Determination of Phenylthiocarbamide Tasting Ability and Genotype by Way of Single Nucleotide Polymorphism

Kevin Reynolds  
School of Natural Sciences at Ferrum College  
kreynolds@ferrum.edu

Abstract

SNPs, or Single Nucleotide Polymorphisms is a slight change in DNA. They have many uses throughout the scientific community. I however used it to figure out my genotype for the PTC, phenylthiocarbamide, gene, which determines whether or not I can taste PTC dust. I sampled PTC on a piece of paper and could taste bitterness, which tell me I am a taster. I then extracted DNA from my check cells, amplified the DNA by PCR, digested my PCR product with HindIII, and analyzed it by gel electrophoresis. When I ran my DNA, I was unable to determine my genotype. I also looked at the human taster and non-taster sequence and compared it to some other primates and determined that primates are tasters for PTC and over time have mutated to give way to non-tasters.

Introduction

Over the course of this lab I hoped to perform a successful Single Nucleotide Polymorphism, or SNP analysis. I will specifically be looking at my gene sequence that codes for the ability or lack of the ability to taste phenylthiocarbamide, or PTC, which is a bitter tasting substance.

A SNP or Single Nucleotide Polymorphism is a small genetic change, or variation, that can occur within a person’s DNA sequence. A single nucleotide polymorphism variation occurs when a single nucleotide, such as A, replaces one of the other three nucleotides letters, C, G, or T. For a variation to be considered a SNP it must occur in at least 1% of the population. In fact, SNPs make up 90% of all human genetic variation, and occur every 100 to 300 bases along the 3-billion-base human genome (3).

Although many SNPs have no effect on cell function, scientists believe that some could lead to disease or influence a how a person responds to a drug. Scientist believe that SNP maps will help them identify the multiple genes associated with complex illnesses such as cancer, diabetes, vascular disease, and some forms of mental illness. While SNPs do not directly cause disease, they can help determine the likelihood that someone will develop a particular illness. SNP analysis is commonly used for pharmacogenomic, diagnostic, biomedical research, gene mapping, and has many other applications (3).

In general SNP analysis consists of a few key steps. First I obtained a DNA source, and extracted my DNA. I then amplified my DNA to insure that I had a sufficient amount. The amplified DNA was then digested with a restriction enzyme, and was then run through an agarose gel by electrophoresis (2).

PTC, or phenylthiocarbamide, was first found to have a bitter taste at DuPont in 1930 when C.R. Noller complained that it tasted bitter when some of the dust accidentally got in his mouth, while his partner Arthur Fox did not taste anything at all. Albert Blakeslee later found that the inability to taste PTC is a recessive trait that varies in the human population. The gene for the PTC taste receptor, TAS2R38, was identified in 2003 (2).

Tasting phenylthiocarbamide is a dominant trait. People who can taste the bitterness of phenylthiocarbamide, or “tasters”, can either be homozygous (TT) or heterozygous (TT) dominant with a gene sequence of 5’GGCGGGCCACT3’. While people who taste nothing, “nontasters”, have the recessive alleles (tt) with a gene sequence of 5’GGCGGGCACT3’ (2).

Methods

To perform my SNP analysis I first isolated my DNA with approximately ten milliliters of 0.9% saline solution. I swished it in my cheeks for thirty seconds. Then it was expelled into a paper cup and swirled to mix...
any cells that may have settled to the bottom. A micropipet with a fresh tip was then used to transfer 1000μL of the solution into a 1.5mL microcentrifuge tube. The microcentrifuge tube was then centrifuged for ninety seconds at full speed. I then removed my tube and poured off as much supernatant as I could without disturbing the pellet of cells at the bottom. I then resuspended the cells in the remaining saline by pipetting in and out of a micropipet set at 30μL. 30μL was then added to a polymerase chain reaction, PCR, tube containing 100μL of Chelex. I then placed my PCR tube into a thermal cycler which was set to run for ten minutes at 99°C. Once it had boiled I shook the tube for five seconds. It was again centrifuged for ninety seconds at full speed. I micropipetted 30μL of the clear supernatant into a clean 1.5mL tube, being careful not to disturb the cell debris or Chelex bead. I then stored my sample on ice at -20°C until I could continue my analysis (2).

The second step was too amplify the DNA using polymerase chain reaction, or PCR. This process is used to amplify a specific sequence of DNA, in this case the PTC gene sequence. To do this I first obtained a Ready-To-Go PCR Bead and added 22.5μL of PTC primer/loading dye mix and 2.5μL of my cheek cell DNA using a micropipet with a fresh tip. My PCR tube was then placed in the thermal cycler which was programmed for thirty cycles for the following: 94°C for thirty seconds-Denaturing Step, 64°C for forty-five seconds-Annealing Step, and 72°C for forty-five seconds-Extending Step. During the Denaturing step the high temperature breaks the hydrogen bonds between the base pairs of DNA. The Annealing step allows the DNA primer to form bonds with the single stranded DNA. Finally, the Extending step allows the polymerase to make a new copy of the DNA. This repeats thirty times. Until I was able to continue to the next step, I placed the tube on ice at -20°C (2).

The next step was to digest the PCR product with HaeIII. This enzyme looks for the 5'GGCC3' sequence, which codes for a "taster", and cuts between the middle G and C. To do this I transferred 10μL of the PCR product into a clean 1.5mL tube, and put on ice for later, to be run as undigested. 1μL of the HaeIII enzyme was then placed into the tube with the remaining PCR product. It was then mixed and pooled by either tapping the tube on the table or placing it in the centrifuge. The PCR tube was placed in the thermal cycler at 37°C for thirty minutes for digestion. The tube was kept on ice until I was ready to continue (2).

The final step was to analyze the PCR products by gel electrophoresis. This step separates the DNA based on size, therefore letting me distinguish between cut ("taster") and uncut ("non-taster") DNA. I used a 2% agarose E-gel, which is a premade gel, and in the first well, on the left, I loaded 20μL of pBR322/BstNI size marker. I then loaded 10μL of my undigested sample into one well, and 16μL of my digested sample into a second well. The gel was then run at 130V for thirty minutes. When it had finished running I looked at the gel using transillumination (2).

Results

When the gel was ran, well 1 was filled with a pBR322/BstNI marker, which has set molecular weight markers to act as a standard. When the gel was ran I was able to see bands at 1875bp, 1058bp, 929bp, 383bp, and 121bp (2).

When I looked at my wells in which my undigested, control, sample (well 11) and digested sample (well 12), I would theoretically have seen one band in the undigested lane, and from one to three bands in the digested sample lane. I would have only seen one band in the undigested lane, at roughly 221bp, because the restriction enzyme was added to this sample.

For the second well, well 12, in which my digested sample was placed, I could have seen one of three possible outcomes. If I was a non-taster, I would have only seen one band at roughly 221bp, just like my undigested sample, as the restriction only cut the gene sequence for a taster, 5'GGCC3'. If I were a taster I would have either seen two or three bands depending on whether or not I am homozygous or heterozygous dominant. If I were heterozygous dominant then I would have seen three bands, one at 221bp, just like the undigested sample, one band just below that at roughly 177bp, and a faint third band at 44bp. The second and third bands were the dominant allele that was cut and the first band would have been the uncut recessive allele. If I were a homozygous dominant taster I would have only had two bands at
roughly 177bp and 44bp, as the restriction enzyme cut for the dominant allele creating two bands.

When my gel was actually run, my wells did my work properly. Picture 1.1 shows my actual gel ran during this procedure. As I saw a streak in the gel I know that I had DNA, however I was unable to see distinct bands.

I had a phenotype for a taster, for when I tasted PTC paper I could taster the bitterness of PTC, so I would have either seen two bands for a homozygous dominant taster (TT), or three bands if I were a heterozygous taster (Tt).

Conclusion

While I was able to determine that I am a taster phenotypically, I could not determine my genotype, as my gel did not perform as expected.

There are now ways to see the differences in the gene sequences from a number of species. Through the human genome project and other gene-mapping project, gene sequences are now available in an online database. I used a database provided by the National Center for Biotechnology Information and the BioServer internet site from Dolan DNA Learning Center to compare and analyze the SNP differences in the TAS2R38 gene in a human taster and human non-taster, as well as chimps, bonobo, and gorillas.

I looked at nucleotide 145, 785, and 886 in the sequences to compare. The SNP at nucleotide 145 changed the C (cytosine) in a taster, to G (guanine) in a non-taster's gene sequence. The original taster codon in the sequence is CCA, and when it is transcribed and translated it codes for the amino acid glycine. The non-taster codon is GCA, which leads to arginine instead of glycine. The second SNP we looked at was at nucleotide 785. The taster sequence has a C (cytosine) where as the non-taster has a T (thymine). The codon for the taster is GTC, which codes for arginine, however the codon for the non-taster is GTT, which codes for glutamine. The last SNP looked at is at nucleotide 886. The taster has a G where the non-taster has an A. The GTC codon, which codes for glutamine, in a taster, changes to the ATC codon, coding for stop, which may explain why non-tasters can not taste PTC as they do not have the additional amino acids that a taster would. Figure 1.2 shows the SNP in the tasters and non-tasters.

I also compared human taster and non-taster to chimp, bonobo, and gorilla. Figure 1.3 shows the nucleotides at 145, 785, and 886 for all five specimens analyzed.

By looking at the nucleotide sequences I can determine that the original state of this gene was the taster sequence. Other primates are tasters, which means that there is most likely a very important function for the gene that gives the ability to taste PTC, most likely for survival. As descendents, we, for some reason, no longer need the ability to taste PTC, whether it is for survival reasons or something different. The only place in the
sequence that does not match up is in the 886 nucleotide for the bonobo, which matches neither the taster nor non-taster.

References


2. “Using a SNP to Predict Better Tasting Ability”, DNA Learning Center, Carolina Biologists


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