B135 A Duplex Real-Time qPCR Assay for the Quantification of Human Nuclear and Mitochondrial DNA in Forensic Samples: Implications for Quantifying DNA in Degraded Samples

Mark D. Timken, PhD, Katie Swango, PhD, Cristian Orrego, PhD, and Martin R. Buoncristiani, MPH, California Department of Justice, Jan Bashinski DNA Laboratory, 1001 West Cutting Boulevard, Suite 110, Richmond, CA 94804*

After attending this presentation, attendees will learn the advantages of using a new duplex qPCR protocol for quantifying nuclear and mitochondrial DNA in forensic sample; the implications of using qPCR versus slot blot quantification methods for successfully genotyping degraded DNA.

This presentation will impact the forensic community and/or humanity by providing a new tool for DNA quantification in forensic samples; to describe quantification differences, especially for samples containing degraded DNA that can be expected when moving from slot blot to qPCR quantitation methods.

This report describes selected results from validation of a duplex realtime qPCR assay that has been developed for the quantification of human nuclear and mitochondrial DNA in forensic samples. This assay was designed to be of general utility for forensic DNA quantifications, but to be particularly useful for the post-extraction analysis of samples that contain degraded DNA. Such samples, though not uncommon in standard casework evidence, are often encountered in evidences from mass disasters, mass graves, and missing persons' cases. Presently, the initial decision as to how to proceed with analysis of such samples, either by nuclear STR or mitochondrial typing, is commonly based on a slot blot quantification approach that (1) has been reported to underestimate the quantity of nuclear DNA in degraded samples and in samples that contain high levels of microbial contamination, (2) provides no information about the quality (fragment length) of the quantified nuclear DNA, and (3) provides no direct information about the quantity of human mitochondrial DNA in the sample. Due to these quantification deficiencies, the actual forensic analysis of challenging samples often proceeds first by obtaining inadequate STR typing results, and then by using any remaining extracted DNA in an attempt to obtain mitochondrial typing results, the latter analysis typically attempted without any direct knowledge of the presence (or absence) of human mitochondrial genome in the DNA extract. The efficiency and quality of this analysis procedure can be improved substantially by obtaining reliable estimates of the amounts of human nuclear and mitochondrial DNA in these samples. Based on such estimates, an optimal analytical approach can be selected at the outset, leading directly to optimal genotyping or haplotyping results and to a concomitant savings in time, in labor, in reagent/kit costs and of extracted DNA.

In the duplex nuclear-mitochondrial qPCR assay developed, the authors quantify a nuclear target sequence that spans the repeat region of the primate-specific *TH01* STR locus, a locus that has been used widely for forensic applications. This target sequence is of direct interest for quantification, considering that a primary reason for quantifying human nuclear DNA in forensic samples is to determine the amount of extract to amplify in a commercial multiplex STR PCR kit. For degraded samples, quantification of the relatively long *TH01* target (~170-190bp) leads to improved STR typing results, compared to typing results based on quantification by slot blot hybridization. For the mitochondrial portion of the duplex qPCR assay, the authors quantify a short target (69bp) in the mitochondrial *ND1* gene. This selection provides a sensitive means for determining the presence of human mitochondrial DNA, degraded or not, in forensic samples. Although the presentation will focus mainly on quantification and analysis of samples containing degraded DNA, the authors will also briefly describe other results from validation work, including studies of precision, reproducibility, sensitivity, species specificity, and applications to casework-type samples.

qPCR, Duplex, Degraded DNA

 ** Presenting Author*