B40 Real Time PCR for Distinguishing Degraded Samples

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After attending this presentation, attendees will develop a real-time PCR assay to detect template degradation in forensic samples.

This presentation will impact the forensic community and/or humanity by outlining a strategy to distinguish intact mtDNA template from challenged mtDNA samples. Obtaining this information prior to amplification would prevent the need to repeat the amplification with dif- ferent primers sets, avoiding excessive consumption of precious evidence.

Forensic mitochondrial DNA (mtDNA) testing is a lengthy and labor- intensive procedure. Tools being developed to assist in mtDNA analysis include real-time PCR assays, primers for amplification of mini-amplicons, and improved extraction methods. For example, there has been developed and validated a real-time PCR assay for quantitation of mtDNA in sample extracts which can be run alongside Quantifiler, a nuclear DNA quanti- tation assay, to determine whether STR analysis of nuclear DNA or sequencing of mtDNA should be performed. Although this simultaneous assay provides a rapid and objective estimate of mtDNA quantity, it does not provide information about mtDNA template size.

The current project focuses on developing an additional assay that can provide information about the size of intact mtDNA in the sample. This real-time PCR assay should be capable of distinguishing samples with degraded DNA from samples with mostly intact DNA. This information about mtDNA template size will allow the analyst to determine which set of amplification primers should be used with extracts from challenged samples, such as hair and bone, which frequently have degraded DNA. Depending on the size of the available template DNA, mtDNA can be ana- lyzed using amplicons of ~400bp, ~200bp, or ~ 100bp. Obtaining this information prior to amplification would prevent the need to repeat the amplification with different primers sets, avoiding excessive consumption of precious evidence.

The assay design involves a partially nested primer design which amplifies two different sized mtDNA control region fragments (99 and 461 bp) and two minor groove binding (MGB) probes, each labeled with a unique fluorescent tag for detection of these amplicons. In addition, the assay includes primers and probe specific for an internal positive control (IPC) to monitor the quality of reagents and equipment, as well as the presence of PCR inhibitors in the sample extract. The absence of the longer amplicon in the presence of both the shorter fragment and the IPC is indicative of degraded DNA, while the occurrence of the IPC amplicon in conjunction with the lack of both mtDNA amplicons signals intense degradation.

Primer and probe concentrations have been optimized with intact mtDNA. Currently, this assay is being evaluated using intact DNA as well as degraded template generated by an array of DNA damage inducing pro- cedures including controlled cleavage by restriction enzymes and exposure to UV, sunlight and bleach. In addition, the sensitivity of this assay has been evaluated.

This assay, in conjunction with Quantifiler, will provide information on nuclear DNA quantity, mtDNA quantity and mtDNA template size. This data will allow the analyst to determine the most efficient and effective method to analyze DNA extracts.

Mitochondrial DNA, Real Time PCR, Degraded DNA

 ** Presenting Author*