Exercise 8:
Forensic Genetics/ Human Phenotypes

Introduction
Genetic analyses are now commonplace in criminal investigations. Forensic genetic labs analyze, for example, samples collected from a victim and potential suspects, and compare them against unknown samples collected from the crime scene (e.g., blood, hair, skin fragments under a victim’s fingernails, etc.). Usually, the process includes extracting DNA from the samples, using PCR (polymerase chain reaction) to make millions of copies of DNA, and using gel electrophoresis to analyze variation in DNA fragment lengths (see Figure 1). Typically, scientists use VNTR (variable number tandem repeat) loci (locus = location of gene on a chromosome) to compare known and unknown samples (see Figure 2). If a suspect matches (shows the same banding pattern on a gel) DNA evidence collected from a crime scene, it can be used to build a case against that suspect (for example, see Figure 3). While it is easy to eliminate suspects due to a non-match (one or more loci from DNA collected at the crime scene do not match a particular suspect), it is harder to “prove” a match. Because there is a decreasingly small probability that a suspect will match each new locus that is added to the analysis (usually 7 or 8 are used), if a suspect matches at all loci, the data are expressed as “probability of accidental match.” It is theoretically possible that someone else out there has the same genotype as one collected from a crime scene, so the police can’t say for sure that “this is the guy/gal,” but they can say that there is a “one in a billion chance that this person matches by accident.” Thus, combined with other evidence in court cases, DNA can be a very powerful tool to prosecute criminals. Conversely, DNA evidence has also been used to free people who have been jailed for crimes they did not commit.

Figure 1. Process of gel electrophoresis
Figure 2. VNTR loci vary in fragment length
Figure 3. Picture of gel. Notice 4 “lanes” with 4 different samples. The second and fourth lanes from the left have the same banding pattern and “match”, while the first and third samples don’t have matches.
Today, you will simulate a forensic murder investigation using agarose gel electrophoresis. Molecules such as DNA, RNA and proteins are commonly separated using gel electrophoresis, a process that separates molecules based on their charge, size and shape (Figures 1, 2, and 3). The basic outline of the process is as follows. First, a gel is prepared by dissolving agarose (a gelatin-like substance) by boiling in an appropriate buffer. The melted agarose is poured into a tray and allowed to cool and solidify. The tray and solid agarose are then placed in a buffer solution inside a chamber with electrodes on either end. A “comb” is removed from the gel, which leaves wells, or holes, in which a solution containing DNA is placed. Electrical current is directly applied to the buffer solution and causes the DNA fragments in each sample to migrate to one end of the gel.

The agarose is a solidified sugar that forms a latticework of microscopic pores when it solidifies; larger DNA molecules move more slowly through the gel than smaller molecules, and compact molecules pass more quickly than less compact molecules (given they have the same molecular weight).

In this exercise, you will load known and unknown samples onto a gel. You have 4 samples collected from a crime scene: 1) from skin under the victim’s fingernails; 2) hair found near the victim; 3) DNA collected from fingerprints on the doorknob and 4) DNA collected from saliva on the edge of a drinking glass at the crime scene. You also have 4 “known” samples that you will compare the DNA to: one sample from the victim and 3 samples from each of three suspects (the suspects were required to submit DNA samples as part of the murder investigation). DNA has already been extracted from these samples, and it is your job, as a forensic geneticist, to run them using gel electrophoresis. Based on the pattern and color of banding after you run the gel, you will make a determination as to the suspect who may have committed the murder.

For this activity, you will employ a micropipettor to obtain tiny volumes of these known and unknown samples measured in microliters. Note the following conversions:

- 1 liter = 1000 ml
- 1 liter = 1,000,000 microliters (µl)
- 1 microliter (µl) = 0.000001 liters

Please read the general information concerning gel electrophoresis and the use of a micropipettor provided in lab.

**Materials for each lab table**
- Gel electrophoresis box with casting tray, dams and comb
- Power supply for each gel box
- 0-20 microliter pipettor with a box of disposable tips
- Tube of melted agarose (25 ml; leave in water bath until ready to pour)
- Electrophoresis buffer
- 8 samples, in tubes numbered 1-8:
  - Tube #1- victim’s DNA
  - Tube #2- suspect #1 (wife)
  - Tube #3- suspect #2 (business partner)
  - Tube #4- suspect #3 (person he recently fired from work)
  - Tube #5- from skin under the victim’s fingernails
  - Tube #6- hair found near the victim
  - Tube #7- fingerprints from crime scene
  - Tube #8- saliva from drinking glass
Procedure

Casting the gel – one per table

1. Place the casting tray in the bottom of the gel box. Insert the dams so that the inward facing sides are vertical. Insert the comb (8 tooth) in center position.

2. Obtain a tube of melted agarose from the water bath. Slowly pour the agarose into the casting tray. Try to avoid getting bubbles in the gel (if you do get bubbles, break them with a pipet tip). Let the gel cool (~10 minutes) - it will be firm, a milky color and look like jello.

3. Remove the dams. Add electrophoresis buffer to the end chambers so that the gel is barely covered with buffer. Carefully remove the comb (the buffer helps lubricate the comb so that it is removed more easily from the gel).

Loading the gel

4. Set your micropipettor to 5 microliters. Be sure you have read the technique and have seen a demonstration by the TA. Practice while the gel is cooling. Firmly push the pipettor into one of the tips in the tip rack (DO NOT touch the tip with your fingers, this will cause contamination of the samples). Read through step 5 about how to use a micropipettor; practice with distilled water before you move to the next step on this handout.

5. a. Push the plunger knob on the micropipettor down to the first stop. Keeping the plunger down, lower the pipet tip into the sample in the bottom of one of the 8 sample tubes and release the plunger after the tip is submerged in the liquid. (Start by loading sample #1 to the hole created by the comb in the far left side of the gel, sample 2 in the next lane or space in the gel, etc until sample #8 is loaded on the right edge of the gel. This order is critical because if you forget the order or mix it up, you will not know which samples are which when you go to analyze your results).

b. CAREFULLY place the pipet tip into the first dry well (hole) in the gel (but not to the bottom). It may help to steady the pipettor with your free hand. Try not to poke the bottom or sides of the well.

c. SLOWLY press the plunger to the first stop and then to the second stop to release the sample into the well. If you do not do this slowly, your sample may shoot up out of the buffer and get lost in the buffer. Before you release the plunger this time, remove the tip from the buffer (or else you will pick up buffer as you take the tip out of the gel).

d. Eject the tip in the waste container using the tip ejector button.

e. Repeat steps a-d for each of the 8 samples, loading all 8 wells in the gel. Start with the sample from tube #1) on the far left and end with sample #8 (from tube #8) on the far right.

Running the gel

6. When all samples are loaded, put the lid on the gel box. Check that the power supply is turned off before connecting to the gel box. Connect the red wire from the red (+) connector on the power source to the red connector on the gel box; connect the black wire from black to black. Turn on power supply and set to about 80 volts. If working properly, you should see bubbles forming in the buffer around the electrodes within a couple of minutes. Observe the movement of the dyes in the gel. Electrophoresis should be complete in 20-30 minutes. While the gel is running, complete the human phenotype exercise and fill out your other assignment sheet, keeping an eye on the gel. Stop the process before any dyes reach the end of the gel.

7. Turn off the power, unplug electrodes, and open the gel box. Lift the gel tray out and set it on the lab table. Investigate each of your 8 samples - some will have single bands and some will have double bands. Fill out the electrophoresis table on your assignment sheet and answer the questions that follow.

8. Pour the used buffer in the “Used Buffer” flask at the end of the table.
Assignment Sheet- Exercise 8- Forensic Genetics

Name____________________________________________________

A table representing the gel is below. In each lane, sketch the position of your band(s) and note the color of each. Answer the questions below based on the appearance of your gel.

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<th>Lane/ Sample #</th>
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Remember, sample #1 is the victim’s DNA, lanes 2-4 are DNA from 3 suspects and 5-8 are samples collected from the crime scene.

Which lanes match?

Can you determine which samples from the crime scene match “known” samples? If so, which ones match?

Of the three suspects, which was the most likely murderer? Why?

Were there any crime scene samples that did not match known samples? If so, why do you think there were?

If this were a real murder investigation, would only one locus (as analyzed here) be sufficient evidence to use in a court case? Why or why not?
Exercise – Human Phenotypes

In this exercise, you will study several patterns as to how genes are inherited and calculate some genetic problems based on the physical appearance (phenotype) of yourself and a lab partner.

Problem #1 – Ability to taste sodium benzoate
Sodium benzoate is a chemical that either tastes bitter to some people or has no taste at all to some people. The ability to taste sodium benzoate is a dominant trait, and thus “tasters” have the genotype TT (homozygous dominant) or Tt (heterozygous). People unable to taste sodium benzoate (“non-tasters”) thus have the genotype tt (homozygous recessive) because non-tasting is a recessive trait and, to be expressed, the allele must be present on both chromosomes.

Obtain a piece of sodium benzoate paper and taste it. Then answer questions 1-3 on your assignment sheet.

Problem #2 – Hereditary traits
You and a lab partner will now investigate aspects of each other’s appearance (phenotypic traits) that are heritable. These traits have no significance on whether we are better or worse people; they just help make us more interesting. For each trait, record your phenotype, genotype and your partner’s phenotype and genotype on your assignment sheet to hand in. Remember, for dominant traits that are observed in a genotype, your genotype could be either homozygous dominant or heterozygous; similarly for recessive traits expressed in the phenotype, the genotype must be homozygous recessive. Observe these 14 phenotypic traits, fill out the table on your assignment sheet and then answer questions 4-6.

Trait #1 – cleft in chin. This trait is recessive. (Use upper & lower case “C”s to represent genotypes). Thus, people who do not have cleft chins are either homozygous dominant or heterozygous.

Trait #2 – tongue rolling. This trait is dominant. (Use upper & lower case “R”s to represent genotypes)

Trait #3- tongue folding. The ability to fold the tongue backwards without touching the roof of the mouth is recessive. (Use “T”s and “t”s to represent genotypes)
Trait #4 – mid-digital hair. The presence of hair between joints on top of the hand is dominant and thus absence of hair is recessive. (Use “H”s and “h”s for genotypes)

Trait #5 – hitchhiker’s thumb. A thumb that is straight when fully bent back is **dominant**, and hitchhiker’s thumb (curved) is **recessive**. (Use “H”s and “h”s for genotypes)

Trait #6 – clasped hands/ thumb up. Clasp your hands interlacing your fingers as shown below. Record which thumb is up. Right thumb up is **recessive**. (Use “L”s and “l”s for genotypes)
Trait #7 – earlobes – Attached earlobes (d) are recessive; detached (D) are dominant.

Trait #8 – freckles – Freckles on face are dominant (F) over no freckles (f).
Trait #9 – dimples – Dimples in cheeks are dominant (D) over no dimples (d).
Trait #10 – Convex and prominent nose is dominant (C) over straight nose (c).

Trait #11 – Hold your hands before your face with the palms toward you. Place the two little fingers side by side and press them together. Do they run parallel their entire lengths or do the terminal digits flare out away from each other? Each bone in a finger is termed a phalanx. Bent phalanx (B) is dominant over straight phalanx (b).

Trait #12 – hairline. Widow’s Peak is dominant (W) to straight hairline (w).

Trait #13 – Eyebrow size. Broad eyebrows are dominant (B) to narrow eyebrows (b).
Trait #14 – Eyebrow shape. Eyebrows separated by a patch of skin on the forehead are dominant (S) to connected eyebrows (s)
Assignment Sheet – Exercise 8 – Human Phenotypes

Name________________________________________________________________

1. Can you taste sodium benzoate?

2. What is your genotype?

3. Suppose Mr. and Mrs. Jones were both able to taste sodium benzoate and further research was done to determine that they were heterozygous (Tt genotype) for the tasting allele. Fill in the Punnett square below.

Then, answer the following questions:
A. What is Mr. Jones’ genotype?______________ Phenotype?
B. How many different kinds of gametes to Mr. and Mrs. Jones each have?_____
C. What are the possible genotypes of the Jones’ children?

D. What are the possible phenotypes of the Jones’ children?

E. What percentage of children are expected to be tasters?_________ Non-tasters?_________

F. Now suppose the Jones were both non tasters.
   a. What are the possible genotypes of the Jones’ children?
   
   b. What are the possible phenotypes of the Jones’ children?
   
   c. What percentage of the Jones’ children are tasters?_________ Non-tasters?_________
Assignment sheet – Page 2 – Human phenotypes

Name ____________________________________

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4. Suppose your mom has freckles and your dad does not. (assume your mom is homozygous dominant for freckles) What is the percent chance that you will have freckles? Your sister/brother?

5. Now assume your mom is heterozygous for freckles. What is the percent chance that you will have freckles? Your sister/brother?

6. Now suppose both your mom and dad have widow’s peaks. Let’s assume your dad is homozygous recessive and your mom is heterozygous. Could you possibly have a widow’s peak? If so, what is the percent chance? How about your sister/brother?