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Lab Report #6 Final

PTC Genetics

**Abstract**

PTC also known as phenylthiocarbamide, determines the ability for a human to have a strong or weak taste. A PTC paper stripe was used to determine if we could taste a bitter taste. To confirm if what we tasted was accurate we used a cytobrush to brush our side cheek to extract our DNA. The DNA was centrifuged and after a thermal cycler was used to identify the banding patterns in the agarose gel to indicate who is a strong, weak or non-taster for PTC gene. The banding patterns on the gel indicate the type of taster, one band is a non-taster, two bands is a strong taster and three bands are weak tasters. After running the gels computer software Finchtv was used to graph the nucleotides. The graph better interpreted w A gel electrophoresis uses an here the Haelll restriction enzyme cleaves the DNA sequence between the GGCC recognition sites.

**Introduction**

 A human's taste system can differentiate between five tastes sweet, sour, bitter, salty and umami ([Melis,](https://onlinelibrary-wiley-com.citytech.ezproxy.cuny.edu/action/doSearch?ContribAuthorStored=Melis%2C+Melania) [Grzeschuchna,](https://onlinelibrary-wiley-com.citytech.ezproxy.cuny.edu/action/doSearch?ContribAuthorStored=Grzeschuchna%2C+Lisa) [Sollai,](https://onlinelibrary-wiley-com.citytech.ezproxy.cuny.edu/action/doSearch?ContribAuthorStored=Sollai%2C+Giorgia) [Hummel](https://onlinelibrary-wiley-com.citytech.ezproxy.cuny.edu/action/doSearch?ContribAuthorStored=Hummel%2C+Thomas) and [Barbarossa](https://onlinelibrary-wiley-com.citytech.ezproxy.cuny.edu/action/doSearch?ContribAuthorStored=Tomassini+Barbarossa%2C+Iole), 2019).

Phenylthiocarbamide is a PTC gene that determines how strong a person's taste is. The ability to taste the PTC gene (TAS2R38) is coded in a single gene for a receptor on the tongue. The tongue has papillae, which contain taste buds filled with gustatory cells that deal with tasting. The gustatory cells have bitter taste receptors that signal to the brain when something has a bitter taste. A strong taste for the PTC gene would be dominant TT, a weak taster has alleles Tt and a non-taster would have the alleles tt. An inability to taste PTC is seen in individuals in a recessive style due to a fixed position in chromosome 7 TAS2R38 (Risso, Sainz, Morini, Tofanelli and Drayna, 2018). Chemicals responsible for the bitter taste in food are thioureas, bioactive compounds, ureas, peptides and some amino acids ([RoldãoLeite](https://www-sciencedirect-com.citytech.ezproxy.cuny.edu/science/article/pii/S027153171730653X%22%20%5Cl%20%22%21), [Santos Júnior,](https://www-sciencedirect-com.citytech.ezproxy.cuny.edu/science/article/pii/S027153171730653X#!), [Silva de Sousa](https://www-sciencedirect-com.citytech.ezproxy.cuny.edu/science/article/pii/S027153171730653X#!), [VasconcelosLima](https://www-sciencedirect-com.citytech.ezproxy.cuny.edu/science/article/pii/S027153171730653X#!), [Miranda-Vilela](https://www-sciencedirect-com.citytech.ezproxy.cuny.edu/science/article/pii/S027153171730653X#!), 2018). Khataan, Stewart, Brenner, Cornelis, El-Sohemy conducted a study to measure the accuracy of the PTC papers and found that the filters can strongly predict the PTC gene and found women compared to men have a stronger genotype for the TAS2R38 gene (2009). In the lab the filters were used to determine if we could taste the bitterness. A thermal cycler was used to amplify pieces DNA by a polymerase chain reaction. The haelll enzyme cuts the alleles after amplification; the sixth position of the nucleotides illustrates the type of PTC taster one is. A non-taster has a G nucleotide in the sixth position of the allele, which is indigestible by the restriction enzyme, while a weak and strong taster has a C in the sixth position. The DNA that is extracted is placed in a electrophoresis to determine the banding patterns, which indicate the strength of the PTC gene. The electric field of the electrophoresis moves DNA by its molecular weight, the larger DNA moves slower compared to smaller DNA fragments (Fang, Spisz, Wiltshire, D'Costa, Bankman, Reeves & Hoh, 1998). A Finchtv computer software is used to further demonstrate the accuracy of the DNA bands.

**Method**

 In lab over the course of several weeks we conducted an experiment to test the gene TAS2R38 and if it is recessive or dominant gene. First we used PTC paper stripes to distinguish who is a strong taster, a weak taster, and a non-taster. While placing the paper stripe to my tongue, there was a strong bitter taste, indicating that I was a strong taster. My other group members were non-tasters and weak-tasters. Next we used a sterile cytobrush to brush the side of our cheek 25 times, to determine if strength of taste was accurately interpreted. We swirled the cytobrush in a tube, which contained 100ul of chelex suspension. It was then placed the tube in a centrifuge on 100-Celsius heat for ten minutes. We then store the DNA in -20 Celsius. We then added 20ul of primer mixer, which contained forward primer, reverse primer and loading dye to the tube, then added 3ul of DNA. We loaded the tube samples in the thermal cycler and ran the “PTC” program. We stored the amplified samples in -20 Celsius. Next we made the agarose gel for the electrophoresis to determine the banding patterns. In order to make the gel 50ml of TBE buffer with 1g of agarose and mixed it and placed it in the microwave for 30 seconds until dissolved and clear. We added 5ul of SYBR safe into the solution once it had cooled down slightly. We poured the solution in the tray and placed the combs into the solution to create the wells. We placed the trays in the refrigerator until it solidified. We placed the gel in the electrophoresis chamber. Each student got two micro centrifuge tubes, one labeled D=digested plasmid and the other labeled U=undigested plasmid. We transferred 5ul of the samples for each member into tubes “U” and “D” and added 1ul of Haelll enzyme to tube “D” and incubated for 10 minutes at 37 Celsius. In groups of 3 we loaded 5ul of the sample from each member into the wells. The first well we loaded the ladder and following for each member we used “D” and “U”and placed each sample in the wells. We plugged in the wires and turned on the electrophoresis for 110V for 30 minutes, and watched as the bands visualized. We observed the results under the UV trans illuminator and recorded the results.

**Result**

 In figure 1, the gel demonstrates the results for my group. The first well indicates the DNA ladder. The second two wells illustrate a student who is a non-taster for PTC gene, which is demonstrated by the one band of fragment DNA. The following two wells were my results. When I tasted the PTC paper stripes I tasted a strong bitter taste. The bands in the gel indicate two solid bands and one faint band. The DNA fragments demonstrate three bands, which indicates that I was a weak PTC taster. We used the Finchtv computer software to illustrate my nucleotides of my allele for the PTC gene. The nucleotides read 5’AGTGCCCGCC’3 and when reversed it reads 3’TCACGGGCGG’5. We look at the sixth position of the nucleotide to illustrate the cut of the restriction enzyme, which will illustrate the strength of the taste for PTC. In my case when the sequence is read from 5’ to 3’ the sixth position is G which means that I was a weak taster of PTC. If the sixth position was a C, it would indicate that I was a strong taster, which I had originally thought. Both the gel and the Finchtv results indicate I was a weak taster for PTC. When looking at the following two wells in the gel, it illustrates a non- taster for PTC due to the single band fragment. The student who placed their DNA in the wells, had slightly tasted the PTC, though the gel illustrates they were a non-taster. The last two wells indicate there must have been an error in DNA or when inserting the DNA in the gel, because it doesn’t follow the PTC pattern. The first bands are supposed to be on the 221bp mark, which is illustrated by the DNA ladder. If the person is a strong taster it will be illustrated with two bands one on the 221bp and the other on 177bp, and if they’re a weak taster the gel will have three bands on 221bp, 177bp, and 44bp.

**Discussion**

 PTC also called phenylthiocarbamide, is a chemical compound that controls the gene TAS2R38 that allows a human to taste food as really bitter or tasteless. This can be found in food such as broccoli, Brussel sprouts, kale, cabbage, cauliflower and etc. Depending on an individual’s taste of PTC chemical in food, this may lead them to the type of foods they prefer to eat. In class as PTC paper stripe was used to indicate the type of taste one is. I had tasted a strong bitter taste when I had placed the stripe on my tongue. The results illustrated that I must have perceived it wrong because it demonstrated that I was a weak taster. The PCR was used to amplify the extracted DNA from our cheek cells. In the reaction a restriction enzyme cleaves the DNA at the recognition site GGCC. When using the electrophoresis, we were able to visualize the DNA fragments that were extracted. The three fragments of DNA on the gel illustrates the amount of cuts the restriction enzyme Haelll was able to make. We used the Sanger sequence to compare our genotype sequence to the DNA fragment results from the gel. Further concluding that the gel and the sanger sequence match, indicating I was a weak taster for PTC. There were some limitations during the experiment, which may have led to errors. Such as not extracting a lot of DNA from our cheeks. I still feel strongly that I tasted the bitterness in the PTC stripe, even though the results indicate I was a weak taster. After the PCR reaction I did not have enough DNA extracted to fill the well, which may have affected the fragment bands. Another limitation is not using the directed amount of samples in the tubes, which may have been due to poor usage of the pipette.

**References**

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