides. Because the restriction sequence is a palindrome (reads the same forward on one strand and backward on the complimentary strand), the cut is "staggered," producing "sticky ends." Restriction digests chop the DNA into small pieces that can then be separated and identified.

Example: BamH1 will cut this piece of DNA as follows:

```
                ↓
DNA Strand: CGTC GGATCC AGGATAGGCTCTA
            GCAG CCTAGG TCCTATCCGAGAT
                     ↑
```

After restriction digest with this enzyme, the following two fragments of DNA will be generated:

```
            CGTCG          GATCCAGGATAGGCTCTA
            GCAGCCTAG          GTCCTATCCGAGAT
```

Because everyone's genetic makeup is different, when any two people's (except identical twins) DNA is exposed to the same restriction enzyme, different lengths of DNA fragments will be generated.

After the digest is complete, you need to separate the DNA fragments so that you can produce a **DNA fingerprint**. The way DNA fragments are separated is through **electrophoresis**, or the movement ("phoresis") of charged molecules through an electric field ("electro"). DNA is negatively charged, due to the many phosphate groups in its backbone. When placed in an electric field, DNA fragments will move toward the positive pole of the field, with fragments of different sizes moving at different rates. Small ones move fast, large ones move more slowly, and medium-sized ones travel at speed in between. Researchers capitalize on this difference in migration rate to separate DNA fragments of different sizes from each other.

(B) Human Genetics and Blood Typing

In 1866, Gregor Mendel presented the results of his experiments on the inheritance of traits in the garden pea. Unfortunately, Mendel's results were not understood by his audience at the time. Almost 150 years later, however, most biologists now take the importance of Mendel's work for granted. Recent advances in molecular genetics and genetic engineering techniques have allowed the "test-tube" production of products such as insulin, human growth hormone, bovine growth hormones, tomatoes that resist rotting, cloned animals, and plants and fish that glow in the dark. These technological advances have been controversial in most cases, and in the future you will be expected to make decisions about issues such as these. To make an educated judgment, you must thoroughly understand the mechanisms of genetics. The genetics problems in this exercise should help take you to that point.

Every sexually reproducing organism has at least 2 units of genetic information for each trait – one piece from the mother and one from the father; these bits of information are **genes**. Recall from your lecture material that most eukaryotic organisms are **diploid**; *i.e.*, they contain pairs of homologous chromosomes, one member of each pair coming from each parent. Since there are at least two different versions of each gene, a new word is needed to distinguish them from the concept of a "gene" – that word is **allele**. So you have at least two alleles for each gene – one on the chromosome you got from your mother and the other on the chromosome you got from your father. A good analogy to this lies in chemistry: alleles are to genes as isotopes are to elements – different versions of the same thing. And to continue the analogy; just as elements can have one, two, three, or more isotopes, genes can have one, two, or three or more alleles. Chemical differences among alleles caused by different sequences of nucleotides along the DNA molecule are responsible for the production of different products that can lead to differences in expression of the trait. For example the alleles that code for blue eye color and those that code for brown eye color result in different pigments produced in the iris of human eyes. Those different pigments are coded for by different eye pigment alleles.

EXERCISES: Work in groups of at least three for the duration of this lab.

A. Gel Electrophoresis

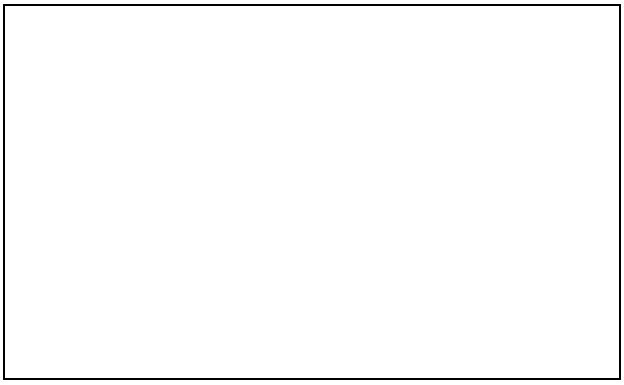
Scenario: You are attempting to solve a crime in which a man was beaten to death. A witness saw a person flee the crime scene, and two possible suspects have been identified. You have been able to obtain the assailant's DNA by collecting blood and skin underneath the victim's fingernails. This is your DNA evidence. You also have a DNA sample from the victim, and DNA samples from the two suspects.

Materials:

- Four DNA samples (evidence, victim, suspect 1, and suspect 2). This DNA has already been digested with a restriction enzyme. The sample appears blue because a dye that has been added to the DNA. This "loading dye" will run ahead of the DNA fragments in your samples, letting you know when it is time to stop running your gel.
- A syringe and four yellow or white disposable plastic tips
- A gel box with a prepared agarose gel, covered with buffer solution
- A power supply (Note: each power supply can run 3 gel boxes simultaneously; you will need to share the power supplies.)

Procedure: (Read the entire section before you start.)

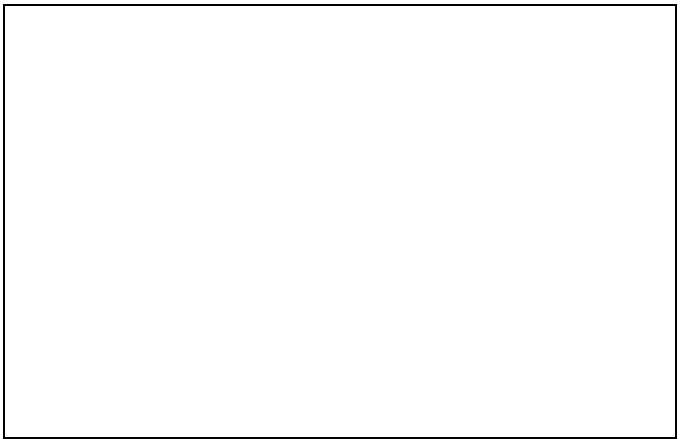
1. Load the DNA samples
   a. Put a clean plastic tip on your syringe. Use a new tip for each sample.
   b. Draw up all of the DNA sample/dye mixture into the pipette; do this slowly to avoid drawing in air. If you draw some air into the tip, slowly depress the plunger on the syringe over the sample vial to expel the air, taking care not to expel the sample, too.
   c. Gently pipette the DNA mixture into one of the wells. Hold the tip just above the well but under the buffer solution; the DNA will sink to the bottom of the well.
   d. Make a "map" of your gel, indicating which DNA samples you placed into which well (fill in the diagram below).



2. Running your gel
   a. BEFORE you hook up your gel box to the power supply, ask your instructor to make sure your setup is correct and safe. You may not start running your gel until your instructor tells you to.
   b. Ensure that the power supply is turned OFF. Place the lid on your gel box, with the red (positive) terminal on the opposite end of the gel from your sample wells. Attach the red wire from your gel box to the positive (red) terminal on your power supply. Attach the negative (black) wire to the negative terminal on the power supply. Wait until all groups have completed their gels and attached them to the power supply to turn on the power supply.
   c. Have your instructor turn on the power supply, setting it to 85 volts and a running time of 1 hour.
   d. Let your gel run for approximately 1 hour. BE CAREFUL not to disturb the apparatus while it runs. At this time, proceed to exercise (B) Human Genetics and Blood Typing and conduct the brief exercises. You should be able to complete this section in the time it takes for your gel to run. Check, periodically, to make sure that the blue dye has not run too far (into another groups wells, or off the end of the gel would be considered too far). If you feel that the samples are going too far, have your instructor check your apparatus.

3. View your gel.
   a. After your gel has run for about one hour, turn off the power supply and disconnect the wires from the power supply.
   b. Carefully take the gel box back to your lab bench. Remove the lid from your gel box, and take out the gel tray. Tilt the gel tray and pour the excess buffer solution back into the gel box.
   c. *Carefully* slide your gel out of the tray and into a plastic box. These gels break easily!
   d. The gel has been prepared with a built-in stain that sticks to DNA and fluoresces in UV light. To view the bands of DNA on your gel, gently place the gel in the light box (transilluminator) and close the lid. SAFETY NOTE: Be sure to close the lid over the gel to prevent exposure to harmful UV light. With the lid closed (and ideally, the lights in the lab room turned off), view the bands on your gel.
   e. When done visualizing the bands on your gel, turn off the light box, open the lid, and place the gel back in the plastic box. Do not throw away your gel, as it can be melted down and re-used.

4. Analyze your results
   a. Draw the DNA bands on the chart on the next page (remember to indicate where the wells are and label the source of DNA for each well).



Discussion Question.

1) Based on your DNA evidence above, did one (or both) of the suspects commit the crime? How can you tell?

_____
_____
_____
_____
_____

B. Human Genetics

Materials:

- For your group, 1 packet each of the following taste papers: Control (white), PTC (blue), Sodium Benzoate (pink), and Thiourea (yellow).
- A sample of any other taste test product that might be available (check with your instructor).
- One bottle each of Synthetic Blood, A-antiserum, B-antiserum, Rh-antiserum
- Plastic blood-typing tray
- 4-5 clean toothpicks
- Laptop

Procedure: (Read the entire section before you start.)

1. Taste tests. The sensation of taste generally is shared by all of us, at least in terms of the ability to taste salt, bitter, sweet, and sour. However, there are some compounds that some people can taste but others cannot. PTC

(phenylthiocarbamide) is a drug that can prevent the thyroid gland from taking in the iodine that is essential for the manufacture of thyroid hormone, and is one of those substances that only some people can taste. The ability to taste this substance is governed by the alleles T and t, where tasting (T) is dominant over nontasting (t).

a. To find out about your phenotype, first taste a strip of paper from the white Control packet. This paper is identical to that in the test packs but doesn't contain any chemicals. Now, taste a paper strip impregnated with PTC. Can you taste it? _____ What is(are) your possible genotype(s)? _____
b. Now try the other test chemicals. What is your (a) phenotype and (b) possible genotype(s) for each of these?

Sodium Benzoate (S=tasting, s=nontasting) (a) _____ (b) _____

Thiourea (U=tasting, u=nontasting) (a) _____ (b) _____

2. <u>ABO blood types</u>. ABO blood types (phenotypes) are determined by three possible alleles; I^A, I^B, and ***i***. This multiple allele example of inheritance means that each of us inherits one pair of the three alleles. I^A and I^B alleles are **<u>co-dominant</u>** (neither one dominates the other) but both are dominant over the ***i*** allele. Make sure you understand this before you proceed.

Blood Type (phenotype)	Genotype
A	I^AI^A or I^Ai
B	I^BI^B or I^Bi
AB	I^AI^B
O	***ii***

a. Is it possible for a child with type O blood to be produced by two AB parents? Explain.

_____
_____
_____
_____
_____

b. In the case of disputed paternity, the child is type O, the mother is type A. Could an individual of the following blood types be the father?

A _____
B _____
AB _____

3. Rh factors. A second way of typing blood is to identify the **Rh factor**. This factor is separate from the ABO blood typing and is inherited as a single pair of alleles, ***Rh+*** and ***Rh-***. ***Rh+*** is dominant over ***Rh-***. Hence you may be A+, A-, B+, B-, AB+, AB-, O+, or O- with the plus or minus signs indicating the Rh factor.

   a) Do you know your blood type? _____ Your Rh blood type? _____ If you are Rh+, you will need to know the phenotypes of your parents and siblings to be sure of your genotype because it could be ***Rh+Rh+*** or ***Rh+Rh-***. If everyone in your immediate family is Rh+, then what is your probable genotype? _____ If you are Rh+ and one of your parents is Rh-, what is your genotype? _____ Can you determine your genotype with certainty? _____

4. Synthetic blood typing activity. Obtain a bottle of synthetic blood. Write down the name on the bottle. Place 3 drops of the blood in the wells of a plastic tray labeled "A," "B," and "Rh". Then, in the well labeled "A", add 2 drops of A antiserum; in the well labeled "B", add 2 drops of B antiserum, and in the well labeled "Rh" add 2 drops of Rh antiserum. A positive reaction for A, B, or Rh proteins occurs when the antibodies in the antiserum cause the blood proteins to clump, giving the blood a thick or grainy appearance. Stir the blood with the plastic toothpicks provided, taking care not to cross-contaminate the wells. Wait for 3-4 minutes for a reaction.
   a. Whose blood did you type (name on bottle)? _____
   b. What blood type was your sample? _____
   c. What is/are the possible genotype(s) of your sample? _____
   d. Have your instructor initial here to check your results_____

5. Online Genetics Tutorial. You probably still have a few minutes until the gels from Part A of this lab are finished. In the meantime, try your hand at a few human genetics problems online. Have someone from your group pick up one of the lab laptops, log in, and then navigate to the following page:

   http://www.biology.arizona.edu/human_bio/human_bio.html

   a. This website has several activities and problem sets to help you understand some basic human genetics, and it even includes some "real-world" situations to work through. Work through the first three genetics topics: *Blood Types*, *Color Blindness*, and *Human Genetics* in class. Blood types should be a review if you made it through #2 above, but the others have a lot of new material. If you come across terms you don't recognize (especially in the *Human Genetics* section), click the tutorial button for an explanation. Your instructor may ask you to demonstrate solutions to some of the problems, so be prepared to answer questions! You might even see some very similar problems on future lab exams…

Notes: