A100 Development and Validation of a Multiplex qPCR System for Male and Total DNA Quantification

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After attending this presentation, attendees will acquire information about the development and validation of a novel multiplex qPCR system simultaneously quantifying total genomic DNA and male human genomic DNA.

The presentation will impact the forensic science community by providing a new total genomic DNA and human male genomic DNA quantification method other than the ABI Quantifiler Duo DNA Quantification kit and Promega Plexor HY kit, assisting other labs in their determination of which kit will most likely fit their current system.

STR analysis, the core technology in DNA identification, requires a defined range of template quantities to produce optimal results. Quantification of both total human DNA and male human DNA in mixed forensic samples provides critical information for the selection of either autosomal or Y STR profiling, and to determine the quantity of extract to amplify. Two commercially available kits commonly used in forensic laboratories to simultaneously measure total and male human DNA are Plexor HY and Quantifiler Duo®. A recently published study compared the two kits in terms of precision, sensitivity and accuracy.[1] The results showed both systems produced linear estimates for DNA quantity over a broad range of input DNA. However, the study also indicated that the Plexor HY kit performed well with low level amounts of DNA, whereas the values for the ratios of total DNA to male DNA were far more accurate for Quantifiler Duo®. One of the most important reasons for the differences in performance between the commercial kits is the Y chromosome target. TSPY is a multi-copy number Y chromosome target used in the Plexor HY kit, with varying copy numbers from 23 to 64 among different populations of males.[2] SRY, used in the Quantifiler Duo® kit, is a single copy target with no copy number variation reported among males. Although increasing the copy number of a target enhances sensitivity, it also results in more variation.

A novel multiplex qPCR method to simultaneously measure total and male only DNA in a more sensitive and accurate manner will be presented. The multiplex qPCR system includes three amplification targets, one of which is located on an autosomal chromosome, and the others are found on the Y chromosome. Three TaqMan probes corresponding to the three targets are labeled with different fluorescence. One of the Y chromosome targets is a single copy gene which is used to quantify male DNA accurately when the male DNA concentration is higher than 16pg/µl. The other Y chromosome target is a multi-copy number gene which was shown to measure as little as 0.32 pg/µL of male DNA. The novel multiplex qPCR system was compared with the Quantifiler Duo® kit and Plexor HY kit regarding accuracy, sensitivity, and precision. Purposeful male and female DNA mixtures and mock evidence samples were measured using the novel Multiplex qPCR system, and the profiles generated with the determined DNA concentrations were assessed. Studies demonstrate that this system overcame the drawbacks of both of the Quantifiler Duo kit and Plexor HY kit as it is more sensitive that the Quantifiler Duo® kit and more accurate than the Plexor HY kit.

References:

1. H E. LaSalle, G Duncan, B McCord, An analysis of single and multi-copy methods for DNA quantitation by real-time polymerase chain reaction. Forensic Sci. Int. Genet.5 (2011) 185–193.
2. Repping S, van Daalen SK, Brown LG, Korver CM, Lange J, Marszalek JD, et al. High mutation rates have driven extensive structural polymorphism among human Y chromosomes. Nat Genet 2006; 38:463–7.

Male DNA, Genomic DNA, Quantification

 ** Presenting Author*