B49 A Comparison of Two Commercial Quantitative Polymerase Chain Reaction (qPCR) DNA Quantitation Kits for Prioritizing Forensic Samples for Downstream Genetic Analysis

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Learning Overview: After attending this presentation, attendees will become familiar with two commercial quantitative Real Time PCR kits (qPCR), Applied Biosystems™ Quantifiler® Trio and Qiagen's Investigator® Quantiplex Pro, and their ability to accurately predict DNA quantity and quality prior to short tandem repeat (STR) analysis. Attendees will also learn how to best apply generated quantitative and qualitative results from these commercial assays to establish sample prioritization workflows, reducing cost and time associated with STR amplification and analysis.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by illustrating the strengths of two commercially available quantitative real time PCR kits supplied by different manufacturers. Both kits assess not only DNA quantity, but quality, enabling prediction of successful STR typing success allowing for optimized genetic workflows. Furthermore, this presentation will illustrate how this information can be applied for screening procedures such as Y-screening approaches for sexual assault samples.

The quantification step is a vital part of the STR profiling process that is not only required but can also be utilized to establish appropriate sample workflows, maximizing the amount of information that can be generated from a sample. Manufacturers in the field continuously optimize their products, such as quantification kits, to reduce processing time and increase reliability of the information generated. Investigator® QuantiPlex Pro (Qiagen) and Quantifiler™ Trio (Applied Biosystems) are two examples. Each assay quantifies four targets, including a large and small human autosomal target that can be used to evaluate the quality of the sample, a male target to evaluate whether the sample is appropriate for STR or Y-STR analysis, and an internal positive PCR control that can be used to identify the presence of inhibitors.

Due to the increase in the submission of evidence to be processed for DNA as well as the complexity of samples submitted, laboratories continue to look to optimize their workflows, striving for efficient but accurate results. To this end, many laboratories have adopted screening procedure for sample prioritization. Quantitation cut-off limit, male:female ratio, and information regarding the quality of a sample can all be used to prioritize samples for downstream analysis. When using quantitative information for workflow decisions, accurate and reliable results are necessary to ensure greatest success for STR genotyping analysis.

In this study, both Investigator® QuantiPlex Pro and Quantifiler Trio™ performance was assessed applying both full-scale and half-scale reactions. As both qPCR assays generated similar results regardless of the volume of the reaction scale, the remaining studies were performed using half-scale reaction volumes. A male and female dilution series of human genomic DNA were prepared using purchased cell lines. Low template samples over a range of male to female ratios were chosen to assess the correlation of low or undetermined quantitative values with the success of generating STR and YSTR profiles. Concentrations below 100 pg were amplified using Applied Biosystem® GlobalFiler Amplification Kit and YFiler® Plus Amplification Kit. The correlation between quantitation value and quantity of STR profile generated was used to determine appropriate decision cut-off points for when to stop a sample at quantitation or continue to amplification. The male:female ratio generated through quantitation was also compared to the STR profile generated in both GlobalFiler and YFiler® Plus. This correlation can be applied to Y-Screening workflows for the analysis of sexual assault samples. The correlation between quality results generated from the IPC and calculated degradation index was also compared to the quality of STR profile generated.

Based on the quantitation values obtained and the subsequent DNA profiles generated, both qPCR kits evaluated in this study performed similarly in terms of accuracy and reliability of the information generated. By implementing either kit with a quantitation-based screening workflow, a laboratory can greatly decrease the amount of time and money spent on performing DNA analysis on samples that may not generate a useful DNA profile.

Quantifiler® Trio, Investigator® QuantiPlex™ Pro, Y-Screen