B99 Development of a Pre-Screening qPCR Mixture Detection Assay Using High-Resolution Melting Curve Analysis of the Short Tandem Repeat (STR) Loci D5S818 and D18S51

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After attending this presentation, attendees will have a better understanding of how a quantitative Polymerase Chain Reaction (qPCR) mixture detection assay can be used to screen low-level touch DNA samples.

This presentation will impact the forensic science community by demonstrating a way to potentially detect the presence of a mixture earlier in the DNA laboratory workflow process.

With the current forensic DNA laboratory workflow process, it is not possible to determine if a sample is a mixture of multiple contributors until after the capillary electrophoresis step has been performed and the analysis of the DNA profile shows the presence of three or more peaks at two or more loci; however, because of the large number of low-level or "touch" DNA mixture samples that forensic laboratories are receiving as evidence today, mixture detection at an earlier step in the DNA workflow process could be a beneficial tool. Receiving this information earlier would allow the analyst to determine if consumption of the sample is warranted or if protocols should be adjusted to allow for the combining of swabs from different areas of the evidence item prior to STR amplification. The quantitation step is the most logical place to add a mixture screening assay as it is a necessary and required step of the DNA analysis process, uses qPCR which can accommodate multiplexing a mixture detection assay, and because it is early enough in the workflow that changes can be made prior to downstream STR analysis.

STR loci D5S818 and D18S51 were chosen as the targets for a mixture detection assay because they are small in size and they are polymorphic enough to be able to readily determine the presence of a mixture. Fluorescent detection of the STR alleles by qPCR is most easily achieved with the use of an intercalating dye and melt curve analysis; the temperature of the STR melt product will be proportional to the number of polymorphic repeats (length) of the STR allele; however, prior to incorporation of the the STR amplification and detection in the quantitation reaction, it was important to demonstrate that the addition of an intercalating dye does not affect the performance of the quantitation kit. Thus, samples were run with and without the addition of SYTO-64® dye on a qPCR platform using a common commercially available human quantitation kit. Data from this study showed that the addition of an intercalating dye does not change the standard curve quality parameters or the quantitation values of unknown samples. Further, all replicates (with and without additional dye) provided values that were well within the normal inter-run reproducibility range (0.0001 to 3.3107). Next, D5S818 STR primers were used to successfully amplify and fluorescently detect standard melt products from both single-source and two-person mixture samples on the same qPCR platform using the same intercalating dye. Three distinct melting curve patterns were identified for the observed genotypes at the D5S818 locus, which was consistent with published studies. A comparison of the melting temperatures for the samples showed that the mixture samples produced melt products from 73.85°C to 74.02°C while the single-source melting curve groups ranged from 73.84°C to 73.95°C. Because there is some overlap between melting temperatures of single-source samples and mixtures, melting temperature alone cannot be used to distinguish between single-source samples and mixtures. Further mixture analysis showed that curve morphologies for the single-source and mixture samples were also very similar; however, it was noted that there was a distinct difference between the peak heights of the shoulder peaks, suggesting that this method could be used to distinguish a mixture from a single-source sample using the D5S818 locus. In order to improve resolution and magnify any melt curve differences between genotypes in future studies, High Resolution Melt (HRM) curve analysis may be more beneficial. Unfortunately, HRM in the available qPCR instrument has a fixed wavelength in the green channel which overlaps with the target fluorophores used in the quantitation kit. Thus, melt curve data from the quantitation experiments with only the intercalating dye added were reexamined. Data from these runs showed there were no melt products detectable after the quantitation amplification reaction. Consequently, it appears that the scorpion molecule used for fluorescent detection of the human quant target prevents the absorption of the intercalating dye. Therefore, high-resolution melt curve analysis, rather than standard melt curve analysis, will be possible. Future experiments will

include demonstrating the ability to detect all common alleles at the D5S818 andD5S818 loci using high-resolution melt curve analysis on the qPCR platform and EvaGreen® as the intercalating dye for both single-source and mixed samples. To date, data show that HRM analysis of highly polymorphic STR loci during the quantitation step may be a viable method for identification of a mixture prior to STR amplification.

Mixture Detection Assay, qPCR, Quantiplex