**SNP :** single nucleotide polymorphism

**PCR:** polymerase chain reaction

Aplifies the TAS2R38

Digested with HaeIII- recognizes one of the SNPs

Saline mouthwash; DNA is extracted by boiling with **Chelex resin** which binds contaminating metal ions

PCR is used to amplify a short region of the **TAS2R38** gene

Amplified PCR product is digested with HaeIII- recognizes one of the SNPs

One allele is cut by the enzyme, the other is not

Produces a **RFLP: restriction fragment length polymorphism**

This can be separated on a 2% **agarose gel**

1. Rinse mouth with saline
2. Transfer saline- for mouthwash
3. Centrifuge
4. Pour off supernatant
5. Resuspend
6. Add chelex
7. Transfer cells suspension
8. Boil in thermal cycler
9. Shake vigorously
10. Centrifuge
11. Transfer supernatant
12. Store on ICE

PCR:

1. Add primer/loading dye mix
2. Add DNA
3. Amplify in thermal cycler

Digest PCR products with HaeIII

1. Transfer PCR product
2. Add HaeIII
3. Mix
4. Incubate in thermal cycler

Analyze PCR products by Gel Electrophoresis

1. Pour gel
2. Set
3. Load gel
4. Electrophorisize

Saline Solution

1000 microliters to a microcentrifuge tube- spin for 90 seconds at full speed

Pour off supernatant in paper cup, there will be a cell pellet

Withdraw 30 microliters of cell suspension- add it to a PCR tube with 100 microliters of Chelex

Place PCR tube in a thermal cycler- vigorously shake

Microcentrifuge for 90 seconds

Transfer 30 microliters of clear supernatant into a clean tube- don’t want cell debris or Chelex beads

PCR

PCR tube with Ready-To-Go PCR Bead

Add 22.5 microliters of PTC primer/loading dye mix, allow beat to dissolve

Add 2.5 microliters of your cheek cell DNA directly into the primer/loading dye mix

Thermal cycling

Digest PCR Products with HaeIII

Transfer 10 microliters of PCR product to the U tube- don’t do anything to it

Use a micropipette with a fresh tip to add 1 microliter of restriction enzyme HaeIII directly into the PCR product

Thermal cycler