A85 A Tetraplex Real - Time qPCR Assay to Quantify Nuclear and Mitochondrial DNA Determine Sex of the Donor and Detect Inhibition

Molly McBeth, MFS, 1501 Crystal Drive, Apartment 220, Arlington, VA 22202; Christina D. Lindquist, MS, One Shields Avenue, Davis, CA 95616; and Daniele S. Podini, PhD, 2036 H Street, Northwest, Samson Hall, Room 301, Department of Forensic Science, Washington, DC 20052*

After attending this presentation, attendees will learn about a methodology for the simultaneous quantitation of human nuclear DNA and mitochondrial DNA, as well as the determination of the sex of the donor and the detection of inhibition using real-time PCR.

This presentation will impact the forensic community by demonstrating a tetraplex real-time PCR assay capable of quantifying both human nuclear and mitochondrial DNA, determining the sex of the donor, and detecting inhibition in a single 30 minute reaction.

The Quality Assurance Standards set forth by the DNA Advisory Board require the quantitation of human DNA whenever possible. To fulfill this requirement the forensic community has been moving towards a real-time PCR technique. Real-time PCR offers several advantages over other methods of quantitation: primers and probes can be designed to target specific areas in the genome, the assays are sensitive, the method is easy, fast, automatable, and allows for the simultaneous amplification of multiple targets in a sample providing more information from a minimum amount of sample. This assay was designed specifically for the assessment of severely compromised samples. Such samples, due to the small quantity or poor quality of the nuclear DNA, may result in partial or no profiles when analyzed using an STR analysis. However, these samples may contain mitochondrial DNA which could provide information through the analysis of the control region. In order to preserve the sample extract, this assay was developed to quantitate nuclear and mitochondrial DNA in a single reaction allowing for the immediate determination of whether STR or mtDNA analysis would be most appropriate for a specific sample.

A multiplex real-time PCR assay was developed utilizing the 5' exonuclease detection assay (TaqMan®) using the Cepheid SmartCycler® (Cepheid, Sunnyvale, California) instrument. To quantitate nuclear DNA, mtDNA, detect male DNA and inhibition four primer/probe sets were designed. For the nuclear portion of the assay, the target was a 114 basepair sequence flanking the CSF1PO STR locus. This area contains no known polymorphisms and shares no homologies to other regions of the human genome or to other species. The area targeted for the detection of male DNA was a 111 basepair sequence of the SRY gene on the Y chromosome, downstream from the polymorphic region of the gene. The mitochondrial portion of the assay targeted a 121 basepair area in the mitochondrial 12S gene, between nucleotide 1212 and 1341, which contains few reported rare polymorphic sites (mtDB: http://www.genpat.uu.se/mtDB/). The portion of the assay designed to detect inhibition utilized an internal control DNA that was synthesized to avoid any homologies to any sequence present in nature. Primers and probe were then designed to amplify the 72 basepair synthetic oligonucleotide.

The four probes designed for this assay were each labeled with a different fluorescent dye tag at the 5' end of the oligo: FAM™ for the nuclear probe, TET™ for the male specific probe, TAMRA™ for the mitochondrial probe, and ROX™ for the internal control probe. Appropriate quenchers were added to the 3' end of each probe in order to inhibit fluorescence.

Standard curves were created using 9948 Male DNA (Promega, Madison, Wisconsin). Reproducible DNA concentrations showed a sensitivity of the nuclear DNA to 0.05 ng and of the mitochondrial DNA to 0.50 pg with a 30 minute run time. The detection of inhibition was demonstrated by an increase in the Ct value of the Internal Control in the presence of hematin. The assay was used successfully to determine the appropriate analytical technique for 16 hair samples, three of which were determined to contain sufficient nuclear DNA for STR analysis and the remaining 13 yielded no nuclear DNA but contained sufficient mtDNA for analysis of the control region. The assay provided an accurate means to assess the amount of DNA within a sample. This allows the selection of the most appropriate downstream analytical approach, whether STR or mtDNA, saving time and resources while minimizing sample consumption.

DNA Quantitation, Real Time PCR, mtDNA

 ** Presenting Author*