SNP rs2741762 PCR Purification, Gel Electrophoresis, and Sequencing

Kaylen Karnes

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Abstract

The purpose of the experiment was to determine the quality of the sequence data and analyze the nucleotide sequence. What were the observations from the DNA sample? In order to determine the sequence of the SNPs amplified by PCR, the amplicon had to be purified to remove the enzyme, dNTPs, genomic DNA, and other components of the PCR reaction. If there was one fragment, then the sequence was homozygous. If there were two fragments, then the sequence was heterozygous. If the expressed allele was G, then the SNP for cilantro aversion was not present. As the number of secondary peaks in the sequence data increased, the trace quality decreased. There was one peak expressed and the expressed allele was G meaning that the phenotype is GG. Since the phenotype is GG, this means that cilantro aversion was not present. The noise throughout the nucleotide sequence was minimal and the area containing the expressed allele was very good quality. Because the trace quality was good, the sequence data was accurate. The allele expressed affects the phenotype expressed. The techniques in this experiment could assist in forensic and paternal pursuits in addition to the explanation of an individual’s phenotype.

Introduction

Polymerase Chain Reaction (PCR) is a method that is used to detect, measure, and analyze DNA. The PCR has three parts: the master mix, the two primers, and the DNA sample. The master mix consists of enzyme (Taq [Thermal aquiferous] Polymerase) which copies DNA and the buffer (salt, pH). One primer is forward and the other is a reverse.

PCR is a method used to amplify a segment of DNA to analyze and can also generate copies. Gel electrophoresis is a procedure that administers an electric current to a gel that the DNA is in where it is separated by electrical charge and size. DNA sequencing is a technique by which precise order of nucleotides in a SNA segment can be determined. rs2741762 is an allele that determines cilantro aversion expression.

PCR was used to determine allele and genotype frequencies for the varying D1S80locus SNP in a Finnish population sample before amplifying the fragment length of the DNA (Sajantila). This experiment also shares the same goal of determining the allele expressed in the SNP. The experiment done by Sajantila, however, uses a different SNP and compares two races.

Muiznieks wanted to prove that specific migration inconsistencies during polyacrylamide gel electrophoresis (PAGE) would be the product of DNA with four bases, all of them being slightly different: 5′-GGCC-3′, 5′-GCGC-3′ or 3′-CGCG-5’. This experiment uses the gel electrophoresis method to gain the necessary results, however, Muiznieks is testing a certain format of DNA and this experiment is not.

This study demonstrated that the reason for bad quality PCR amplification was caused by fragmented DNA from tissue and also because of the lack of intact template molecules. DNA fragmentation was analyzed through agarose gel electrophoresis (Dietrich). Although PCR and gel electrophoresis techniques were used for both experiments, the experiment done by Dietrich tests the reason for bad PCR amplification quality and this experiment is not.

The purpose of the experiment was to determine the quality of the sequence data and analyze the nucleotide sequence of the SNP rs274162. What are the observations from the DNA sample? This is important because the DNA sample has the ability to show the phenotype of the individual. PCR, gel electrophoresis, and sequencing techniques were used.

If there is one fragment, then the sequence is homozygous. If there are two fragments, then the sequence is heterozygous. If the expressed allele is G, then cilantro aversion is not present. As the number of secondary peaks in the sequence data increases, the trace quality decreases.

Materials and Methods

A 1.5 mL tube that contained 250 ul of Binding buffer was needed for every PCR reaction. The appropriate PCR reaction sample (45 ul) was added to the tube containing the Binding buffer. It was mixed well by pipetting up and down. Then the PCR sample + Binding Buffer mixture was transferred to a labeled spin filter column. The tube was transferred to the centrifuge and “balanced” with other tubes. The sample was centrifuged for 1 min at 13,000 rpm (16,000 x g). The DNA will stick to the column filter, and the Binding Buffer and PCR reaction components passed through into the tube below. The flow was poured through into a sink. 200 uL of DNA Wash Buffer was added to the spin filter column and spun for 1 min at 13,000 rpm (16,000 x g).

The DNA remained on the filter and the wash buffer was passed through. Proteins, salts, and other contaminants were carried away by the wash buffer. The flow was poured through into a sink. The last two steps were repeated one more time. A clean, sterile 1.5 ml microcentrifuge tube was labeled. The spin filter column was transferred to the labeled tube. 30 uL of elution buffer was added to the center of the white filter, without touching it directly. The column was allowed to sit for 1 minute in order to allow the elution buffer to rehydrate and suspend the DNA. The sample was centrifuged for 1 min at 13,000 rpm (16,000 x g). The microcentrifuge tube will collect DNA at the bottom. The nanodrop was used to measure the DNA concentration (ng/uL) and A260/A280values of 2 uL of purified product (flow through). (A260 corresponds to the absorbance of light at a wavelength of 260 nanometers. The nanodrop was “blanked” using loading buffer and NOT water.

Results

As seen in Figure 1, the cilantro aversion bp is located on the image of the gel along with the sample for the individual.

According to Figure 2, there is one fragment. If there is one fragment, then the sequence is homozygous.

If the expressed allele is G, then cilantro aversion is not present. In Table 1, it states that AA is less likely to like cilantro. According to Figure 3, the expressed allele for the nucleotide sequence is G.

According to Table 2, the 260/280 value is 2.

There are no secondary peaks seen in Figure 4.

Figures

sample of individual



S6 S7 L S8 S9

A screen shot of a computer

Description automatically generated



5000

3000

2000

1500

1000

750

500

339

300

100

**Figure 1:** Image of Gel taken through UV light. This shows the bp and

the cm length can also be taken from this image.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Phenotype (trait)** | **SNP ID** | **Alleles** | **Association** | **Primers (5’ to 3’)** | **Expected size amplicon** |
| Cilantro aversion ***SNPC*** | rs2741762 | G/A | AA less likely to like cilantro | Forward:  CTGGGCAGATCTGACTCTACA  Reverse:  ATTCTGGAGCTTTGTTCTCATCA | 339 bp |

**Table 1:** The table shows the information pertaining to the cilantro aversion SNP.

|  |  |  |
| --- | --- | --- |
| Sample ID | Nucleic Acid Conc. | 260/280 |
| 8 | 31.3 ng/ul | 2 |

**Table 2:** The table lshows the information pertaining to the SNP nucleic acid concentration and the 260/280

>Biol250Spring21\_PREMIX Sample\_Name=Biol250Spring21\_PREMIX Chromat\_id=11308018 Read\_id=11184561 Version=1 Length=300

GTNAATACAAANGNACAGGAACAAAGAATNNNGTTCTTCANGGCTCTNNGTGTCNGATCCAAGAGGCGAGGCCAGTTTCATTTGAGCATTAAGTGTCAAGTTCTGCACGCTATCATCATCAGGGGCCGAGGCTTCTCTTTGTTTTTAATTAATTGTTTTTAACTGTGAGTTTATATACACTTGAAGCAGTATACATTTAGAAATGGTCTACTTGTCGTTTCTTTGATTACTACCCATGAGACAGTATTAGTAATTCTGGCCTATGAAATTGGCAAAGAAAACTACCAGTGGTGGGGAGGG

**Figure 2:** This figure shows a fragment of the sequence that contains the expressed allele.

**SNPC:** Cilantro aversion, **Forward Primer used in sequencing Rxn**

TCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCGCGCCTGGCCGGTGTTTATATTTCTGTGTGACGTGCATTGTGCACAATATGAAACTGCTAGTCATTATAACATGGGGGCACACATAGTAACAGAGGGTAAAACCCAATAAAAATCATCTTGTCACTTTTCCTCTT**CTGGGCAGATCTGACTCTACA**TTCATGACAGAAAATCTCATGATCTTTCCCAACACATAACTCTCCTCACCATCTTACTTCATTCTCATGCCCTTAAGCAGTGTTAGTTCTTTTAAGCTTACTGGTATGCAGTATGCATGTATGAGAGTAGATCAAAAGTTCACATGCATATGCCTTTATAACCCCAATAATTATTTCCTTCACACTAGATTCTTCCCAAGAAAAACAACCTGAGCTTCCCTAAGATCTCTATTCCATATTCA**G**GTTCTTATGTTTTGTTGAAGTAATATCGTAAATTTCAGTCATAGCTTTGCTT**TGATGAGAACAAAGCTCCAGAAT**AAGTCTTCTTTAACCACAAGATTTCTCCTTCTTATAATCTGGTAGTACTCTATTTGTAGATGTGCCCTAATTTATTTAGCCAGTCTTTTACTACTACAAATCATGCTGTAAATAATGGACTTATCCATATATCTGTTTTTATATTTTTGCTAGTGTACCTGACAAATAGAAATCTAGAAATTGAGTTTCTTGGGCAAAAGTTAAATACATACATAATGTTGCTATATATATTGATTGTCTATCTAGCTCCCCTCTGAACTTATTGCACCATTTTATGCTCCCACCAGCAATTTATGAGGTGTTTGTTTCCCCACAACTCAACAATAACATTTCTCATGAAATATTTAGAATTTCGATTATTTAAT

**Figure 3:** This figure shows the forward and reverse primers as well as the expressed allele.

Forward Primer

Expressed Nucleotide in the Allele

Reverse Primer

Nucleotide Sequence in the Sample



122

**Figure 4:** This figure shows the trace quality and the position of the expressed allele.

Discussion

What are the observations from the DNA sample for the PCR, gel electrophoresis, and sequencing techniques? If there is one fragment, then the sequence is homozygous. If there are two fragments, then the sequence is heterozygous. If the expressed allele is G, then cilantro aversion is not present. As the noise in the sequence data increases, the trace quality decreases.

The hypothesis was supported. The individual does not have cilantro aversion and the allele happens to be G. This is supported by good trace quality and nucleotide sequence analysis.

High-throughput sequencing allows allelic transcription to be studied at a genomic scale for basically any species. Estimates of allelic expression can be directly obtained by deriving sequencing transcript pools from heterozygous individuals (Fontanillas, 2011). Sequencing is very helpful in regard to identifying the expression of alleles.

Population genetic problems for VNTR loci can be predicted by the analysis of D1S80 and similar VNTR loci by fragment length polymorphism (Budowle). The analysis of an SNP can determine the expressed allele which can identify differences between a normal and mutated nucleotide. The allele expressed affects the phenotype expressed.

A limitation in this experiment was that because of COVID-19 preventing people from interacting in person, the lab was completed online. This might result in confusion or misinterpretation of the material being taught.

The techniques in this experiment such as PCR, gel electrophoresis, and sequencing can assist in forensic and paternal pursuits in addition to explaining an individual’s phenotype.

References

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