

Lab 5

What was cleared up?

Larger strands have a harder time moving further through the gel matrix while smaller strands are able to move more easily. Molecular weight markers of known DNA lengths are also run on the gel to be used as standards; these can be used by an experimenter to compare and identify as accurately as possible the sample DNA sequences. The DNA gel is then stained, and the bands created on the DNA gel are seen when the DNA gel is placed under UV light ("Gel Electrophoresis").

While this is a highly accurate manner to analyze the genes coded for by the DNA sequence, bioinformatics have also become popularized in the field of biology. Genetic code can be entered into the BLAST program, available through the National Center for Biotechnology Information, and analyzed to determine which stored DNA sequences it most closely resembles. This method increases the expediency of genomic investigations, and connections can be made based upon the similarity of one gene or sequence from another. Specific sequences of DNA can also be entered into Bioservers, a program from the Dolan DNA Learning Center which stores imported sequences of DNA and allows the user to pick specific imported sequences to compare to each other. Through this program, the nucleotides of one entered sequence could be compared to the nucleotides of another entered sequence in a more time-efficient manner ("Part 2: Bioinformatics of the TAS2R38 gene" 5-9).

Why did we do bioinformatics? What did we need to learn?

The broad question being asked in this experiment is whether one is a taster or non-taster of PTC based upon his or her genotype. The formulated hypothesis is based upon the determined phenotype of the individual found through a taster stick. Because I could taste the bitterness of the taster stick, I hypothesized that my TAS2R38 gene is either homozygous dominant or heterozygous dominant. Based upon the experimentation performed, I will be able to determine what my genotype is for this gene.

#### Method:

The phenotype of an individual was tested using a taster stick and recorded to later compare to genotype data. To test one's genotype, cheek cell samples were collected from each subject using saline mouthwash. The collected cheek cells were then lysed upon using multiple rounds of centrifugation which results in the separation of the supernatant (DNA) and the pellet (cell wall debris). From each sample, DNA was extracted and thus isolated for analysis. The sample was prepared for Polymerase Chain Reaction (or PCR). PCR was used on each sample to amplify a specific sequence of the TAS2R38 gene through completion of the three temperature cycles. After PCR, the specific sequence (GGCGGGACT for taster or GGCGGCCACT for non-taster) should be amplified multiple times in DNA fragments ("Polymerase Chain Reaction" 1). A restriction enzyme HaeIII was added to the sample to cleave the desired nucleotide sequences from the DNA fragments. HaeIII acts as an endonuclease, cutting specific nucleotides and creating fragments to run through gel electrophoresis. It cleaves one allele (DNA strand) anywhere where 5'GGCC 3' occurs on the leading strand and 3'CCGG 5' occurs on the lagging strand. This is known as restriction digest; it creates a restriction fragment length polymorphism which aids in determining whether the DNA codes for a taster gene or a non-taster gene. The restriction enzyme cuts the DNA of a taster gene in a different location than it would for DNA of a non-taster gene producing fragments of different lengths based upon the gene one possesses ("Restriction Enzymes"). Another allele was kept undigested to compare in size to the digested DNA fragments. The digested DNA and undigested DNA were loaded onto a 2% agarose DNA gel and underwent gel electrophoresis. Gel electrophoresis produced bands in the gel that coincided to markers in the first and second lane, which helped in determining whether the digested allele was a taster or non-taster gene ("Restriction enzyme digestion and gel electrophoresis of your PTC gene PCR products" 1). Based upon the position of the band from the digested DNA, the subject was able to determine his or her genotype.

Intro or Discussion just the Methods are