B84 The Integration of a High-Resolution Melt (HRM) Curve Short Tandem Repeat (STR) Assay Into a Commercial Quantification Kit for Preliminary Mixture Detection: Getting More Information Earlier in the DNA Workflow

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Learning Overview: After attending this presentation, attendees will be aware of a newly-developed modified quantification assay with high resolution melt curve analysis, coupled with back-end statistical analysis, that can be used to distinguish between single-source and mixed DNA samples early in the forensic DNA workflow.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a way to detect mixtures, and potentially provide early exclusionary data, prior to multiplex Short Tandem Repeat (STR) amplification and Capillary Electrophoresis (CE) analysis.

Currently, the forensic 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. 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 of 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. To this end, a high-resolution melt (HRM) curve assay was designed that could be performed on common qPCR platforms utilizing STR loci D5S818 and D18S51 as targets. The authors previously reported that analysis of HRM data from single-source samples using linear discriminant analysis (LDA) provided higher classification rates than a principle component analysis (PCA)-based method for STR genotyping and geno-grouping using the Rotor-Gene Q (RGQ) platform. Further, that work showed that the higher resolution of the RGQ improved genotyping and geno-grouping prediction accuracy rates using this method (58.9% and 81.0% for D5, respectively) when compared to the more common ABI 7500 qPCR platform (23.9% and 65.4% for D5). Thus, the current study focused on integration of the STR HRM assay into the Quantiplex® quantification kit utilizing the RGQ platform and subsequent testing of the integrated assay using mixture samples. Prior to any sample testing, the amplification reaction conditions and amplification/melt parameters for the integrated assay were tested and optimized. The final protocol for the integrated quantification/HRM assay included a reduced-volume Quantiplex® reaction, modified Quantiplex® amplification parameters, and the addition of a transition cycle and melt step (60-95°C) following amplification.

Initial testing confirmed that when the HRM dye is added to the Quantiplex® amplification reaction using the transition and melt parameters no interfering melt products are observed and that, when STR primers are added, the melt curves produced are within the expected temperature ranges and exhibit minimal background noise. Additionally, no significant differences in primary peak temperatures were noted when the samples amplified using the integrated Quantiplex®/HRM assay were compared to the optimized singleplex STR amplification/melt reaction. Interestingly, the variation of the primary melt peak temperature observed was lower using the integrated Quantiplex® /HRM assay than what was previously noted from the inter-run variation observed when singleplex amplification and melt was performed. Further, when Quantiplex® standard samples were amplified with the added STR primers and HRM dye, the human DNA quantification standard curves produced were within the acceptable quality ranges and no significant changes were noted when sample quantification values were compared to the expected values (17.74% variation from expected vs. 20.72% inter-run variation for standard Quantiplex® reactions). Thus, the integrated quantification/HRM assay was tested using 56 single-source samples and ten 2-person 1:1 mixtures. The integrated assay was able to accurately identify 100% of the mixture samples tested and 92.9% of single-source samples tested. Taking all tested samples into account, the integrated STR melt-curve quantitation assay was able to properly distinguish between single-source and mixture samples in 62 of 66 samples tested for an overall accuracy rate of 96.43%. Overall, this work provides a viable qPCR-based integrated HRM + quantification assay that can provide an analyst with indication of a mixed forensic sample early in the forensic DNA workflow.

DNA Mixtures, qPCR, HRM