## B138 Application of Real Time qPCR Multiplex Results to Downstream Decision Making in Samples Containing Mixtures of Male and Female DNA

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The goal of this presentation is to describe the results of Real Time PCR quantitation of male/female mixtures and the effect of this data on decision making at the point of PCR amplification for STR loci.

This presentation will impact the forensic community and/or humanity by providing data from a real time qPCR multiplex for simultaneous quantitation of total genomic and male DNA which can be used to improve amplification decisions. This will assist analysts in determining which DNA samples are likely to provide useful information when amplified for autosomal STR and/or YSTR loci.

The availability of real time PCR (qPCR) methods capable of determining total genomic and male DNA in forensic samples has changed and improved the decision making process for STR testing. Other quantitation methods, such as the slot blot, do not provide information regarding the relative amounts of male versus total genomic DNA and are much less sensitive than real time PCR. Knowledge of the male DNA concentration can be used to determine if the sample should be amplified with autosomal and/or Y specific STR loci.

In this study, a real time qPCR multiplex capable of detecting male and total genomic DNA in one reaction was used. This assay utilizes Taqman® probe chemistry and contains a primer/probe set for the TPOX locus and a primer/probe set for the sex-determining region of the Y chromosome. The information gleaned from validation of this real-time multiplex was used in concert with validation data from ABI Profiler Plus™, COfiler™, and Promega PowerPlex Y® testing systems to make decisions regarding the most effective approach for sample analysis.

Experiments were designed with the purpose of determining if real time results are reliable predictors of both Y STR and autosomal STR amplification results. For this study, several dilution series of male/female mixtures were quantified for total genomic and male DNA using the real time multiplex. After quantitation, all of the samples were amplified using the PowerPlex Y® kit and a subset was amplified using the Profiler Plus kit as well. The PowerPlex Y® amplifications were based on the male specific qPCR results, whereas the Profiler Plus amplifications were based on the total genomic DNA qPCR results. The samples were then analyzed using an ABI Prism 310 Genetic Analyzer.

The results showed that the real time qPCR multiplex used in these experiments provides valuable quantitation data for both total genomic and male DNA in mixed samples. The real time qPCR results for the male/female dilution series confirmed that this assay detects male DNA in a wide variety of male/female DNA mixtures. The lower range of detection is approximately 12.5pg of male DNA in a 1:5000 male:female mixture. For both PowerPlex Y® and Profiler Plus™, the amplifications were successful using the real time qPCR data to calculate the volume to be added to the PCR reactions. When run on the ABI Prism 310, the amplifications resulted in peaks heights within the laboratory's analysis parameters. Additionally, the real time data proved to be a good indicator of the ratio of male to female DNA. The Profiler Plus peak height ratios for the male/female mixtures were reasonably consistent with the male to total DNA ratios calculated from the qPCR data. These results were also similar to the known male/female ratios in the actual dilutions.

From these data and data from the previous validations of Profiler Plus™, COfiler™, and PowerPlex Y®, it is possible to draw important conclusions regarding mixed samples prior to STR amplification. Because this assay can accurately detect male DNA in the presence of overwhelming amounts of female DNA, it is possible to decide how much sample to use for PowerPlex Y® amplifications. In addition, since the assay can reasonably determine the ratio of male to female DNA in a mixed sample, it is possible to reasonably deduce which samples will result in a useful secondary male profile when amplified for Profiler Plus™.

**DNA Quantitation, Real-Time PCR, Y STRs**

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