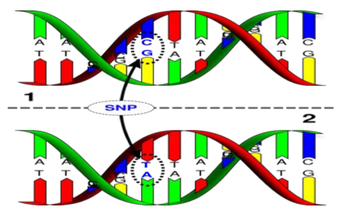
DNA Sequencing: SNP2 and SNPB: Determining Genotype

By: Davice Jones and Jeri Tyler

Biology 250:50, Longwood University

Background

- Molecular biology provides insight on the function of the macromolecules essential to life.
- Single Nucleotide Polymorphism is a fragment of a DNA strand that either is homozygous or heterozygous for a certain trait.
- Gel electrophoresis, PCR reactions, 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 Sneeze Reflex) and **SNPBH** (Blue/Brown Eyes).



Specific Aim

Research Question: Individual One: Determining the genotype of SNP2 and SNPB of one individual.
Individual Two: Determining the genotype of SNP1 and SNPBH of one individual.

Hypothesis: Individual One: It was hypothesized that the SNP's SNP2 and SNPB would be homozygous.
Individual Two: It was hypothesized that the SNP SNP1 would be heterozygous and SNPBH would be homozygous.

Importance: Diagnosing genetic-related illnesses and solving murder cases.

Methods

PCR Reaction
Sterile swab each individual's cheek cells. Kept on ice. Each SNP had specific primer. Master mix and water added.

Gel Electrophoresis
Electrophoresis chamber filled with 2% agarose gel with .25 TAE buffer to cover gel. Ladder sample inserted and 5 ml of each SNP inserted. Power was fun at 300 V for 15 minutes and picture was taken.

PCR purification using NEB BioLabs Monarch kit
250 uL Binding buffer added to each PCR reaction. Solution mixed then transferred to spin filter column and centrifuged at 13,000 rpm. Extra liquid discarded spun again. Repeated three more times. 25 uL of elution buffer added. Centrifuged again for 1 minute. Nanodrop technology used.

Sequencing
Ten uL purified amplicon sample sent to Eurofins Genomics. Sequencing as text and SNP chromatogram obtained to be analyzed.

Results

- Conclusions:
  - Individual 1:
    - Genotype for SNP2 was homozygous
      - Concentration: 8 ng/uL / A260/280: 0.074
    - Genotype for SNPB was homozygous
    - Concentration: 13 ng/uL / A260/280: 0.097
  - 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

All hypotheses, except for SNP1, were supported. SNP1's hypotheses was not supported due to use of control.

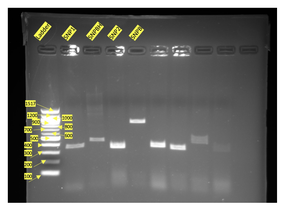


Figure 1. Gel Electrophoresis with ladder and samples labeled. Gel electrophoresis run with SNPs showing the number of base pairs each SNP contained.

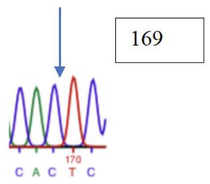


Figure 2. SNP2 Chromatogram. The figure shows the sequenced data of the Curly Hair SNP, with the arrow being the SNP studied.

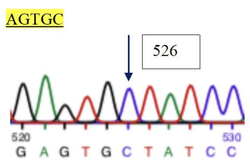


Figure 3. SNPB Chromatogram. The figure shows the sequenced data of the Bitter Tasting SNP, with the arrow showing the SNP studied.

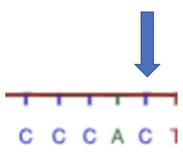


Figure 4. SNP1 Chromatogram. This figure shows the low and small quality peaks due to the mixed pool of SNP1.

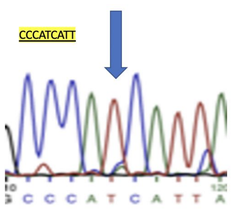


Figure **5. SNP1 Chromatogram of Control.** The figure shows the sequenced data of the control for the Photic Sneeze Reflex SNP, with the arrow showing SNP studied.

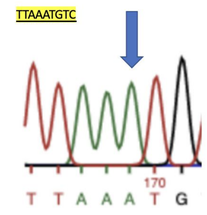


Figure 6. SNPBH Chromatogram. The figure shows the sequenced data of the Blue/Brown eyes SNP, with the arrow showing the SNP studied.

	4c1	4c2
Concentration	8 ng/ul	13 ng/uL
A260/A280	0.074	0.097

Figure 7. Nanodrop Technology for Individual 1`. The table shows the two nanodrop measurements for the SNP2 and SNPB.

Sample	A260	A260/280
3c1	0.137	1.43
3c2	0.253	2.51

Figure 8. Nanodrop Technology for Individual 2. The table shows the two nanodrop measurements for SNP1 and SNPBH.

Conclusions

- Individual 1
  - SNP2 was homozygous for Curly Hair
  - SNPB was homozygous for Bitter Tasting
- Individual 2
  - SNP1 could not be determined due to a mixed pool
    - Control showed to be homozygous for Photic Sneeze Reflex
  - SNPBH was homozygous for Blue/Brown Eyes
- Limitations:
  - Not enough DNA in sample for Individual 2 leading to mixed pool
- Future Experiments:
  - Determining whether or not an individual carries a specific genetic-related illness
  - Using genetic sequencing to determine genetic related illnesses and murder cases (4 & 5)

References

1. Slater, G.W. 2009. DNA gel electrophoresis: the reputation model(s). Electrophoresis 30:S181-S187.
2. Borkowska, Edyta Marta, Traczyk-Borszynska, Magdalena, Kutwin, Piotr et al. 2019. Usefulness of droplet digital PCR and Sanger sequencing for detection of FGFR3 mutation in bladder cancer. Urologic Oncology-Seminars and Original Investigations, 37(12):907-915.
3. Abdulgader, Shima M, Lentswe, Tshepiso, 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.
4. 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.

5 . Jung, J.Y., Kim, S., Oh, Y., Lim, S., Lee, Y.H., Hwang, J.H. 2018. A simple method of VNTR D1S80 locus allelic ladder construction for capillary electrophoresis-based genotyping. Journal of Forensic Sciences, 63(2): 526-529.