**PTC GENOTYPING KIT TUTORIAL**

You can find this and other more detailed tutorials at <http://the-odin.com/tutorials/>

This kit contains everything you need to genotype samples for the bitter taste receptor.

Background Reading

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1181941/>

<http://www.ncbi.nlm.nih.gov/gene?cmd=retrieve&dopt=default&rn=1&list_uids=5726>

**Protocol**

1. Rinse your mouth with water and then swab inside of cheek.
2. Take cheek swab and place in 300uL 50mM NaOH in 1.5mL microcentrifuge tube
3. Stir swab in tube for 1 minute until solution is cloudy
4. Heat tube in almost boiling water for 10 minutes (95C)
5. Add 300uL of 50mM Tris
6. For a 50uL PCR reaction
   1. 1uL of cheek cell solution for PCR reaction template
   2. 1uL of PTC primer
   3. 10uL of 5x Master Mix(or 25uL of 2x Master Mix)
   4. 38uL water with 5x Master Mix(or 23uL with 2x Master Mix)
7. See PCR temperatures and times below
8. Included in the kit is a taste test strip that contains [phenylthiocarbamide](http://en.wikipedia.org/wiki/Phenylthiocarbamide)(PTC) use it to determine if you can taste bitter. Taste the control test strip first and then the PTC test strip and see if you can tell the difference.
9. After you run your PCR reaction remove 20uL from each reaction and place them in separate tubes. Add 1uL Fnu4H1 or HaeIII restriction enzyme to each reaction in each of these extra tubes and let sit for 30-45 minutes.

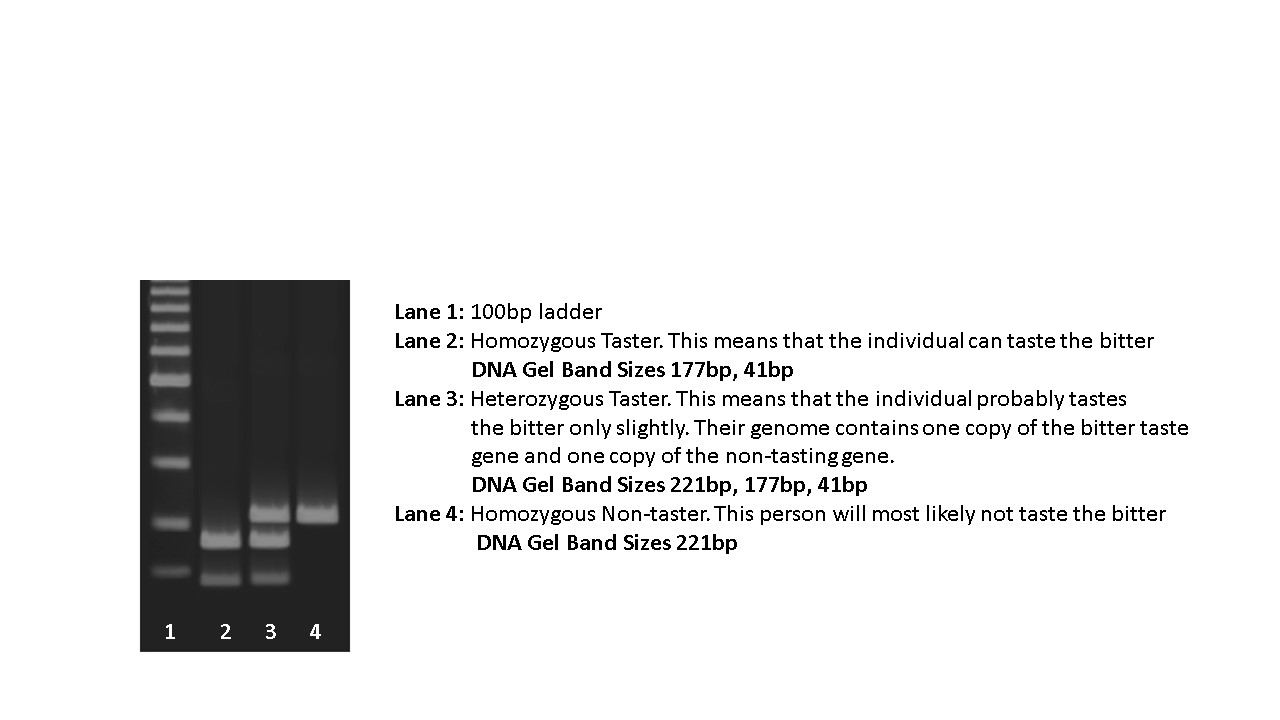
You can find how to cast and run a gel here: <http://the-odin.com/tutorials/>

1. First, make TAE if you don’t have it by adding 5g of TAE mix to 1L of distilled water or 50 to 1L to make a 10x stock and then dilute that down to 1x(i.e. 100mL 10x and 900mL water)
2. Next, add 0.5g of agarose to 50mL of TAE and microwave it on high for 1 minute or until all agarose is dissolved(the solution should be clear)
3. Add 5uL of Gel Green to the still hot molten agarose solution and swish around to mix
4. Dump the molten agarose into your gel mold and let set (this usually takes around 20-30 minutes but can be sped up by placing it in the fridge)
5. Add 3.5uL of 6x loading dye to your digest and add each of your digest.loading dye solutions to the individual wells on the gel (one well for one reaction)
6. Add 4uL of 100bp ladder to the first or last lane of the gel

**Polymorphic Sites in the PTC Taster Gene**

|  |  |  |
| --- | --- | --- |
| **Nucleotide position** | **Taster** | **Nontaster** |
| 145 | CCA  Pro | GCA  Ala |
| 785 | GCT  Ala | GTT  Val |
| 886 | GTC  Val | ATC  Ile |

HaeIII restriction enzyme only cuts the DNA of the taster allele at the sequence GGCC and creates two bands. When someone has a polymorphism or mutation at position 145 the sequence reads GGGC and so there should be no cut and only one DNA band. Sometimes individuals will have one copy of the taster gene and one copy of the non-taster gene and will have 3 DNA bands(one that is cut into two bands and one that cannot be cut).



**PCR Reaction**

**1 x** 95C 10 Minutes

**35 x**

95C 30s

55C 1 minute

72C 1 minute

**1x** 72C 10 minutes

**Primers:**

Forward PTC Primer

AACTGGCAGATTAAAGATCTCAATTTAT

Reverse PTC Primer

AACACAAACCATCACCCCTATTTT

**PTC Gene** <http://www.ncbi.nlm.nih.gov/nuccore/30230490?report=fasta>

ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTATGAAGTCAGGAGTACATTTCTGTTCATTTCAGTCCTGGAGTTTGCAGTGGGGTTTCTGACCAATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCAGGCACTGAGCAACAGTGATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCTTTTCCTGCATGGACTGCTGTTCCTGAGTGCTATCCAGCTTACCCACTTCCAGAAGTTGAGTGAACCACTGAACCACAGCTACCAAGCCATCATCATGCTATGGATGATTGCAAACCAAGCCAACCTCTGGCTTGCTGCCTGCCTCAGCCTGCTTTACTGCTCCAAGCTCATCCGTTTCTCTCACACCTTCCTGATCTGCTTGGCAAGCTGGGTCTCCAGGAAGATCTCCCAGATGCTCCTGGGTATTATTCTTTGCTCCTGCATCTGCACTGTCCTCTGTGTTTGGTGCTTTTTTAGCAGACCTCACTTCACAGTCACAACTGTGCTATTCATGAATAACAATACAAGGCTC**AACTGGCAGATTAAAGATCTCAATTTAT**TTTATTCCTTTCTCTTCTGCTATCTGTGGTCTGTGCCTCCTTTCCTATTGTTTCTGGTTTCTTCTGGGATGCTGACTGTCTCCCTGGGAAGGCACATGAGGACAATGAAGGTCTATACCAGAAACTCTCGTGACCCCAGCCTGGAGGCCCACATTAAAGCCCTCAAGTCTCTTGTCTCCTTTTTCTGCTTCTTTGTGATATCATCCTGTGTTGCCTTCATCTCTGTGCCCCTACTGATTCTGTGGCGCGAC**AAAATAGGGGTGATGGTTTGTGTT**GGGATAATGGCAGCTTGTCCCTCTGGGCATGCAGCCATCCTGATCTCAGGCAATGCCAAGTTGAGGAGAGCTGTGATGACCATTCTGCTCTGGGCTCAGAGCAGCCTGAAGGTAAGAGCCGACCACAAGGCAGATTCCCGGACACTGTGCTGA