Determining Genotype of Two Specific Traits on Two Individuals Using SNPs

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Background

- Molecular biology is the study of composition, structure and interactions of cellular molecules that help carry out the biological processes in cells
- Singular Nucleotide Polymorphisms (SNPs) are an abundant form of genome variation that act as biological markers which can be used to track the inheritance of genes (1).
- The genotype of individual SNPs were analyzed through the methods such as gel electrophoresis, polymerase chain reactions and DNA sequencing (2).
- These methods have been used to predict an individual's response to certain drugs and inheritance of disease genes as well as determining the presence of phosphorus in amplified DNA sequences. (2&3).
- This experiment focuses on:
  - Individual 1: **SNP1** (Photic Sneeze Reflex) and **SNP2** (Curly Hair)
  - Individual 2: **SNPBH** (Blue/Brown Eyes) and **SNPC** (Cilantro Aversion)

Specific Aim

Research Question: Can SNPs Be Used to Determine Genotype?
Hypothesis: Individual 1 would be homozygous for SNP2 (Curly Hair) and heterozygous for SNP1 (Photic Sneeze Reflex) and Individual 2 will be homozygous for both traits, SNPBH (Blue/Brown Eyes) and SNPC (Cilantro Aversion)
Importance: Using SNPs to identify forensic cases and genetic medical illnesses and to predict genotype of individuals.

Methods

Sample Collection
Each individuals cheek cells were swabbed and mixed in sterile water.

PCR Reaction
Each SNP had specific primers, and sterile water, primer mix, cheek cells, and master mix with Taq DNA polymerase, buffer for salt and pH, and dNTPs were added to PCR tube.

Gel Electrophoresis
Electrophoresis chamber with 2% agarose gel, 0.25X TAE buffer to cover the gel was added. DNA ladder and samples were loaded and ran at 300 V for 15 minutes.

PCR Purification
DNA was purified with binding buffer, elution buffer, and DNA wash buffer. Nanodrop was used to determine DNA concentration and A260/A280 values.

DNA Sequencing
DNA was sent to Eurofins Genomics Company to be sequenced. Text file and SNP trace file was obtained from the company to be analyze.

Results

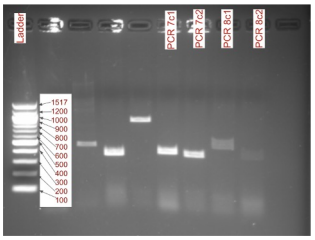

Figure 1. Gel Electrophoresis.
Gel electrophoresis for PCR 7c1 (SNP1), 7c2 (SNP2), 8c1 (SNPC), and 8c2 (SNPBH). The DNA ladder is on the far left with the numbers labelled representing number of base pairs.

Table 1. Nanodrop Measurements.
This table represents the Nanodrop measurements for DNA concentration and A260/A280 values for PCR 7c1 (SNP1), 7c2 (SNP2), 8c1 (SNPC), and 8c2 (SNPBH).

	7c1	7c2	8c1	8c2
DNA Concentration	11.4 ng/µL	16.6 ng/µL	39.1 ng/µL	32.1 ng/µL
A260/A280 Value	1.78	1.77	1.88	1.94

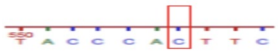

Figure 2. SNP1 Chromatogram.
The chromatogram shows the data for SNP1 representing photic sneeze reflex for PCR 7c1. Boxed area represents the location of the SNP.

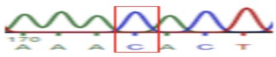

Figure 3. SNP2 Chromatogram.
The chromatogram shows the data for SNP2 representing curly hair for PCR 7c2. Boxed area represents the location of the SNP.

Figure 4. SNPC Chromatogram.
The chromatogram shows the data for SNPC representing cilantro aversion for PCR 8c1. Boxed area represents the location of the SNP.

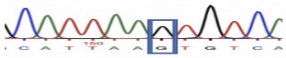

Figure 5. SNPBH Chromatogram.
The chromatogram shows the data for SNPBH representing Blue/ Brown Eyes for PCR 8c2. Boxed area represents the location of the SNP.

Individual 1: PCR 7c1 and 7c2
- SNP1: N/A
- SNP2: Homozygous

Individual 2: PCR 8c1 and 8c2
- SNPC: Homozygous
- SNPBH: Homozygous

Conclusions

- Individual 1:
  - SNP1: Hypothesis was inconclusive due to lack of data.
  - SNP2: Hypothesis was supported for homozygous (only 1 peak).
- Individual 2:
  - SNPC: Hypothesis was supported for homozygous (only 1 peak).
  - SNPBH: Hypothesis was supported for homozygous (only 1 peak).

- Limitations:
  - Gel could be pierced which could affect visibility when inserting the DNA into gel electrophoresis wells.
  - Genotype for SNP1 undetermined due to no peak on trace sequence
  - If filter was touched by the elution buffer during purification, results may be altered
- Future Experiments:
  - SNPs could be used to determine if an individual is susceptible certain diseases (4).
  - Predict the genotype of a criminal involved from DNA left at a scene.

References

1. Ramensky, V., Bork, P., & et. al. (2002). Human non-synonymous SNPs: server and survey. *Nucleic Acids Research*, *30*(17), 3894–3900.
2. Garibyan, L., & Avashia, N. (2013). Polymerase Chain Reaction. *Journal of Investigative Dermatology*, *133*, 1–4.
3. González, T. I., Espina, M., & et. al. (2014). Enhanced Detection of DNA Sequences Using End-Point PCR Amplification and Online Gel Electrophoresis (GE)-ICP-MS: Determination of Gene Copy Number Variations. *Analytical Chemistry*, *86*(22), 11028–11032.
4. Duncan, G., Balamurugan, K., Budowle, B., Smerick, J., Tracey, M. 1996. Microvariation at the Human D1S80 Locus. *Int J Legal Med. 110*(3): 150-154