**BIOL 104 Forensic Biology**

**Lab 9 Pipetting and PCR**

1. **Introduction**

Before we begin this procedure, we must learn to use a micropipette in order to transfer the small (micro!) amounts of liquids necessary when working with DNA.

Kary Mullis developed the Polymerase Chain Reaction (PCR) in 1983 as a way of making copies, or amplifying, pieces of Deoxyribonucleic Acid (DNA) in a test tube. PCR has revolutionized forensic science, allowing small samples of DNA to be analyzed in the lab. Typically, a DNA polymerase enzyme that was isolated from the bacterium *Thermus aquaticus, Taq* DNA polymerase, is used for PCR*.* Since these bacteria are found in hot springs, their enzymes can survive the higher temperatures required for the first step of PCR, denaturation.

During denaturation, hydrogen bonds between the base pairs are broken, separating the two strands of DNA and exposing the sequence of nucleotides to be copied. Like other DNA polymerases, *Taq* DNA polymerase requires a 3’ hydroxyl group to begin synthesis of a new strand of DNA. This 3’ hydroxyl group is provided by two primers. During the second step of PCR, annealing, the forward primer will form base pairs with one strand of the DNA and the reverse primer will form base pairs with the opposite strand. Annealing requires a temperature between 50°C and 65°C, depending on the sequence of the primers. In the third step of PCR, synthesis, the *Taq* DNA polymerase will catalyze the formation of phosphodiester bonds between the deoxyribose sugar of one nucleotide and the remaining phosphate group of the next deoxynucleotide (dNTP), synthesizing a new strand of DNA in the process. The process of denaturation, annealing and synthesis will be repeated 40 times. PCR is considered a “chain reaction” because the new strands of DNA produced in one round, or cycle, of PCR are used as reactants in the next cycle, providing additional strands of DNA for copying. In this manner, the desired sequence of DNA is amplified exponentially.

In this exercise, we will use PCR to amplify three loci, or locations, in DNA isolated from your cheek epithelial cells. The first loci we will amplify lies within a non-coding region of the amelogenin gene (Amel) whose protein is part of tooth enamel. The product is 6 base pairs (bp) shorter on the X chromosome than the Y chromosome, allowing it to be used for sex determination. The other two loci are Short Tandem Repeats (STRs), or microsatellites. STRs are sequences of between three and seven nucleotides repeated in tandem different numbers of times in different individuals. The von Willebrand factor type A (vWA) is involved in blood clotting; depending on the number of repeats of the sequences TCTA and TCTG, the products may range from 123 to 181 bp. The second STR, D13S317 (or s317), is located on chromosome 13; depending on the number of repeats of the sequence TATC, the products may range from 157 to 201 bp. Both vWA and D13S317 are part of the thirteen standard STRs reported in the Combined DNA Index System (CODIS). All three loci are currently used to generate DNA fingerprints, or profiles.

1. **Materials & Methods**

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

1. **Using a Micropipette**
2. We have two types of micropipettes—one that will dispense

volumes between 1 μl and 10 μl (P10) and another that will dispense volumes between 100 μl and 1,000 μl (P1000). Choose the appropriate micropipette based on the volume required.

1. Adjust the volume by turning the knob. Be careful to not turn the

knob on the P10 so that the volume shown is below 1 μl or above 10 μl. On the P1000 the volume shown should not be adjusted below 100 μl or above 1,000 μl.

1. Place the bottom of the micropipette into the top of one of the

plastic tips in a box and tap the micropipette to make sure the tip is firmly attached.

1. Press the control button to the first stopping point and put the micropipette tip into the liquid.
2. Slowly release the control button, keeping the tip in the liquid. The selected amount of liquid will be lifted into the tip.

6. Pull the tip out of the liquid. The selected amount of liquid will stay

 in the tip.

1. Hold the micropipette tip at an angle against the inside of the tube

where you want to transfer the liquid.

1. Press the control button to the first stop to release most of the liquid in the tip. Do not release the control button yet.
2. When the liquid stops flowing, press the control button to the second stop to completely empty the tip. Do not release the control button yet.

10. Pull the tip out of the tube. Then finally release the control button.

1. If you are finished with the tip, press the ejector button and it will be ejected from the micropipette.

12. Perform the following exercise to practice your pipetting skills.

a. Record the weight of an empty microcentrifuge tube in your

Results section.

b. Pipette the following volumes of dH2O into the microcentrifuge

tube, one at a time:

402.6 μl

317.4 μl

194 μl

 60 μl

 18.8 μl

 5.2 μl

 2 μl

 c. Add up the total volume of dH2O you pipetted into the

microcentrifuge tube. Close the lid and label your tube with

your lab number. Record the weight of your microcentrifuge

tube containing the dH2O.

 d. Subtract the weight of your empty microcentrifuge tube

from the weight of your microcentrifuge tube containing the dH2O to find the weight of the dH2O.

 e. In theory 1000 μl (1 ml) of water weighs 1 g. How

accurate was your pipetting? Calculate your percent

error according to the formula below:

**% Error = Experimental Value – Theoretical Value x 100**

 **Theoretical Value**

 f. Show this calculation in your Results section.

1. **Polymerase Chain Reactions**
2. Retrieve the microcentrifuge tube labeled with your assigned

number and “DNA.” This contains the DNA you extracted from your cheek cells in last week’s lab; we will use it as the “DNA template” to amplify by PCR.

1. Add the following reagents to a 0.2 ml tube labeled with your

assigned number and “A” for Amel:

dH2O 16 μl

2x PCR Master Mix

(containing buffer, dNTPs and Taq DNA polymerase) 25 μl

DNA template 5 μl

Amel Forward Primer (25 ng/ μl) 2 μl

Amel Reverse Primer (25 ng/ μl) 2 μl

 50μl

1. Add the following reagents to a 0.2 ml tube labeled with your

assigned number and “v” for vWA:

 dH2O 16 μl

2x PCR Master Mix

(containing buffer, dNTPs and Taq DNA polymerase) 25 μl

DNA template 5 μl

vWA Forward Primer (25 ng/ μl) 2 μl

vWA Reverse Primer (25 ng/ μl) 2 μl

 50μl

1. Add the following reagents to a 0.2 ml tube labeled with your

assigned number and “S” for D13S317:

dH2O 16 μl

2x PCR Master Mix

(containing buffer, dNTPs and Taq DNA polymerase) 25 μl

DNA template 5 μl

s317 Forward Primer (25 ng/ μl) 2 μl

s317 Reverse Primer (25 ng/ μl) 2 μl

 50μl

1. Make sure the reagents are mixed together at the bottom of your tubes by pipetting them up and down several times.
2. Place your tubes in a thermal cycler with the following settings:

 95° 11 min

 96° 1 min



 94° 30 sec

 60° 30 sec 10 cycles

 70° 45 sec



90° 30 sec

 60° 30 sec 30 cycles

 70° 45 sec

 60° 30 minutes

 4° hold

1. **Paper PCR**

Use the sample primers and DNA sequences to simulate three rounds of PCR in order to better understand what is going on the microliter amounts of clear reagents.

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

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

1. **Results**
2. **Using a Micropipette**

 Weight of tube plus dH2O \_\_\_\_\_\_\_\_\_\_ g

- Weight of empty tube \_\_\_\_\_\_\_\_\_\_ g

 Weight of dH2O

% Error =

The reactions will be stored in a freezer at -20°C and analyzed by gel electrophoresis in the next lab.

1. **Conclusions**
2. How accurate is your pipetting? On what, did you base this conclusion?

1. What are some common errors that can occur when using a micropipette?
2. Why must micropipette tips be changed between pipetting different liquids?
3. Why is PCR used in forensic science?
4. Why do we need to add primers to the PCR?
5. Why do we need to add both a Forward and a Reverse primer to the PCR? What would happen if we left one out of the reaction?
6. Why do we need to add a DNA polymerase to the PCR?
7. How does *Taq* DNA polymerase make PCR more efficient? What would we have to do if the DNA polymerase we were using was not heat-resistant?

1. Why do we need to add a buffer to the reaction? What do buffers do?
2. What are dNTPs? Why do we need to add dNTPs to the reaction?
3. What happens at temperatures between 90° and 96° in the PCR? What happens at 60°? What happens at 70°?
4. Assuming you start with only one copy of the template DNA, how many copies of the product will you have after one cycle of PCR? After two cycles? After three cycles? After 40 cycles? Write an expression that predicts the number of DNA copies generated from one copy of template DNA after **n** rounds of PCR.
5. What are STRs? How many standard STRs are used by CODIS?