A91 Internal Validation of a Real-Time Quantitative Polymerase Chain Reaction Assay for Human Mitochondrial DNA

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The goal of this presentation is to inform the attendees of an internal validation performed on a human mtDNA real-time quantitative PCR assay at the University of North Texas Center for Human Identification. Attendees will gain a practical working knowledge of the assay's design, chemistry and performance. In addition, attendees will learn of this assay's potential for improving the work-flow efficiency and success rate of mtDNA sequence analysis.

This presentation will impact the forensic science community by exploring ways in improving human mtDNA sequence analysis.

An internal validation was performed on a human mitochondrial DNA (mtDNA) real-time quantitative PCR (qPCR) assay following standard 8.3 of the "Quality Assurance Standards for Forensic DNA Testing Laboratories." The purpose of this internal validation was to demonstrate that the developed method performed as expected and is suitable to be used at the University of North Texas Center for Human Identification (UNTCHI). The internal validation studies conducted include: (1) precision and reproducibility; (2) sensitivity; (3) inhibition: and, (4) known and non-probative evidence samples. Data collected from 18 separate qPCR runs demonstrate this assay has a high degree of precision and reproducibility evidenced by consistent cycle threshold values of the quantification standards and controls. The mtDNA target in the lowest quantification standard was detected in each experiment indicating the assay's sensitivity of detection is extremely low at 0.0001 pg/µL or approximately six human mtDNA copies per microliter. The assay successfully produced results at various levels of template mtDNA when challenged with various concentrations of three different PCR inhibiting compounds. Finally, reportable mtDNA sequence data were obtained for forensic casework sample types, including whole blood and skeletal remains, which had been tested with the human mtDNA qPCR assay. The successful completion of this validation study demonstrates the suitability of the human mtDNA qPCR assay for use in forensic casework and identification of human remains. Using this assay can assist an analyst in determining: if a sample contains sufficient human mtDNA to proceed with downstream sequence analysis; the amount of sample to be used for mtDNA amplification; and, if a sample with inhibitors requires dilution or additional purification measures.

The University of North Texas Center for Human Identification is an accredited laboratory which performs nuclear and mitochondrial DNA analysis. MtDNA sequence analysis is especially useful for challenging samples, such as telogen hairs, teeth, and older skeletal remains where the amount of genetic material and its quality vary greatly and often fail to produce nuclear DNA typing results. Implementation of a sensitive quantitative assay for mtDNA will improve work-flow efficiency and increase success rate of these sample types. The amount of mtDNA used for amplification and the quantity of amplified product for sequencing is critical for obtaining high quality data. Too much product added to the cycle sequencing reaction results in noisy data and too little product generates low sequence signal. With an optimal amount of product added to the cycle sequencing assay, clean data are obtained. High quality sequence data which exhibits good signal intensity and very little baseline noise is critical for efficient interpretation of data and high throughput sequence analysis. Additionally, if an optimal amount of DNA is added to the amplification reaction, then downstream cycle sequencing procedures can be standardized. Using an optimized quantity of mtDNA in front-end amplification reactions for sequence analysis also preserves precious sample extract. This facilitates judicious use of the amount of sample extract consumed which is a principal concern when analyzing forensic samples. Although several methods have been developed to quantify DNA, real-time quantitative PCR (qPCR) assays offer great advantages such as a high degree of specificity, sensitivity and precision.

Quantitative PCR, Mitochondrial DNA, Internal Validation

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