Yu Tun Ng

BIO 2450L

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Final Lab Report: PTC

Abstract

The purpose of this experiment was to allow students familiarize themselves with different techniques used in molecular genetics to analyze genetic variation. The technique that was used to assess genetic variation was known as Polymerase Chain Reaction (PCR). PCR is a type of fragment-based method used to amplify a specific fragment of DNA. Two experiments are performed over the course of three weeks. The first week entailed the extraction on the DNA of interest, setting up the PCR, and sending the amplicons for sequencing. The second week entailed the casting of gels for gel electrophoresis, the use of restriction enzyme digestion, and the analysis of gel electrophoresis. The third week entailed data analysis using FinchTV. The goal of this experiment was to help students determine whether they are a taster or non-taster. DNA from cheeks cells was extracted and PCR was used to amplify 221 bp fragment of TAS2R38 gene. The amplification of TAS2R38 gene with PCR is followed by DNA restriction analysis with *H*aelll restriction enzyme. This cleaved DNA at GGCC recognition site. Then, gel electrophoresis is used to observe the fragments following digestion. As a result, YN (Yu Tun Ng) is a weak PTC taster with heterozygotes alleles (Tt).

Introduction

The first experiment tested for a genetic taste receptor that regulates the bitterness of a substance. To test whether individuals have this specific receptor, an artificial, non-toxic compound known as phenylthiocarbamide (PTC) was used to mimic the taste of bitterness (Leite, Júnior, Sousa, Lima, & Miranda-Vilela, 2018). The ability to taste for PTC is correlated to genetic linkage and inheritance (Wooding, et al., 2004). Individuals will inherit two copies of this gene, resulting in various bitter taste gene variation. Thus, individuals may have different levels for tasting PTC. Some individuals may perceive a strong taste for PTC, while others may perceive a weak taste to PTC or none. The receptor protein that detects for bitterness is encoded by an autosomal gene known as TAS2R38. This gene is represented by two alleles, ***T***, as the dominant tasting allele, and ***t***, as the recessive non-tasting allele. Homozygous dominant recipient (***TT*)** will have a strong sense of bitter taste; heterozygote recipient (***Tt***) will have a mild sense of bitter taste, and homozygous recessive (***tt***) will not sense any bitterness. To

examine the genotypes with the different phenotypic tasting abilities, PCR technique was used to amplify the 221 bp fragment of the PTC gene. Polymerase Chain Reaction (PCR) is a type of quantification method widely used by scientists to generate many copies of a section on the DNA of interest (Carr & Moore, 2012). Following PCR analysis, a gel electrophoresis and Sanger sequencing were performed at last to analyze the bitter taste allele sequences. The gel electrophoresis is used to observe the separation of banding DNA pattern based on their size (Brody & Kern, 2004). Meanwhile, the Sanger sequencing is used to determine the sequences of a individual fragments on the DNA of interest (Totomoch-Serra, Marquez, & Cervantes-Barragán, 2017).

Materials and Methods

In the first week, students were given a control paper and a synthetic PTC paper for tasting. Students were instructed to taste the control paper first, and then the PTC paper. After tasting, students were instructed to quantify the class data and determine the ratio between strong PTC taster, weak PTC taster, and non-tasters among each other. Following PTC tasting, students were then instructed to extract DNA from their cheek cells by using a sterile cytobrush. The cytobrush was inserted into the mouth and brushed the inside of the cheek 25 times. After brushing, the cytobrush was swirled in 100 uL of Chelex suspension. The centrifuge tube was placed on 100 °C heat block for ten minutes. Following heating, the tube was centrifuged at a maximum speed for five minutes. The top layer, known as the supernatant, is composed of the DNA extracted from the cheek cells. The bottom later consisted of beads and was avoided. The tube with the DNA was stored in -20 °C.

In the second week of the experiment, students were instructed to set up restriction digests on the PCR products to quantify allelic polymorphisms. First, students in groups of four prepared a 2% agarose gel. Students combined 50 mL TBE buffer with 1 g agarose. The mixture was heated until all the agarose has dissolved in the buffer solution with a clear looking appearance. The flask was then removed from the microwave and 10 uL of SYBR safe solution was added to the flask and swirled. The SYBR will stain the DNA and help see the DNA in the gel under UV light. The solution was left to cool down for approximately 3 minutes, until it was no longer hot. The solution was gently poured into a gel casting tray

and two plastic combs with 10 wells were placed at each end of the gel. The gel tray was placed in the refrigerator for approximately 20 minutes to allow the gel to solidify. After the gel solidified, the combs were removed, leaving small wells behind. Following gel casting, students obtained two new microcentrifuge tubes. Both tubes were labeled with the corresponding initials of our names. The first tube was labeled with the letter "U" for undigested and the second tube with the letter "D" for digested. Students pipetted 5 uL of PCR product twice and transferred into both tubes. Then, 1 uL of *H*aell enzyme was pipetted and inserted into tube "D". Both tubes were vortex to allow the mixture to homogenate, and then incubated for 10 minutes at 37 °C. The remaining 15 uL of PCR product was saved for DNA sequencing. Afterwards, both samples were removed from the incubation and was loaded into the gel that was prepared beforehand. The U (undigested) same was loaded on the first lane, and the D (digested) sample) was loaded on the second lane. A 5 ul of DNA ladder was included at the last well of the gel to help size fragments. Finally, the gel was set for 20 minutes at 120 V. Picture were taken and analyzed.

In the third week of the experiment, student's amplicons that were sent for sequencing were observed and analyzed by using the software FinchTV. Various chromatograms were observed to edit the sequences.

Results

Week 1: DNA Extraction – Yu Tun Ng is a Weak PTC taster with heterozygote alleles (Tt).

Week 2: Gel Electrophoresis

YN (U, D) – SC (U, D) – S (U, D) – Kb Ladder

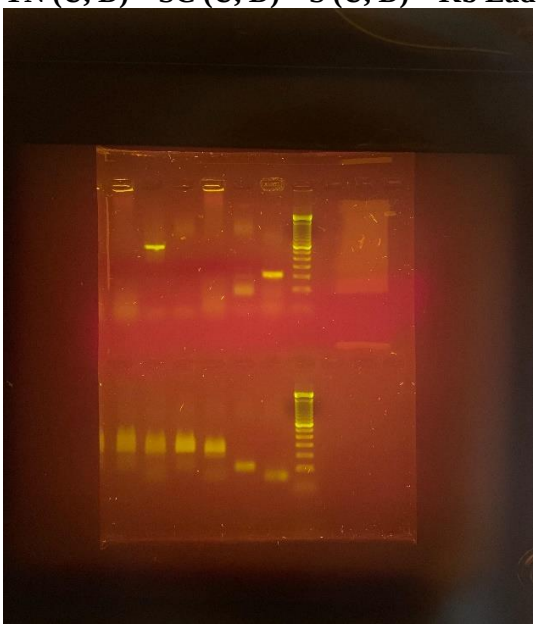

Figure I. Group (12-14)

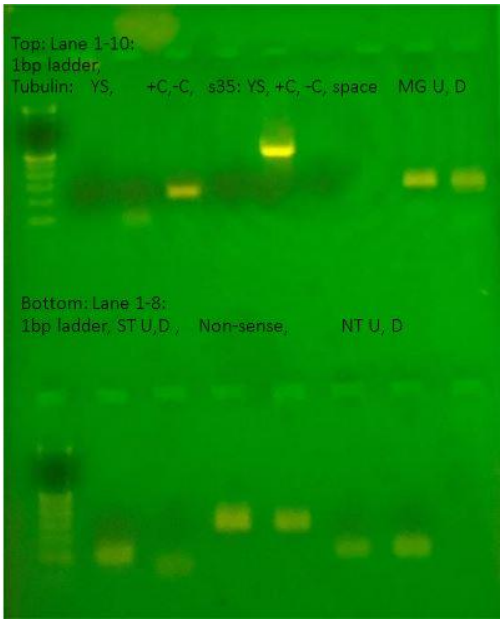

Figure II. Professor Gotesman Sample

Week 3: PTC Chromatogram: Highlighted pattern (AGTGGCCGCC)

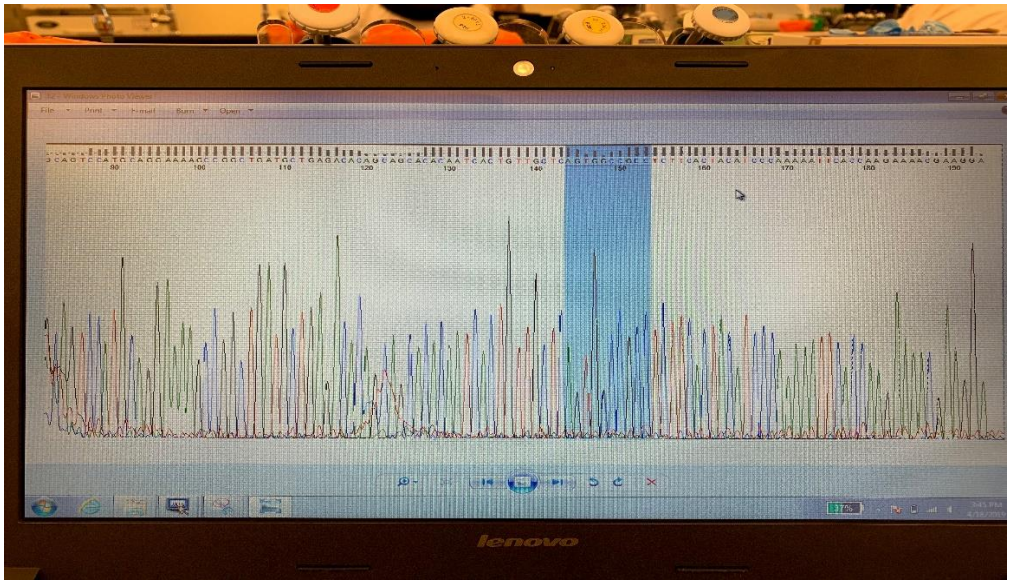

Figure III.

Discussion

In this experiment, the amplified PCR product was digested with the restriction enzyme, *H*aelll. The mixture of this restriction enzyme with the taster (TT) allele resulted in 44bp and 177bp. Meanwhile, the non-taster (tt) allele was not cut and stayed as 221 bp fragment. Based on the gel electrophoresis, strong PTC taster exhibited two bands, weak PTC taster exhibited three bands, and non-tasters exhibited one band. As displayed on Figure I., three patterns bands are observed on the first two lanes for YN. The first lane is the undigested DNA and has a line around 221 bp. The second lane is the digested DNA and has three lines around 221 bp, 177 bp, and 44 bp. This implies that YN is a weak PTC taster with heterozygote alleles (Tt) as it shows three fragments. If individual was a strong PTC taster, the combination of GGCC recognition sequence would be cut for both homozygous dominant alleles (TT), and none for homozygous recessive alleles (tt). However, for individuals with heterozygote alleles (Tt), only one allele has been digested and the other allele has not. As displayed on Figure III., the highlighted section of the chromatogram presented the allele sequence AGT**GGCC**GCC. The complementary base pair of this sequence would be TCA**CCGG**CGG, so the reversed sequence would be GGC**GGCC**ACT. This confirms that YN had at least one allele, in this case Allele 1, that makes her a weak taster. Therefore, it is evident that the trait for bitter taste of PTC is in fact passed on from parents to offspring.

References

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