Stephanie Cabrera April 25, 2019

Lab Report: Molecular Genetics, PCR and Genotyping

Abstract:

The purpose of this experiment was to understand molecular genetics using polymerase chain reaction (PCR). PCR amplifies a specific DNA fragment from a DNA sequence. The first part of this experiment focuses on testing the phenotype of bitterness using the phenylthiocarbamide (PTC) strips. The second portion of this experiment consisted in amplifying the 221 bp fragment of the PTC gene to determine if the results from the first part of this experiment match with the second portion. In week three of this experiment using the software FinchTV allele sequence was able to be compared and contrasted. In conclusion, the results obtained for this experiment concluded that DNA sample #13 is heterozygous thus a weak taster. This experiment demonstrates the use of PCR analysis in genetics to determine taster and non-taster genes.

Introduction:

Taste composes one of the six senses in humans, thus it plays an important role in allowing humans to know whether something that can harm their digestive system or pleasure their stomach. In this experiment we analyze the effects of taste in human by performing two different types of analysis. For this experiment the use of phenylthiocarbamide (PTC) was used to determine the level bitterness in humans. This was then confirmed by performing a polymerase chain reaction to determine if in fact we have the gene which tastes for bitterness. This was measured through the receptor of TS2R38. TS2R38 belongs to the gene family which bind to different molecules to distinguish bitterness however, TS2R38 encodes seven transmembrane G protein receptors such as PTC (Deshaware, S., Singhal, R., 2017). This protein receptor has various variants and alleles where; *T* is the dominant gene which is responsible for the taste of bitterness and *t* is the non-tasting allele. The differences between two common alleles

are due to single nucleotide polymorphism (SNP) (Blair,C., 2018) . The possible combinations that humans can inherit are as follow homozygous dominant (TT) which would have a strong taste for bitterness homozygous recessive *(tt)* which would not taste any bitterness and heterozygotes *(Tt)* which can taste bitterness mildly.

Polymerase chain reaction was discovered in 1980's and used to amplify a specific DNA fragment in a DNA sequence. PCR's are described as an exponential amplification where one template becomes two or more templates during a cycle (Carr, A.C., Moore, S.D.,2012). During the PCR technique the DNA fragment that is going to be amplified is determined usually ranges from 500-100 bp. For a PCR reaction to occur primers are needed to identify the DNA fragment. Another important component of PCR is the master mix which consists of PCR buffer, nucleotides (dNTPs), both primers, taq DNA polymerase and the template DNA. DNA polymerase is an important enzyme in PCR since it links the nucleotides together to form the product of PCR (Garibyan, L., Avashia, N.,2013). PCR are usually ran in a machine called thermal cycles which use different temperatures to amplify the DNA. There is a series of steps in order to amplify the desired DNA fragment. First the DNA fragment is denatured this allow the DNA strand to separate. Then annealing occurs where which allows the primers to attach to the target site. And the last step is extension where the DNA polymerase binds to add nucleotides to the developing DNA. After this is done the next step is to remove the primers and the unincorporated nucleotides. In PCR analysis restriction enzymes are used to cut DNA fragments at specific binding sites. Restriction enzymes have been able to transform genetics and molecular biology by allowing DNA recombination to occur. In this experiment HaeIII restriction enzyme is used which is able to cleave DNA at their recognition sites thus producing cuts in order to able to analyze these smaller fragments closely. (Loenen, W. A., Dryden, D. T., 2013). The use of this

restriction enzyme allows for the correct cuts to be made thus allowing for the fragments to be broken down by size when running on the gel electrophoresis.

In addition to performing a phenotypical assessment and PCR a DNA chromatogram is performed in experiments such as these. DNA chromatogram allows for DNA/ allele sequencing to be analyzed in at specific sites. Sanger sequencing is one method used to analyze large files of chromatogram. Sanger DNA was developed in 1970 and allows to large DNA sequences to be analyzed downstream rather than doing this manually (Stucky, B.J, 2012). Although Sanger sequencing has been around for many years it does provide an essential tool for biologist studying genetics by saving valuable time however, Sanger sequencing does not detect sequences out of regions that contemplated (Totomoch-Serra, A., 2017). Thus, it analysis the sequence that is being specifically fragmented. Molecular biology and genetics are constantly improving by providing new technology in order to ease and provide accurate results for society. This experiment provides a sense of how genetics has evolved by simply testing whether individuals are tasters or non-tasters. This was accomplished by performing a phenotypical assessment first then by performing a PCR analysis and then by using DNA chromatogram to visualize allele sequencing.

Methods:

In week 1 of this experiment PTC strips were handed out to each student to determine whether they were strong PTC taster, weak PTC taster and non-taster. Afterwards a sterile cytobrush was given to each student in order to extract DNA from their cheek cells. With the cytobrush each cheek was struck about 25 times in order to extract DNA. Then the cytobrush was swirled in 100 ul Chelex suspension in a microtube. This was then centrifuged and then placed in a cell suspension of 100 °C for 10 minutes. This was then placed in the centrifuge for 5 minutes in high

speed and then in a -20 °C bath. While the samples where in the bath, PCR with PCR beads and DNA sequencing were prepared. 22 ul of primer mixer which consisted of forward, reverse and loading dye) was placed in each of the PCR tubes containing PCR beads. Once these PCR tubes were ready 3 ul of DNA samples where loaded into each corresponding tube. These samples where then loaded into the PTC program and where left to run. Once the PCR program ran the PCR tubes these were then sent for DNA sequencing using the sanger method.

Week two of this experiment consisted using restriction enzyme digestion and gel electrophoresis. Each group of 4 students prepared 2% agarose gel by mixing 50 ml of TBE buffer and 1g agarose. This was then placed into the microwave for 30 seconds and then continuously mixed until the agarose was completely dissolved. Once the solution was fully mixed 10 ul of SYBR Safe DNA was added into the flask and mixed gently. The gel apparatus was set up using the comb which contained 10 wells. The solution was then poured into the apparatus and allowed to cool by placing the apparatus into the refrigerator for no more than 20 minutes. As the gel apparatus cooled down, new micro centrifuge tubes were obtained and labeled with a U for undigested and D for digested along with each student's initials. Then 5 ul of the PCR product from the previous week was transferred to each micro centrifuge tube. 1ul of HaeIII enzyme was added to the micro centrifuge labeled D. This was then briefly vortexed/ mixed with a pipette. After this was done both micro centrifuge tubes were incubated for 10 minutes in a 37 °C. The remaining 15 ul of PCR product was saved for DNA sequencing. The gel apparatus was then removed from the refrigerator, the comb was gently removed as well and then it was placed in the gel electrophoresis apparatus where the wells face the negative electrode. Gel electrophoresis apparatus runs from negative to positive when DNA fragments are being broken up based on size. Once the gel was loaded TBE buffer was added to cover the gel

electrophoresis. The gel was then loaded and so where wells as follows. Each student loaded two wells one with 5 ul of their Digested DNA and the other well with 5 ul of their Undigested DNA. The last well was loaded with 5 ul of the DNA ladder presented for this experiment. Once all wells were loaded then gel was ran at 120 V. When the gel finished running the gel electrophoresis were interpreted under UV light.

Week three of this experiment consisted of data analysis using a software called FinchTV. Using this software each student imported their DNA sequence into the software in order to edit the sequence and analyze it using a chromatograms. This analysis was used to compare and contrast whether if the banding patterns from the gel match the results from the first week of the experiment.

Results:

Week 1:

Stephanie's PTC phenotype is **weak taster**. Stephanie was able to taste the bitterness however, the taste was not as strong. Thus her phenotype is **heterozygous (Tt)**.

Week 2:

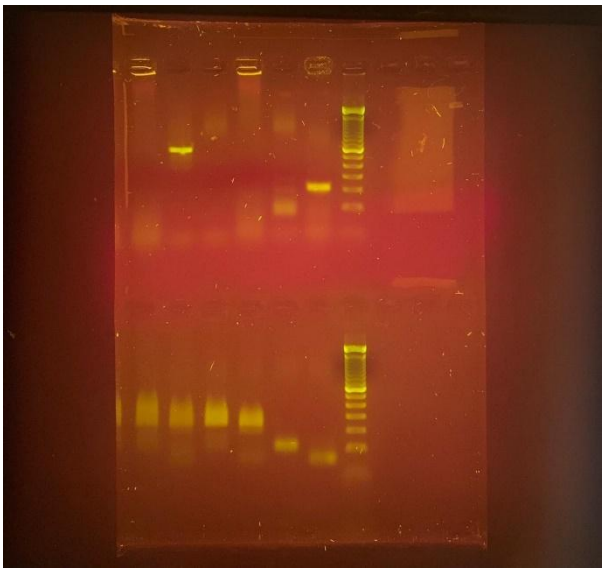

Figure 1: The gel electrophoresis from group #12-14.

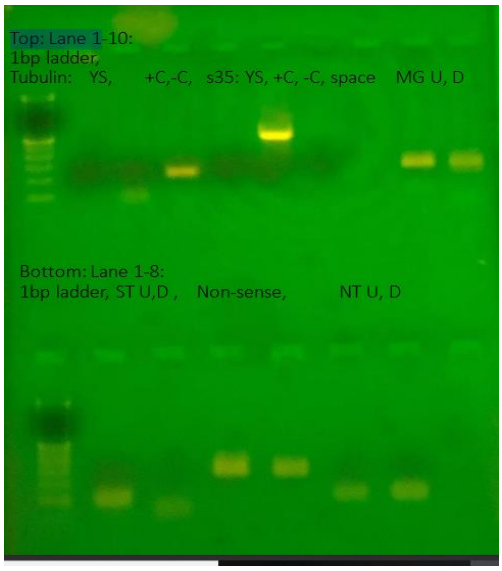

Figure 2: Gel electrophoresis of PTC from Professor Gotesman..

Figure 1 shows two PCR gel electrophoresis ran however for this portion of the experiment only the second gel was analyzed. The bottom PCR shows the banding patterns for DNA samples #12-14. Wells #3 and #4 were loaded with sample #13 which consisted of Stephanie's undigested and digested DNA sample. Based on the results presented in the first portion of this experiment her sample should have showed a 3 bands in order to demonstrate she is heterozygous (weak taster). Although the bands are not clear there does seem to have three blurry banding patterns confirming the analysis from week one. Figure 2 demonstrates a clear visual of 3 banding patterns from a heterozygous thus one can conclude that Professor Gottesman is a heterozygous, weak taster. Figure 1 also demonstrates DNA sample #14 which are wells #5 and #6 as a strong taster only two bands were present. The first two wells from this PCR also demonstrate a heterozygous where three blurry bands were present in digested sample.

Week 3:

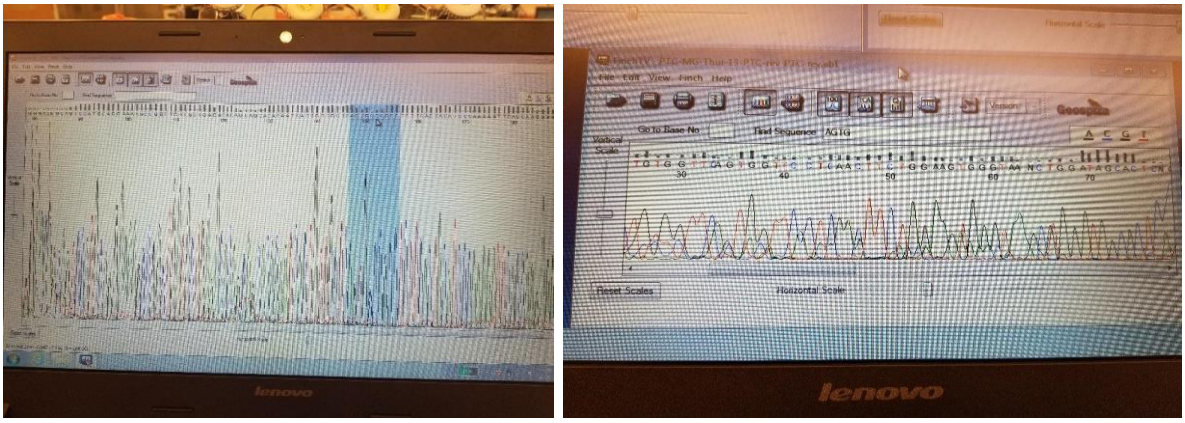

Figure 3 &4: Show the analysis of DNA sequencing using the software FinchTV. Figure 3 shows the complete allele sequence of the sample. Figure 4 shows the GGCC cut for this DNA sequence at around position 146.The results presented from Stephanie's DNA sequence presented as follows: AGTGCCCGCC which gives for a complementary of TCAC**G**GGCGG. Which is the reverse sequence of GGCGG**G**CACT. This confirms with the sequence of a weak

taster. Thus, at least one allele is present that demonstrates that Stephanie is a heterozygous/ weak taster.

Conclusion/ Discussion:

In conclusion polymerase chain reactions help quantify a DNA fragment thus it can lead to future benefits such as analyzing alterations of gene expression in tumors, microbes and other diseases (Garibyan, L., & Avashia, N.,2013). In this experiment the amplification of fragment 221 bp for the taster gene demonstrates whether each student was a homozygous dominant/ recessive or heterozygous taster. In order to demonstrate if one was a heterzgous taster three bands needed to present thus three cuts needed to be made at 221 bp, 177 bp and 44 bp. In DNA sample #13 which consisted of Stephanie's DNA one is able to see cuts made however, although they are blurry one can conclude that three bands are present thus confirming she is a heterozygous. If students where a strong taster then two bands would be present at the 177 bp and 44 bp thus indicating that they are homozygous dominant. If a student was a homozygous recessive then they would have presented with only one band at the 221 bp fragment. The enzyme used to perform these cuts was HaeIII, which looked for the taste allele sequence of GGCC. If cuts were made then these individuals are non-taster thus do not present with the allele sequence of GGCC. The software of FinchTV allowed students to visualize the allele sequence in order to compare and contrast the results obtained from the gel electrophoresis and from week one. Polymerase chain reactions allow scientists to quantify a DNA fragment and analyze it to determine if phenotypical results match genetics results. Overall, this experiment demonstrated a full understanding of restriction enzymes, PCR and the benefits it presents in molecular biology and genetics.

References:

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