

**Genetics in Forensic Science:  
Human DNA Fingerprinting**  
Results will be discussed in class Nov. 16.

**Readings:**

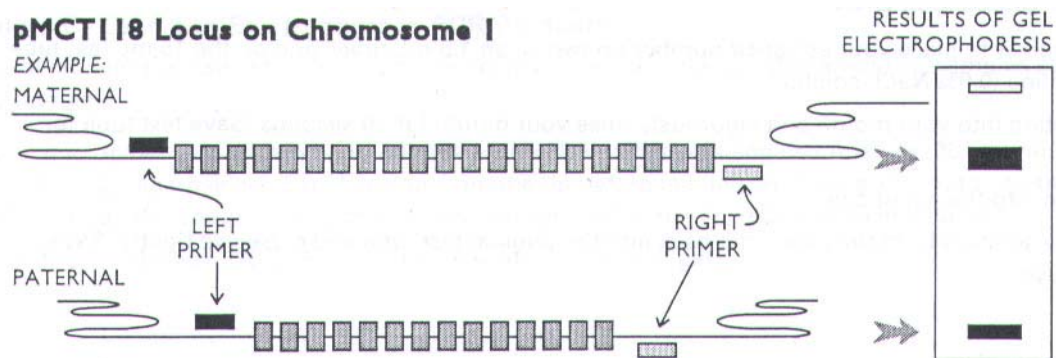
Hartwell Chapter 9 pp. 327-330.

Nakamura Y., Carlson, K. Krapco, and R. White 1988. Isolation and mapping of a polymorphic DNA sequence (pMTC118) on chromosome 1p. *Nucleic Acids Research* 16: 9364.

**Objective:** In this lab exercise, the polymerase chain reaction (PCR) will be used to amplify a locus from your own DNA to create a personal fingerprint. Although DNA from different individuals is 99.9% alike, there are variable regions that are polymorphic and provide the basis for genetic disease diagnosis, forensic identification, and paternity testing. You will work in groups as usual but you will each extract and amplify your DNA.

**Introduction:**

In this experiment, students will amplify a non-coding region of chromosome 1 containing the VNTR (minisatellite) sequence designated pMCT118. It has a repeat unit of 16 base pairs. Most individuals have between 14 and 40 copies of the repeat on each of their copies of chromosome 1. An individual's two copies of chromosome 1 usually have different numbers of copies, as do the chromosomes from two different individuals. The different versions of the pMCT118 polymorphism are referred to as alleles and are inherited in a Mendelian fashion. The pMTC118 locus has no known relationship to any disease state or any other phenotype.



The source of template DNA is a sample of several thousand cheek cells obtained by saline mouthwash (bloodless and noninvasive). The cells are collected by centrifugation and heated in a buffered solution containing beads of the resin Chelex. Chelex binds (chelates) metal ions that act as PCR inhibitors and as catalysts in the breakdown of DNA at high temperatures. Lysis of the cells and release of DNA is accomplished by a

boiling water bath and the alkalinity of the Chelex suspension. This is a "quick and dirty" extraction method (the DNA will probably be unamplifiable after a year at  $-20^{\circ}\text{C}$ ). PCR is then performed with the DNA-containing supernatant.

You can visit the DNA learning center at <http://DNALC.org>. Click on Resources then [Biology] Animation Library to view or download animations on PCR and DNA fingerprinting using VNTRs

### **Materials:**

Gloves

Tip disposal

Boiling water bath (Hot plate and large glass beaker)

Floating tube racks

Forceps

Microfuge

1.5 ml tubes

Paper cups

Eppendorf pipet aids P1000, P200, P20

Blue and yellow tips (autoclaved)

Kimwipes

Ice buckets

TA needs an ice bucket to store samples when ready for PCR.

0.9 % Saline solution (autoclaved) in 15 ml capped centrifuge tubes at 5 ml per tube.

Water (autoclaved) for Chelex solution.

Chelex resin 10% slurry in autoclaved  $\text{H}_2\text{O}$

PCR reaction beads in 500  $\mu\text{l}$  tubes (1 for each person)

PCR cyclers

2% Agarose gel (1/2 nusieve:  $\frac{1}{2}$  normal agarose with ethidium bromide)

Gel electrophoresis apparatus

DNA size markers (pBR322 digested with BstN1, sizes are:

1857 bp, 1058 bp, 929 bp, 383 bp and 121 bp) use 20  $\mu\text{l}$  per gel..

Video camera

### **Method:**

Assuming the protocol is rigorously followed, amplification problems in this lab probably relate to poor oral hygiene and inhibitors present in food particles and bacteria.

**Before you come to class, make sure there are no food particles in your mouth.** (If possible, brush your teeth and rinse thoroughly with water.)

**Wear Gloves to avoid contamination of the solutions with cells from your hands**

1. Your TA will give you a 0.5 ml tube with a PCR bead. The tube will be labeled with an identification number for your DNA. This will be the tube for your PCR reaction. Label three sterile 1.5 ml eppendorf tubes with the same number that is on your PCR tube. These tubes will be for your DNA preparation. Each person will prepare one sample of their own DNA.
2. Pour 5 ml of saline solution (0.9% NaCl) into a cup, then into your mouth. Swish it vigorously around your mouth for 10 seconds. Then expel the fluid back into the cup.
3. Pipette 1.5 ml of the solution back into the labeled 1.5 ml eppendorf tube.
4. Place your tube, in a **balanced configuration** with others, in a microcentrifuge and spin for 30 seconds at full speed.
5. Remove the supernatant from the tube using your P1000 pipette and a sterile blue tip. Do not disturb the pellet of cells at the bottom. You can dispose of the supernatant in your paper cup.
6. Use your P1000 pipette and a clean blue tip to transfer 500  $\mu$ l of Chelex slurry into your 1.5 ml eppendorf tube. Be sure the Chelex resin is well re-suspended in the water before pipetting.
7. Draw the liquid in the tube up and down with your pipette and the blue tip several times to re-suspend the cell pellet. Be sure the pellet is completely re-suspended before proceeding.
8. Transfer 500  $\mu$ l of the Chelex-cell suspension to your second labeled 1.5 ml eppendorf tube. Be sure the Chelex beads remain well suspended in the transferred sample. Use your pipette to pipette up and down to be sure the cells and Chelex are well suspended.
9. Place your sample in a floating tube rack in a boiling water bath for 10 minutes. The TA will collect the tubes and put them in the boiling water. After your tube has been incubated 10 minutes, use forceps to remove it and cool the tube on ice for 30 seconds.
10. Place your tube in a **balanced configuration** with others in a microcentrifuge, and spin at full speed for 30 seconds.
11. Set your P200 pipette for 200  $\mu$ l and transfer 200  $\mu$ l of the clear supernatant to the third clean *labeled* 1.5 ml eppendorf tube. Be very careful not to take any of the pellet. Store your sample on ice.
12. Dispose of the yellow tip in the container provided.

## PCR reactions

Set up the PCR reactions as follows:

1. Use a pipettman with a fresh tip to transfer 22.5  $\mu$ l of pMTC118 primer/loading buffer mix (red) to your labeled PCR tube containing a PCR bead. Tap the tube with a finger to dissolve the bead.
2. Use a fresh tip to add 2.5  $\mu$ l of your DNA prep to the reaction tube, and tap to mix. Pool reagents by tapping on the lab bench.
3. Put your sample in an ice bucket for your TA to take to the thermocycler.

Your PCR reaction will have the following final components:

- 10 mM Tris-HCl (pH 9.0 at 25° C) - provides optimal pH for enzyme
- 50 mM KCl - maintains ionic strength for Taq
- 1.5 mM MgCl<sub>2</sub> - cofactor required by Taq
- 5  $\mu$ M dNTP's (1.25  $\mu$ M of each) - dATP, dGTP, dCTP, dTTP
- 5  $\mu$ M of oligonucleotide primer
- 1.3 units Taq DNA polymerase - thermostable DNA synthesizing enzyme from *Thermus aquaticus*

The PCR reactions will be run in a thermal cycler as follows: Your TA will run the thermal cycler.

1. 94°C 4 min
2. 94°C 30 sec- denaturation of template
3. 65°C 30 sec- annealing of primers
4. 72°C 30 sec- synthesis of complimentary DNA strands (repeat from step2 x30 cycles)
5. 72°C for 10 minute final extension.
6. 10°C until samples are removed.

### Results:

Your TA will use gel electrophoresis to analyze the PCR reactions on a 2% agarose gel in TAE buffer along with 20  $\mu$ l of the pBR322-*Bst*NI size marker in one lane. The gels will contain the intercalator ethidium bromide which causes fluorescence of ethidium-DNA complexes under UV light and hence allows the DNA bands to be visualized.

The TA will electrophorese the samples at 100 volts until the cresol red dye in the mixture has moved to within one inch of the bottom of the gel.

They will take a photo of each gel with a ruler.

Gels of the entire class's results will be posted on the web page.

We will discuss the results in the lecture period following the lab.