* **Materials:**

Microfuge tubes

5% Chelex

Toothpicks

PCR tubes

1M NaCl

95% ethanol

TBE buffer

Master Mix

Primer Mix

Agarose Powder

Loading Dye

Molecular Weight Marker

Ethidium Bromide

Distilled Water

Micro Centrifuge

Thermal Cycler

Gel Box

Power Box

UV Light Box

Micropipeter

Micro pipettes

* **Procedure (Toothpick)**

1. Add 200 mL of 5% Chelex to a labeled microfuge tube.

2. Gently scrape the inside of cheek 3 times with toothpick.

3. Twirl toothpick in Chelex to release cells.

4. Cap and place tubes in a heat block or bath for 10 minutes at 99oC.

5. Vortex, or finger flick, each for 5- 10 seconds.

6. Spin tubes in centrifuge for 1 minute.

7. Remove 75 mL of supernatant and place in a new tube.

8. Add 25 mL of cold 1M NaCl to the 75 mL of supernatant.

9. Add 200 mL of cold 95% ethanol to tube. Invert tube 5 times to mix.

10. Spin in a centrifuge for 5 minutes at 10,000 rpm.

11. Pour off supernatant.

12. Place open microfuge tubes in heat block until ethanol evaporates.

13. Locate the smear of DNA along the side of the wall. Using 25 mL of TE buffer, slowly pipet up and down many times to wash off the DNA smear.

**Polymerase Chain Reaction**

1. Dispense 20 mL of Master Mix to a new PCR tube.

2. Add 20 mL of Primer Mix to the PCR tube

3. Add 10 mL of your purified DNA to the PCR tube.

4. Place tube into thermal cycler

5. Run program.

**Electrophoresis of Amplified DNA**

1. Retrieve PCR tube and spin briefly in centrifuge

2. Add 5 mL of loading dye to PCR tube.

3. Load 15- 20 mL of your amplified DNA into a well in the prepared 2% agarose gel. Also load 5 mL of molecular weight marker into one of the wells of each gel.

4. When the samples are loaded attach the electrodes to the power supply and electrophorese the samples at 125 volts for 45-50 minutes.

5. Stain gels with 0.5mg/mL ethidium bromide.

Data

* **Procedure (Saline Solution)**