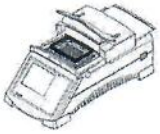
PTC PCR FLOW CHART

I. ISOLATE DNA

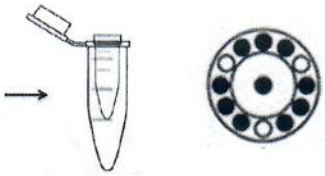
SCRAPE inside of cheek cells with flat end of toothpick for 30 sec

Directly place tooth pick with cheek cells into chelex; mix for 30 sec

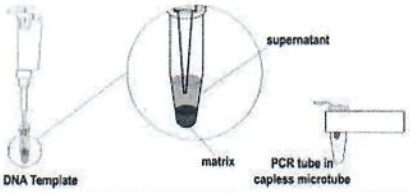
Boil: 99^0 C for 10min in thermal cycler.



*CENTRIFUGE; Full speed; 90 sec
Use capless microtube adapters



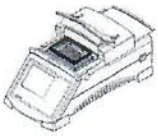
Keep tube on **ICE**; DNA in supernatant; if not proceeding to Part II. Aliquot supernatant and refrigerate



II. AMPLIFY DNA BY PCR - in PCR tube (keep on ice)

Add 22.5 µL of master mix (MM) to labeled (your initials) clear PCR tube. Pipet 2.5uL of your DNA in the supernatant to this tube with the master mix in it. **Mix with Pipet.**
Total vol=25µL

AMPLIFY in thermal cycler



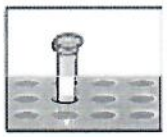
PCR will run through 35cycles cycle of denaturation, annealing and extension. (~ 1hr 25 min). Refrigerate for next day.

III. Restriction Digest with HAEIII

Add2µL Hae III to tube from thermal cycler

MIX

Incubate b@37^{0}C for 5 min in thermal cycler.



Add 2uL of Loading Dye (LD) to your sample. MiX with pipet.

IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

POUR gel (3% agarose with 1;10,000 dilution of Sybersafe dye in 1X Sodium Borate buffer)

Let gel set

Load 20 µL of your sample into the gel (1 sample per well) Include10 µL of 100bp DNA ladder and 20µL undigested control.)

Run 120-130 V for 30 min