B106 High Resolution Melt (HRM) Curve Analysis for Preliminary Screening of Short Tandem Repeat (STR) Loci at Quantification — Obtaining More Information Earlier in the DNA Workflow

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After attending this presentation, attendees will better understand how HRM curve analysis may be used with various statistical tools to screen forensic DNA samples for determination of specific genotypes or geno-groupings.

This presentation will impact the forensic science community by providing a method to detect exclusionary data as well as potentially detect the presence of a mixture prior to multiplex STR amplification and analysis.

Currently, the DNA laboratory workflow does not allow the possibility of quickly identifying exclusionary contributors or determining whether a sample is a mixture until end-point DNA profile interpretation and identification. This presents a problem for low template DNA samples, particularly those from touch DNA samples where numerous small areas of a touched item are individually swabbed to avoid inadvertently creating mixtures. Thus, those samples with low DNA yields may not produce full or even partial STR profiles and since this is determined at the end-stage of the DNA workflow, the possibility of combining multiple single-source extracts may be lost. A screening assay at an earlier step could be a beneficial tool, allowing an analyst to determine if consumption of the sample is warranted or if swabs from different areas of the evidence item should be combined prior to STR amplification. The quantification step is the most logical place to add this screening assay, due to the multifaceted capabilities of quantitative Polymerase Chain Reaction (qPCR) instruments and human DNA quantification kits. Previous work demonstrated that commercially available quantification assays did not themselves produce melt curve products nor was their quantitative accuracy altered by the presence of an additional HRM dye. These data also indicated that genotyping or geno-grouping single-source samples and identification of mixture samples is possible using HRM curve analysis of the D5S818 and D18S51 STR locus using EvaGreen® intercalating dye and a QIAGEN® Rotor-Gene® Q. The current data focuses on a statistical approach for examining duplexed HRM data from two STR amplicons (D5S818 & D18S51) using two different qPCR platforms.

Buccal swab DNA was obtained from individuals who expressed one of seven desired D5S818 and D18S51 genotypes (n=20 for each genotype at each locus). An optimized amplification reaction was used to amplify both STR targets followed by HRM analysis on the QIAGEN® Rotor-Gene® Q and the ABI® 7500 qPCR platforms. Quantitative examination of all peaks identified in the melt curve (including shoulder peaks) revealed that while temperature shifts alone were not significant enough to determine genotypes, the additional differences in the number of peaks present and the primary peak:shoulder peak height ratios were significant enough to allow statistical assessment. Statistical modeling of the resulting data was accomplished using the Principle Component Analysis (PCA) -based Rotor-Gene® Q ScreenClust HRM® software, ABI® 7500 instrument Sequence Detection Systems (SDS) software, and Linear Discriminate Analysis (LDA) approach using R statistical software. Each statistical model evaluates a set of known samples to create training groups for each genotype, which are then used for comparison against each unknown for identification of the most closely related and/or least dissimilar genotype or genotype grouping. From those groupings, the accuracy of this prediction was calculated for each approach. At the D5S818 locus, the ScreenClust HRM® software provided an accuracy of 23.9% when classifying the data between seven genotypes. To improve the predictive power using the PCA-based model, geno-groupings were created from the seven genotypes by combining genotypes with similar melt patterns together into groups. The accuracy was re-evaluated and demonstrated improvement to 46.6% with three geno-groups. The same set of samples was re-evaluated using LDA in R. This resulted in an accuracy of 58.9%. Geno-groups were created again to improve predictive value and the resulting three groups yielded an accuracy of 81%.

These data support the use of an LDA-based statistical model of HRM data from highly polymorphic STR amplicons for identification of a geno-group. Incorporation of this amplification and melt analysis into a commercially available quantitation kit could provide early exclusionary information and potentially provide detail about the number of contributors to a DNA sample *prior* to STR multiplex amplification and CE analysis. Future experiments will include integration of this HRM duplex into commonly used human DNA quantification chemistries and evaluation of both single-source and mixture samples.

High Resolution Melt Curve, qPCR, STRs

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