

DNA Sequencing: SNP1, SNP2, SNPB and SNPBH: Determining Genotype

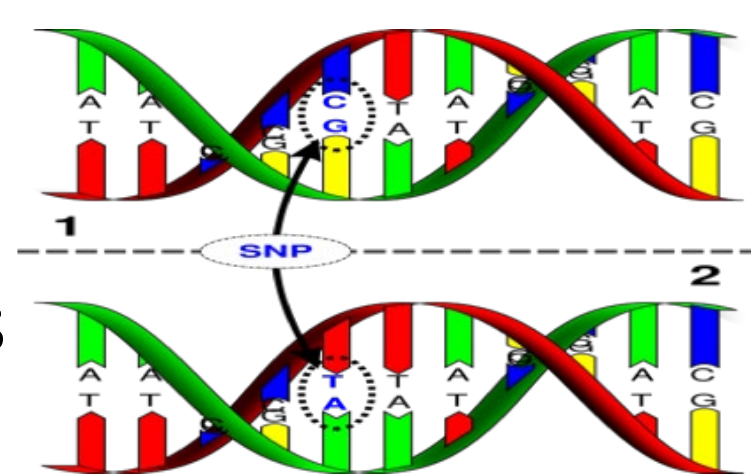
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Biology 250:50, Longwood University

Background

- Molecular biology provides insight on the function of the macromolecules essential to life
- Single Nucleotide Polymorphism (SNP) is a fragment of a DNA strand that either is homozygous or heterozygous for a certain trait
- Polymerase chain reactions (PCR), gel electrophoresis, and genomic sequencing are used to determine genotypes of individual SNPs (1)
- A few examples of application of this method includes Borkowska's experiment on mutations in bladder cancer and Abdulgader's study on medicine resistance in Staphylococcus aureus infections (2 & 3)
- This experiment focuses on
 - Individual One: **SNP2** (curly hair), **SNPB**(bitter tasting)
 - Individual Two: **SNP1**(Photic Sneez Reflex) and **SNPBH** (Blue/Brown Eyes)

Figure 1. SNP Location. This figure depicts a random SNP is a section of DNA.



Specific Aim

Research Question: Individual One: Determining the genotype of SNP2 and SNPB

Individual Two: Determining the genotype of SNP1 and SNPBH

Hypothesis: Individual One: It was hypothesized that the SNP's SNP2 and SNPB would be homozygous

Individual Two: It was hypothesized that the SNP's SNP1 would be heterozygous and SNPBH would be homozygous

Importance: Diagnosing genetic-related illnesses and solving murder cases

Methods

Sample collection of cheek cells

PCR

Gel electrophoresis

Purification

Nanodrop

Sequencing : Eurofins Genomics

Analyze Sequences

Results

Individual 1:

- Genotype for SNP2 was homozygous
 - Concentration: 9.1 ng/uL / A260/280: 1.87
- Genotype for SNPB was homozygous
 - Concentration: 13.8 ng/uL / A260/280: 2.27

Individual 2:

- Genotype could not be determined for SNP1
 - Concentration: 6.9 ng/uL / A260/280: 1.43
- Genotype for SNPBH was homozygous
 - Concentration: 12.6 ng/uL / A260/280: 2.51

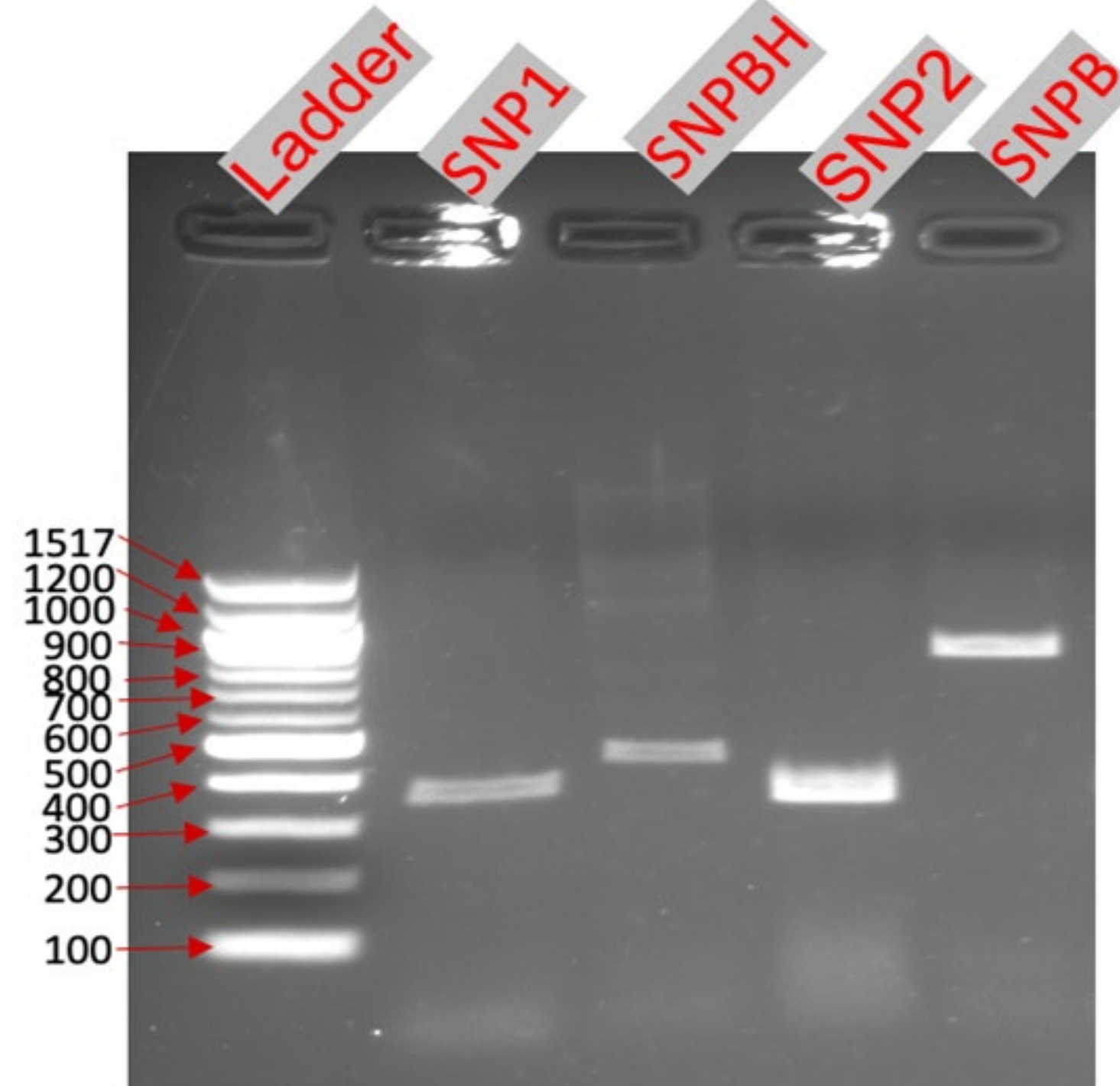


Figure 2. Gel Electrophoresis with ladder and samples labeled. Gel electrophoresis run with SNPs showing the number of base pairs each SNP contained.

	SNP-1	SNP-BH	SNP-2	SNP-B
Concentration	6.9 ng/uL	12.6 ng/uL	9.1 ng/uL	13.8 ng/uL
A260/280	1.43	2.51	1.87	2.27

Table 1. Nanodrop Technology for Individual 1 and Individual 2. The table shows the two nanodrop measurements for the SNP1, SNPBH, SNP2, and SNPB.

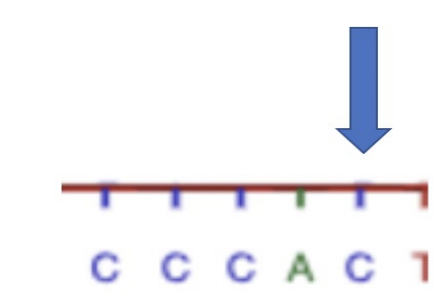


Figure 3. SNP1 Chromatogram. This figure shows the low and small quality peaks due to the mixed pool of SNP1.

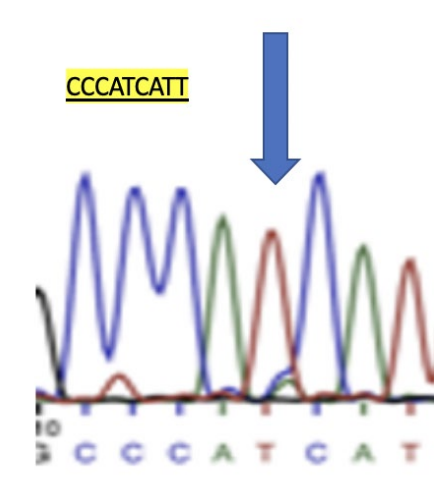


Figure 4. SNP1 Chromatogram of Control. The figure shows the sequenced data of the control for the Photic Sneez Reflex SNP, with the arrow showing SNP studied.

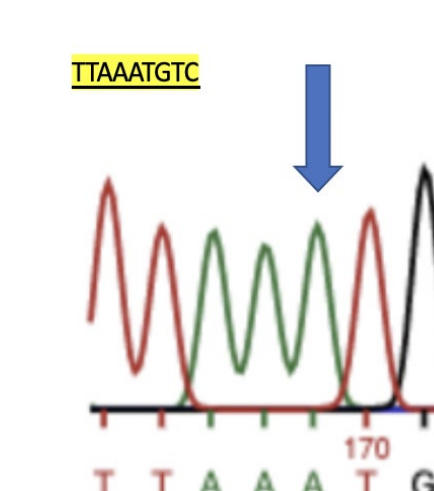


Figure 5. SNPBH Chromatogram. The figure shows the sequenced data of the Blue/Brown eyes SNP, with the arrow showing the SNP studied.

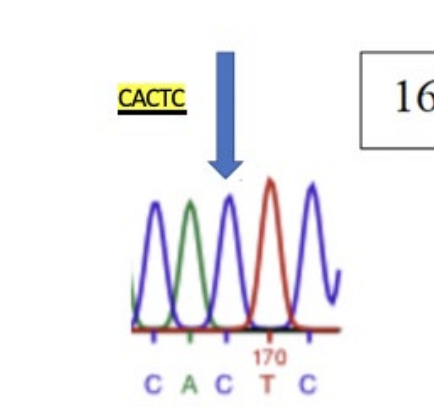


Figure 6. SNP2 Chromatogram. The figure shows the sequenced data of the Curly Hair SNP, with the arrow being the SNP studied.

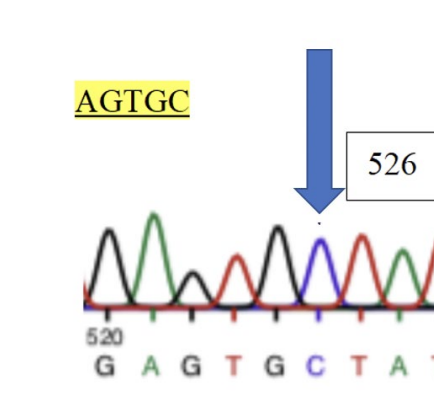


Figure 7. SNPB Chromatogram. The figure shows the sequenced data of the Bitter Tasting SNP, with the arrow showing the SNP studied.

Conclusions

Individual 1:

- SNP2 was homozygous for Curly Hair supporting hypothesis since there was 1 peak
- SNPB was homozygous for Bitter Tasting supporting hypothesis since there was 1 peak

Individual 2:

- SNP1 could not be determined due to a mixed pool (concentration was too small on Nanodrop)
 - Control showed to be homozygous for Photic Sneez Reflex
- SNPBH was homozygous for Blue/Brown Eyes supporting hypothesis since there was 1 peak

Limitations:

- Not putting drop in center of Nanodrop machine and the possibility of the PCR or the gel electrophoresis being contaminated

Future Experiments:

- Determining whether or not an individual carries a specific genetic-related illness (4)
- Using genetic sequencing to solve murder cases (5)

References

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3. Abdulgader, Shima M, Lentswe, Tshepisio, Whitelaw, Andrew, Newton-Foot, Mae. 2020. The prevalence and molecular mechanisms of mupirocin resistance in Staphylococcus aureus isolates from a Hospital in Cape Town, South Africa. *Antimicrobial Resistance and Infection Control*, 9(1):47.
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5. AL-Eitan, L.N., Rababa'h, D.M., Alghamdi, M.A., and Khasawneh, R.H. 2019. Genetic association of XRCC5 gene polymorphisms with breast cancer among Jordanian women. *OncoTargets and Therapy*, 2019(12):7923-7928.
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