B137 Development of a Human-Specific Real Time PCR Assay for Simultaneous Quantitation of Total Genomic and Male DNA

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The goal of this presentation is to describe the development and validation of a duplex real time PCR method for simultaneous determination of total genomic and male DNA concentrations in a forensic sample.

This presentation will impact the forensic community and/or humanity by demonstrating the real-time PCR assay which was developed for simultaneous quantitation of total genomic and male DNA should be utilized in forensic DNA analysis as a replacement to slot-blot quantitation. Increased sensitivity, decreased analysis time, specific quantitation of male and total genomic DNA make the assay superior to the slot-blot assay. In addition, the multiplex assay results in time and cost savings when compared to the ABI Quantifiler kits.

Historically, the slot-blot method has been used to quantitate human DNA in forensic samples. However, the slot blot method is labor intensive, subjective in interpretation, and not highly amenable to automation, which has led to the development of new methods for quantitating human DNA. Current research[1,2] has focused on the use of Real-Time PCR as a more accurate, more sensitive, and less labor intensive method. Real time PCR has the capability of absolute quantitation based upon measurement of the increase in fluorescence signal with each cycle and comparison to a standard curve. A number of detection chemistries are currently available including SYBR® Green detection, fluorogenic probes, and molecular beacon technology. Fluorogenic probe chemistry was utilized in this study, with spectrally distinguishable reporter dyes for each target sequence.

With the growing capabilities for discrimination based upon Y-chromosome STR typing, the need to quantitate the male contribution to a sample is becoming increasingly evident. Commercial kits (Applied Biosystems) for human DNA and Y-chromosome DNA quantitation with real-time quantitative PCR are available in singleplex reactions. However, multiplex PCR has the advantage of consuming half of the DNA as compared to the commercial kit in addition to reducing the cost of the analysis. Therefore, a multiplex real time PCR assay has been developed for simultaneous quantitation of both the total human genomic DNA concentration as well as the male DNA component. Primer/probe sets were designed using the Primer Express® (Applied Biosystems) software. For total human genomic DNA quantitation, the amplicon is a 63bp fragment of the TPOX locus. The Y-chromosome amplicon is a 70bp fragment of the sex-determining region (SRY) of the Y-chromosome. Both primer sequences were determined to be human (or higher-primate) specific with a BLAST search as well as tested experimentally with common DNA sources including yeast, *E Coli*, and mouse.

The lower limit of quantitation was determined to be approximately 10 pg for total genomic DNA and approximately 20 pg for male DNA. The upper limit of the quantitation was set at 50 ng, which is appropriate for typical casework samples. This linear range for the real time PCR quantitation is significantly larger than that of the 125 pg to 10 ng range of the slot-blot assay. In direct comparison to a modified QuantiBlot® (Applied Biosystems) assay followed by BioImage® analysis, the average relative error of the multiplex assay was comparable to the slot-blot assay. The average percent error was 11.6% for total genomic DNA and 12.1% for male DNA, compared to 12.8% for the total genomic DNA quantitation by slot blot. The slot-blot analysis is unable to specifically detect the concentration of male DNA in a mixture. A mixture-challenge study indicated the quantitation of 25 pg male DNA was accurate in mixtures of up to 1:5000 male:female DNA. Additional validation experiments included optimization of Mg^{2+} concentration, comparison of quantitation accuracy to the Quantifiler□ and Quantifiler□ Y kits, and assay reproducibility.

References:

1. Nicklas JA, Buel E. Development of an ALU-based , Real-Time PCR Method for Quantitation of Human DNA in Forensic Samples. *J Forensic Sci.* 2003; 48(5): 935-44.
2. Richard ML, Frappier RH, Newman JC. Developmental Validation of a Real-Time Quantitative PCR Assay for Automated Quantification of Human DNA. J Forensic Sci. 2003; 48(5): 1041-6.

DNA Quantitation, Real-Time PCR, Slot Blot

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