B143 Real-Time mtDNA Specific Quantitation

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After attending this presentation, attendees will learn by incorporation of a mtDNA control region TaqMan assay into mtDNA sequence analysis could be extremely beneficial to the forensic community.

A mtDNA control region TaqMan assay could reduce pre-amplification assay time and enhance casework results which is desirable because forensic mtDNA testing is a lengthy, tedious and labor-intensive procedure.

Many laboratories that perform forensic mitochondrial DNA (mtDNA) analysis use quantitation of nuclear DNA to estimate the amount of mtDNA present in a DNA extract. Since mtDNA can be analyzed successfully from samples with little to no detectable nuclear DNA, extracts are routinely processed even if the nuclear DNA quantitation is inconclusive. In addition, the slot blot hybridization technique that is currently employed by many laboratories to quantitate extracted DNA is time-consuming and relies on subjective interpretation of the quantity of DNA. Alternatively, quantitative real-time PCR provides a rapid, objective estimate of DNA quantity and is amenable to automation. Importantly, quantitative real-time PCR provides the opportunity for custom quantitation. The TaqMan assay uses oligonucleotide probes that hybridize to specific DNA sequences. The probes are designed with a reporter fluorophore on the 5' end and a quencher fluorophore on the 3' end. During PCR, the probe hybridizes to the template DNA downstream of the primer and the 5' to 3' exonuclease activity of the DNA polymerase separates the reporter molecule from the quencher molecule during template extension. Cleavage of the reporter molecule results in a measurable increase in fluorescence. As each DNA strand is extended an increase in fluorescence is observed. This presentation will describe the development of a TaqMan based assay for quantitation of mtDNA.

While others have described real-time PCR assays for quantitation of mtDNA, these assays have targeted areas of the coding region of the mtDNA genome. Previous work by our group has produced a real-time PCR based assay for quantitation of mtDNA based on amplification of region HVIA within the control region. As an extension of our previous project, this work focuses on another, less polymorphic, mtDNA target that lies in the control region of the mitochondrial genome. Since forensic DNA analysis requires human specific DNA quantitation, it is necessary to choose a target region that is conserved among humans but does not amplify DNA from other species. Primers and probes were designed to interrogate a region between HVI and HVII and are specific to human or higher primate mtDNA.

Amplification reactions were optimized for amplification temperature, MgCl2 concentration, primer concentration and probe concentration. Once the conditions for optimal amplification were determined, sensitivity and reproducibility studies were carried out under these conditions. The HL60 mtDNA control region was cloned into pCR2.1 (Invitrogen, Carlsbad, CA) to create a reagent for use as a mtDNA standard to prepare standard curves. This allows the user to compare the fluorescence generated by the sample to a standard curve and determine the amount of mtDNA present in the extract. In some DNA extracts, quantitation by methods such as slot blot hybridization reveals that DNA is present but the DNA does not amplify under standard amplification procedures. Two possible causes for this observation could be DNA degradation or the presence of PCR inhibitors in the extract. The ability to distinguish between degraded DNA and PCR inhibition has been explored. In addition, the effect of various PCR inhibitors on the ability to quantify mtDNA using real-time PCR has been studied. Finally, a validation study on evidentiary-type samples is underway.

Improvements that reduce assay time and enhance casework results are desirable because forensic mtDNA testing is a lengthy, tedious and labor-intensive procedure. Incorporation of a mtDNA control region TaqMan assay into mtDNA sequence analysis could be extremely beneficial to the forensic community. This work has generated a reagent that allows easy creation of standard curves for quantitation of mtDNA by PCR as well as a TaqMan based quantitative PCR assay to quantify mtDNA. This protocol has been optimized and the sensitivity and reproducibility of the method as well as the effect of PCR inhibitors on the assay have been assessed. Finally, a study to validate this assay for use in pre-amplification mtDNA quantitation has been undertaken.

Mitochondrial DNA, Pre-Amplification Quantitation, Real-Time PCR

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