Genotype Analysis of Photic Sneeze SNP

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Abstract

The objective of this study was to determine what the genotype of individuals with photic sneeze reflex has. This was determined using PCR purification to send the samples for sequencing. The samples were then analyzed in SnapGene to determine the genotype using the SNP ID. Four DNA samples were analyzed, sample 1 was inconclusive but the other samples were high quality. The data revealed that photic sneeze reflex was a dominant trait and found as CC or Cc.

Introduction

Identity tests, as performed in the fields of paternity and forensics, rely on the detection of genetic differences among individuals.(Budowle, 1991) This can be found using a few scientific techniques such as gel electrophoresis and PCR(Polymerase Chain Reaction).

Materials and Methods

A 1.5 milliliter microfuge tube was obtained and 60 microliters of sterile water were added to the tube. Cheek cells were collected using the stick side of a sterile swab and swirled in the water. Seven and a half microliters of primer mix were added to the PCR tube and then 12.5 microliters of master mix were added. Five microliters of the liquid with cheek cells were added to the PCR tube. The PCR tubes were placed in the PCR machine and began thermocycling. Initial denaturation was performed at 98 degrees celsius for 5 minutes. The tubes were run through 30 cycles starting at 98° C for 5 seconds, 67° C for 5 seconds, and 72° for 20 seconds. Final extension was run at 72° C for 1 minute and held at 4° C. The 2% agarose gel was covered by an inch in 1X TAE buffer. Five microliters of each sample were loaded into the wells and the gel was run at 300 V for 15 minutes. The gel was viewed using a visualizer.45 microliters of the PCR reaction sample were added to a 1.5 mL tube that contained 250 microliters of binding

buffer. The liquids were mixed by pipetting up and down. The solution was transferred to a labeled spin filter column. The sample was centrifuged for 1 minute at 15,000 rpm. The flow through was disposed of in the sink. Two hundred microliters of wash buffer were added to the spin filter column and were centrifuged again. The flow through was disposed again and a wash buffer was added again and the sample was centrifuged. The filter column was transferred to a clean 1.5 mL microcentrifuge tube. Thirty microliters of the elution buffer were added to the center of the filter. The sample sat for 1 minute and was then centrifuged for 1 minute at 15,000 rpm. The nanodrop was used to measure the concentration and A260/A280 values of 2 microliters of purified product.

Results

As seen in figure 1, there was an amplicon to be sequenced because it has base pair lengths between 400 and 500. Sample B was found to be heterozygous which means that the individual does not have photic sneeze reflex. This was determined by the chromatogram in figure 2, it shows two peaks at the SNP. The peaks are C and T which mean the genotype for the individual is CT.

Discussion

The individual was found to have the CT genotype which is heterozygous. This was expected as the predicted genotype of an unaffected individual was CT. If the individual did have photic sneeze reflex then the genotype would be CC or TT. The reason it could be C or T is because SNP is a single nucleotide polymorphism so it could change and still provide the same result. The individual was predicted to not have photic sneeze reflex from self observation and it was proven correct.

Figures and Tables

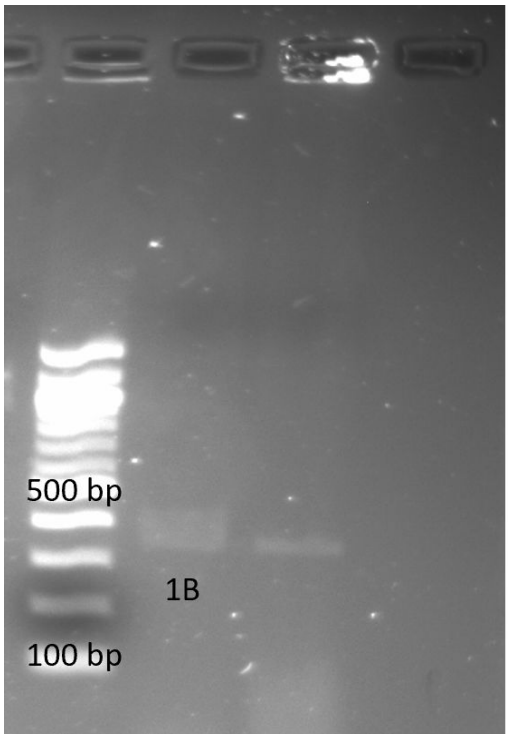

Figure 1. Gel Electrophoresis of Amplicon. L is the 100 base pair ladder. 1B is the individual that was sampled. The bands are in between 400 and 500 base pairs.

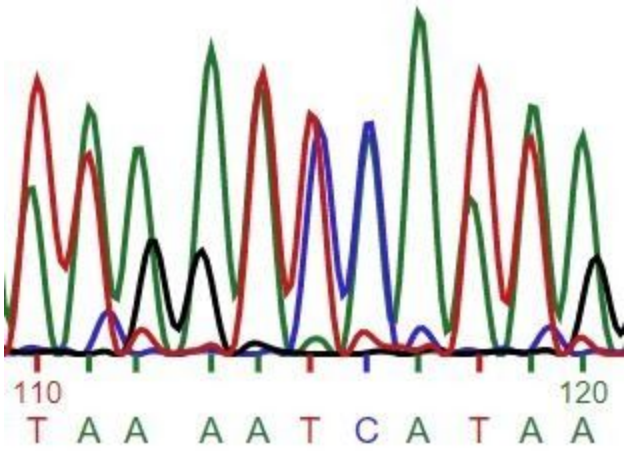

Figure 2. Chromatograph of the DNA sequence of Subjects. The DNA was sequenced and viewed. The SNP is at the middle T. The peaks show that the sample was heterozygous with C and T peaks meaning the individual does not have photic sneeze reflex.

References

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