**BIOL 104 Forensic Biology**

**Lab 10 DNA Fingerprinting**

1. **Introduction**

Gel electrophoresis is a process used to separate molecules based on size and charge. DNA is negatively charged due to the phosphate groups in its backbone, so pieces of DNA will travel through an agarose gel toward a positively charged electrode. Shorter pieces of DNA will be able to more easily travel through the spaces in the agarose gel and will therefore migrate further through the gel, while longer pieces of DNA will stay closer to the wells where the DNA is loaded into the gel. Sizes can be estimated by comparison to a DNA ladder, or marker, of standard DNA fragments.

Recall that the amelogenin (Amel) locus is shorter on the X chromosome than the Y. Using our primers the predicted product sizes are:

 Male Male Female

 Male = 218 bp (higher band

due to 2 different sized

fragments)

Female = 212 bp (lower band

 due to 2 same-sized

 fragments)

In our Polymerase Chain Reaction (PCR) the Short Tandem Repeat (STR) of TCTA and TCTG within the von Willebrand factor type A (vWA) can range from 123 bp to 181 bp, but is sometimes not present at all. With good separation, some students may see two bands indicating they have a longer repeat on one chromosome than the other:

 Subject 1 Subject 2 Subject 3



The same is true for the second STR, D13S317 (or s317). Depending on the number of repeats of the sequence TATC, the products may range from 157 bp to 201 bp:

 Subject 1 Subject 2 Subject 3



Differences in the sizes of the DNA samples amplified by PCR and separated by gel electrophoresis determine the DNA fingerprint.

1. **Materials & Methods**

**Wipe down your lab bench and wash your hands. Be sure to wear your gloves and safety glasses.**

1. **Practice Gel Loading**
2. Use your micropipette to practice loading the wells of an agarose gel with dye.
3. **Gel Electrophoresis**

\* CAUTION: The 6x Loading Dye contains Ethidium bromide, a known mutagen, or reagent that can cause mutations or changes in DNA.

Gloves MUST be worn!

1. Retrieve your three PCR products from the last lab.

2. Add the following reagents to a microcentrifuge tube marked “Amel”:

 PCR product marked “A” 10 μl

 6x Loading Dye\* 2 μl

 12 μl

 Mix by pipetting up and down.

3. Add the following reagents to a microcentrifuge tube marked “vWA”:

 PCR product marked “v” 10 μl

 6x Loading Dye\* 2 μl

 12 μl

 Mix by pipetting up and down.

4. Add the following reagents to a microcentrifuge tube marked “s317”:

 PCR product marked “s” 10 μl

 6x Loading Dye\* 2 μl

 12 μl

 Mix by pipetting up and down.

5. Gels made of **2% agarose in Tris-Borate-EDTA (TBE) buffer** have been poured and allowed to polymerize. Each gel is placed in a chamber with the same buffer surrounding it.

6. Your instructor will load 10 μl of a 100 bp DNA ladder into the first well of each gel. You will then be assigned to gels to load your samples. Record which gel and well where you loaded each sample in the Results section. Gels should be loaded by the specific locus being analyzed. For instance, there should be at least three separate gels: 1 for Amel, 1 for vWA, and 1 for s317. Additional gels may be required to accommodate larger class sizes. All gels should include a negative control and the reference sample.

7. When everyone has finished loading their samples into their respective gels, we will turn on the gel electrophoresis equipment and send a current through the gel. In a 2% agarose gel the 3 markers in the 6x loading dye will migrate at approximately 4 kilobases (kB) or 4,000 base pairs (bp, xylene cyanol FF), 300 bp (bromophenol blue), and 50 bp (orange G).

8. After the gels have been run long enough for the DNA to separate, they will be viewed and photographed. Ethidium bromide will intercalate into the DNA, allowing it to be visualized under ultraviolet (UV) light.\*

\*CAUTION: Skin and eyes must be protected from exposure to UV light, another mutagen capable of damaging DNA!

1. **Forensic DNA Typing**

Read the handout and complete “Exercise 1. A Mix-Up at the Hospital,” “Exercise 2. A Paternity Case,” and “Exercise 3. The Case of the Bloody Knife.”

Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Score:

Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. **Results**
2. **Gel Electrophoresis**

Amel Gel: \_\_\_\_\_ Well: \_\_\_\_\_

vWA Gel: \_\_\_\_\_ Well: \_\_\_\_\_

s317 Gel: \_\_\_\_\_ Well: \_\_\_\_\_

On the photograph of each gel, mark the sizes of the standard DNA fragments in the ladder (100 bp, 200 bp, 300 bp, 400 bp, **500 bp**, 600 bp, 700 bp,

800 bp, 900 bp, **1 kB**)

1. **Conclusions**
2. Toward which electrode will DNA migrate? Why?
3. Which will travel farther from the well, a smaller piece of DNA or a larger piece of DNA? Why?
4. We are using Ethidium bromide to stain the DNA in our gels. Why must gloves be worn when handling Ethidium bromide?
5. We must use UV light to visualize the DNA in our gels. Why must skin and eyes be protected from exposure to UV light?
6. Do you have a product in the correct lane of the gel where you loaded your Amel sample? If so, can you estimate the size? If not, what may have gone wrong?
7. Do you have a product in the correct lane of the gel where you loaded your vWA sample? If so, can you estimate the size? If not, what may have gone wrong?
8. Do you have a product in the correct lane of the gel where you loaded your s317 sample? If so, can you estimate the size? If not, what may have gone wrong?
9. What is the purpose of the negative controls?
10. What is the difference between the analysis of minisatellites and the analysis of microsatellites, or STRs? What is the advantage of using STRs in DNA typing?
11. Can DNA typing be performed on non-humans? Give an example.
12. In “Exercise 1. A Mix-Up at the Hospital” which baby belongs to which couple?
13. In “Exercise 2. A Paternity Case” could Megabucks have fathered X, Y, or Z’s child?
14. In “Exercise 3. The Case of the Bloody Knife” should Smink be charged with the murder of Milhouse? What evidence exists?