A44 Quantitative PCR for Rapid Gel Electrophoresis-Based Pre-STR Mixture Detection

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After attending this presentation, attendees will learn a potential method for a screening technique to identify mixtures at the qPCR stage of DNA analysis.

This presentation will impact the forensic science community by introducing a quick screening method to identify a mixture DNA profile prior to STR amplification.

Forensic DNA units currently do not have a way to accurately determine if a mixture is present prior to the CE analysis stage. This can be detrimental to labs, like the ATFE, that only have a limited sample amount with which to work. When DNA samples are below 2ng at ATFE, half of the sample is used for amplification. If the Identifiler™ STR analysis shows a mixture profile with all the allele peaks below threshold, then the lab does not have enough remaining sample to re-amplify using increased DNA input. If the lab had a procedure to suggest a sample is a mixture early in the process, then ATFE would be able to make key adjustments to their amplification technique in advance. For example, the amount of DNA sample amplified could be increased in order to raise the minor contributor alleles above thresholds. Additionally, examiners could further concentrate samples or increase injection times on the genetic analyzer to allow for improved results. This study is aimed to develop a modified quantitation assay that would allow for mixture detection pre-STR amplification. If successful, identifying a mixture at the quantitation stage would allow for adjustments to be made with a sample prior to amplification and analysis, such as using the full sample elution instead of the standard half sample or combining swabs taken from the same surface.

In this study, the goal was to develop a multiplex qPCR assay that would incorporate either two Single Nucleotide Polymorphism (SNP) markers or D1S80 into the existing Investigator® Quantiplex quantitation reaction for mixture identification. SNPs were chosen that have a minor allele frequency above 10% to increase the likelihood of detecting the minor component of a mixture. These SNPs included rs5030240, rs385780, rs433342, and rs4540055. Primers were designed using Primer BLAST through NCBI. These primers were designed such that the three different alleles would be able to be distinguished from one another when run on a gel. This was achieved by adding poly-T tails of varying lengths to the end of the primer containing each of the different alleles. D1S80 was also chosen based on its well-established PCR chemistry and primer sets. The 16 bp repeat with over 22 alleles together with a high level of heterozygosity across populations increased the likelihood that a mixture DNA profile would be seen. The selected primers were tested for visibility and discrimination with ten DNA samples using the Lonza FlashGel® system. The Lonza FlashGel® system using precast 2.2% agarose DNA cassettes can run at high voltages, and complete DNA migration within 2 - 7 minutes.

Product gels showed bands at the expected sizes for all four SNP markers, and all reagent blanks were clear. However, resolution of the alleles was not sufficient for allelic discrimination. The D1S80 gels showed bands at expected sizes and alleles could be discriminated from one another. Mixture samples amplified with D1S80 could also be discriminated on the gel system; however, the D1S80 primers appeared to interact unfavorably with the Investigator® Quantiplex reaction when incorporated into the qPCR reaction. Further studies should be conducted to look for alternate ways of incorporating these or other markers into the quantitation reaction. Additionally, primer redesign or new detection methods are possible development techniques to improve current allele discrimination. Ultimately, this identification would result in a decrease in sample consumption issues as well as a time savings for forensic DNA labs when analyzing casework contact DNA.

qPCR, DNA Mixtures, SNP Markers

 ** Presenting Author*