Detection of Single Nucleotide Polymorphisms in the Beta-Hemoglobin Gene of Sickle Cell DNA

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Introduction

A single nucleotide polymorphism (SNP) is a change of a single nucleotide in a DNA sequence that results in genetic variation within a population. These specific mutations can often lead to diseases, including sickle cell anemia, which is caused by a SNP in the sixth codon of the beta-hemoglobin gene at nucleotide 20 in which the nucleotide is changed from adenine (A) to thymine (T). Because of this coding change the shape and structure of hemoglobin molecules becomes modified, thereby transforming red blood cells from normal biconcave discs to sticky, "sickle"-shaped crescents (Figure 1). These new cells tend to cluster together and adhere to blood vessel walls, preventing vital organs from receiving sufficient levels of oxygen and potentially leading to organ damage, strokes, heart attacks, or even death.

Figure 1. Red Blood Cells

In order to better identify the presence of SNPs within sickle cell DNA, a new technique involving PCR using HotStarTaq d-Tect Polymerase was used. Unlike normal DNA polymerase, which has the ability to proofread and correct any mismatched base pairs (Figure 2), HotStarTaq d-Tect Polymerase will not extend a primer with a mismatch base pairing between its 3' end and the template DNA strand (Figure 3).

Figure 2. DNA Polymerase

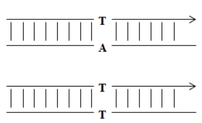
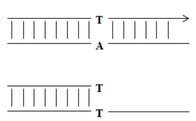


Figure 3. HotSatarTaq d-Tect Polymerase



Based on this principle, a primer designed to perfectly pair with normal DNA would only create a PCR product for samples using normal DNA, whereas no product would form for samples using sickle DNA. The same concept applies to a primer designed to match perfectly with sickle DNA. In this way, it would be possible to quickly and easily detect SNPs within any DNA sequence.

Materials and Methods

To examine the effectiveness of HotStarTaq d-Tect Polymerase, four thin-walled PCR tubes were set up as follows:

	Tube 1	Tube 2	Tube 3	Tube 4
Epitect Master Mix	12.5 µL	12.5 µL	12.5 µL	12.5 µL
dH_2O	10.0 µL	10.0 µL	10.0 µL	10.0 µL
mtDNA Primers	0.5 µL	0.5 µL	0.5 µL	0.5 µL
"A" Primer	1.0 µL	1.0 µL	-----	-----
"T" Primer	-----	-----	1.0 µL	1.0 µL
Normal DNA	1.0 µL	-----	1.0 µL	-----
Sickle Cell DNA	-----	1.0 µL	-----	1.0 µL

These tubes were placed in a thermocycler to undergo PCR with an initial activation step of 10 minutes at 95 °C, followed by 32 cycles of 15 seconds at 94 °C, 30 seconds at 54 °C, and 30 seconds at 72 °C. The final extension step took place for 10 minutes at 72 °C, after which the tubes were held at 4 °C until ready for further use. The samples were each injected with loading gel and set to electrophorese in a 2.0% agarose gel at approximately 120 V.

Results

The results of the PCR can be seen on the electrophoresed agarose gel below (Figure 4). Lane 1 contains normal DNA with "A" primer (Tube 1). Lane 3 contains sickle DNA with "A" primer (Tube 2). Lane 5 contains a marker at 1000, 750, 500, 300, 150, and 50 base pairs. Lane 7 contains normal DNA with "T" primer (Tube 3). Lane 9 contains sickle DNA with "T" primer (Tube 4). The brighter band is a PCR product for the mtDNA internal standard, whereas the primary products can be seen at approximately 239 base pairs.

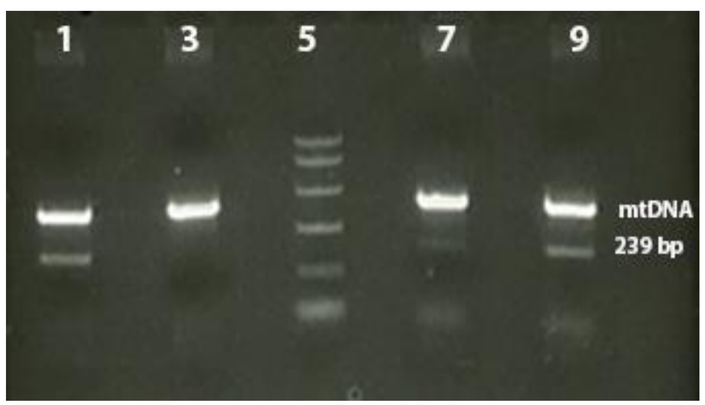


Figure 4. Agarose Gel of PCR Products

Conclusions

This experiment successfully identified single nucleotide polymorphisms within sickle cell DNA, substantiating the efficiency of HotStarTaq d-Tect Polymerase to pinpoint base pairing mismatches between primers and their target sequences. Because of its success, this method could eventually be used as an easier and more cost efficient way to genetically screen for sickle cell disease. In addition, this model could potentially be utilized in future research using different diseases involving SNPs at other genetic locations.

References

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