A129 Real - Time Quantitative PCR Assay for Mitochondrial DNA Quantification

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After attending this presentation, attendees will be informed regarding a sensitive and accurate method for determining mitochondrial DNA (mtDNA) copy number in forensic DNA extracts and for assessing if such extracts contain PCR inhibitors. Attendees will also gain knowledge regarding the minimal number of mtDNA copies required for successful mtDNA amplification and subsequent sequencing.

This presentation will impact the forensic community by potentially increasing the success rate of mtDNA amplification and sequence analysis and by reducing unnecessary consumption of forensic DNA samples so that retesting may be possible if desired.

MtDNA sequence analysis is a useful analytical tool for analyzing limited quantity and/or highly degraded samples, such as hair shafts, bones, and teeth, as well as being informative in maternal lineage cases. There have been efforts to improve the sample preparation portion of mtDNA analysis by attempting to determine the amount of mtDNA contained within a sample. In addition, a better appreciation of the minimum amount of mtDNA required for successful typing would minimize consumption of evidence. Such knowledge would enable an assessment of the likelihood of generating mtDNA profiles from forensic samples. This presentation describes a highly sensitive real-time quantitative PCR (QPCR) assay which was developed to accurately quantify mtDNA for these purposes.

The target sequence for the assay is located within the mtDNA NADH dehydrogenase subunit 5 gene. The chosen sequence possesses minimal sequence homology to the mtDNA of other forensically-relevant species. In addition, the primers and probe utilized in the QPCR hybridize to invariant regions within the human mtDNA genome (based on current population data). The amplicon generated is small in size (105 bp) making the assay more amenable to quantifying samples which contain degraded DNA. The assay is based on absolute quantification and exhibits high sensitivity enabling the detection of as few as 10 mtDNA copies (0.17 fg). Quantification by this method covers a wide dynamic range up to seven orders of magnitude (i.e., 100 million mtDNA copies or 1.7 ng of mtDNA).

To increase the quality and robustness of the assay a novel, synthetic DNA positive control standard was employed in lieu of a plasmid generated standard. The synthetic standard was designed to contain a unique short sequence so if there was contamination due to the control sample it would be readily detectable. Using a synthetic standard instead of a plasmid-generated standard has several benefits, including enhanced quality control, greater purity, lower cost, higher yield, and easier and timelier production. The assay requires only 2µl of sample and results can be obtained within 40 minutes. An internal positive control to detect the presence of PCR inhibitors can be readily incorporated into the assay. Results of validation of the QPCR assay will be presented.

Experimental studies which correlated mtDNA quantities to mtDNA hypervariable (HV) region amplicon yields revealed that as few as 1,000 copies of mtDNA are required for successful downstream HV analysis. This observation may serve as a guide for minimizing sample usage during HV amplification thus conserving DNA samples where possible.

The QPCR assay described is reliable, robust, and reproducible and will enable the accurate and precise quantification of mtDNA for use in downstream analysis.

Mitochondrial DNA, QPCR, Synthetic Standard

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