Testing rs1821380 SNP Marker on four Human Cell Lines

Tiare Z. Brown, Deysha Miller, Charmaine Lindsay, Dr. Jianguo Chen

Department of Biology, Claflin University Orangeburg, SC 29115

Abstract

Single Nucleotide Polymorphism (SNP) is one of the most common polymorphisms found in the human genome and can provide a powerful tool for human identification. SNP technology has been used to reduce high mutation rate occurrences; it can detect shorter DNA fragments. One of the challenges of SNP assay development is accessibility to unlimited amount of DNA with consistent genotypes which represents diverse human cell lines. This research is aimed to generate genotypes for rs1821380 (SNP) in four human cell lines by using DNA amplification, which can be used to develop 100-plex SNP assay. This research was done in three steps: a) DNA samples and the TaqMan probe were diluted to desired concentrations; b) preparation of the real-time PCR was performed by mixing TaqMan master mix, the diluted TaqMan probe, and diluted DNA samples; c) Applied Biosystems 7500 Systems SDS software was opened and the PCR protocol setup was used for DNA amplification. The TaqMan probe consisted of a quencher and a reporter molecule; during separation of these molecules, the reporter gives off a fluorescence. The reporter dyes FAM [G] and VIC[C] and the passive reference dye ROX were selected in order to test the fluorescence of hybridization during amplification. Our result shows that after DNA amplification, the genotypes of each cell lines were determined; African American cell line samples were homozygous [G/G] and Caucasian, Chinese, and Japanese cell lines were heterozygous [C/G]. These results suggest that DNA from human cell lines can provide useful information to construct and develop DNA panels for identification of degraded DNA samples in the forensic field.

Introduction

Along with Short tandem repeat (STR) there is also another type of DNA testing using Single Nucleotide Polymorphism (SNP) markers as a replacement in forensics (Butler *et al.* 2013). SNP is a variation in a single base pair in a DNA sequence. SNPs are currently the most abundant type of genetic variation and the principal study of genetics. SNPs act as a biological marker, helping scientist identify humans.

Real-time Polymerase Chain Reaction (RT-PCR), used for SNP, is a technique consisting of denaturing, annealing and extension that allows the exponential amplification of DNA fragments to lengths of approximately 10,000 base pairs (Freire-Aradas *et al*. 2012) In PCR, a single copy of a DNA fragment could be amplified to millions of copies within hours and is beneficial in the amplification of minute amounts of degraded sample.

TaqMan probes are hydrolysis probes that are designed to increase the specific quantity of PCR. The TaqMan probe consist of an amino group at the 3', the reporter dye and a 5' end as the quencher (Freire-Aradas *et al*. 2012). TaqMan probes work by finding the primer and then begins the extension phase of PCR by creating new complementary DNA by basically 'eating it.'

Materials and Methods

Materials: The materials used to perform the experiment was probe (rs1821380), TaqMan Genotyping Master Mix, DNA samples: NA17119, NA17210, NA17018, NA17060, Eppendorf tubes, centrifuge, micropipettes and Real-Time PCR Machine.

DNA Dilution: Eppendorf tubes were properly labeled. The Human DNA samples were then diluted to 10ng/µL from the original concentration. The volume of distilled water was then calculated by using a simple dilution equation. The new concentration contents were vortexed and centrifuged within an Eppendorf tube.

Probe Dilution: The TaqMan probe (rs1821380), was diluted from it's original concentration by pipetting 2.5µL of the probe and 47.5µL of water into an Eppendorf tube until the content was needed for PCR Amplification.

Preparation of PCR: After completing DNA and Probe dilution, the PCR tubes were gathered and labeled. 10µL of probe (rs1821380) was added to a new Eppendorf tube along with 2µL of the DNA sample. Next, 10µL of TaqMan Master Mix was added to the tube. The solution was then centrifuged before placing it into the Real-Time PCR Machine.

PCR Protocol: The 7500 System Software was opened. The dyes FAM, VIC, and ROX (passive reference) were selected in order to detect the fluorescence of hybridization. The chosen sample volume was 20µL. Finally, The number of cycles chosen was 60 reps in stage three. The DNA amplification process lasted approximately 2 hours.

Results

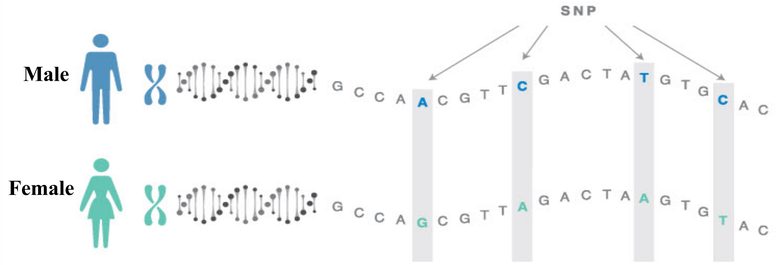

Figure1: Single Nucleotide Polymorphism (SNPs)

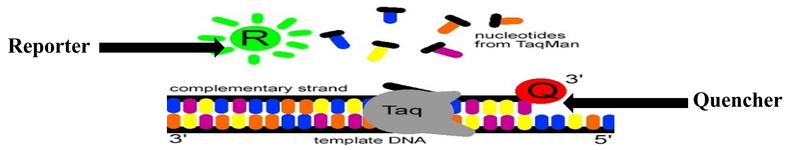

Figure 2: Extension phase by TaqMan probe

Reference SNP Number (rs#)	Nucleotides Affected [VIC/FAM]	SNP Sequence
rs1821380	[C/G]	GACATTCTCCTTCTTCTAT CTGTAT[C/G]CCTTACTGC ATTTTTGCACTGCAGT

Figure 3: rs1821380 Allele Information

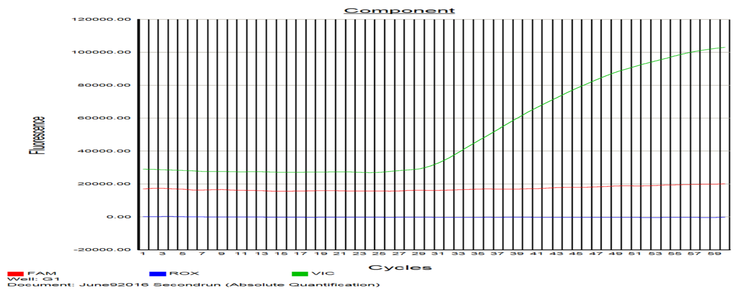

Figure 4: Depicts the results of the PCR reaction between probe rs1821380 and African American DNA sample NA17119.

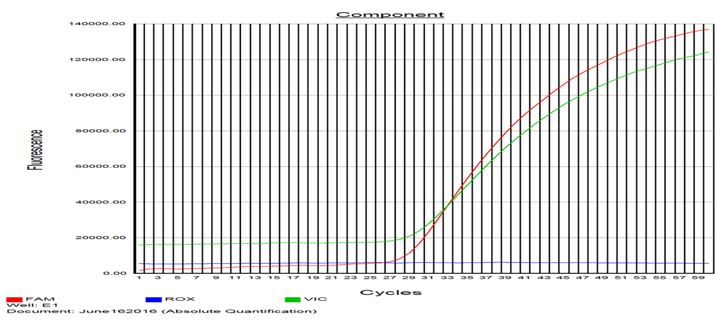

Figure 5: Depicts the result of the PCR reaction between probe rs1821380 and Caucasian DNA sample NA17210

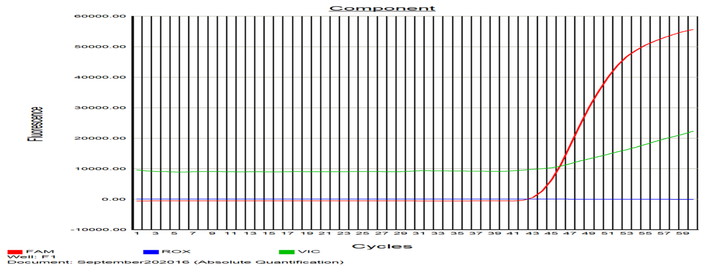

Figure 6: Depicts the results of the PCR reaction between probe rs1821380 and Chinese DNA sample NA17018

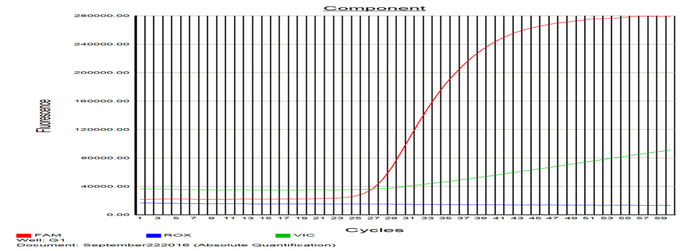

Figure 7: Depicts the result of the PCR reaction between probe rs1821380 and Japanese DNA sample NA17060

Conclusion

Based on the results obtained, it was determined that the genotype for Chinese, Caucasian, and Japanese cell line samples were to be heterozygous for both major and minor alleles. However, African American cell line sample were homozygous.

Future Plans

Design a primer and Taqman Probe for deltaF508, which is a mutation that occurs within the gene of Cystic Fibrosis.

References

C.R. Hill, D.L. Duewer, M.C. Kline, M.D. Cobie, J.M. Butler, U.S. population data for autosomal STR loci, Forensic Sci. Int. Genet. 7 (2013)e82-e83.

Freire-Aradas, et al, A new SNP assay for identification of highly degraded human DNA, Forensic Sci. Int.: Genetics 6 (2012) 341-349

J.M. Butler, M.D. Coble P.M. Vallone, STRs vs SNPs: thoughts on the future of forensic DNA testing. Forensic Sci. Med. Pathol. 3 (2007) 200-205.

"National Center for Biotechnology Information." National Center for Biotechnology Information. U.S. National Library of Medicine, n.d. Web. 26 June 2016.

"Thermo Fisher Scientific." Thermo Fisher Scientific. Web. 26 June 2016.

Acknowledgements

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number **R25GM113740**. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors would also like to thank Dean Dr. Verlie Tisdale, Mr. Sajid Saleem, Dr. Gloria S. McCutcheon (NIH-HIGMS: RISE-Principal Investigator) and Dr. Samina N. Assanie-Shivji (NIH-HIGMS: RISE-Co-Investigator), Department of Biology, School of Natural Science and Mathematics, Claflin University, for their support.