DEVELOPMENT OF A MULTIPLEX QUANTITATIVE PCR (qPCR) ASSAY FOR SIMULTANEOUS QUANTIFICATION OF HUMAN NUCLEAR AND MITOCHONDRIAL DNA

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In forensic casework, multilocus short tandem repeat (STR) typing is often the preferred method of analysis due to its high power of discrimination. However, many evidentiary samples contain low amounts of DNA, or degraded DNA that is not suitable for STR typing. In these cases, mitochondrial DNA sequence analysis is typically performed. Determining which investigative approach is most suitable can be challenging, especially in cases where the sample or extract is limited. Here, we describe a powerful multiplex 5' nuclease real-time PCR assay that enables simultaneous quantification of both human nuclear and mitochondrial DNA from a sample extract. This tool provides specific quantitative data that can be used to determine the most appropriate analytical workflow without consumption of additional sample or increase in labor compared to methods currently used in crime laboratories.

The nuclear target for this custom qPCR assay is the 275 bp *Alu* Yd6 mobile element originally described by Xing et al.[1] High sensitivity of nuclear DNA quantitation using the multicopy *Alu* Yd6 marker has previously been reported, and a qPCR assay designed for this target has been used successfully.[2,3] The mtDNA target sequence corresponds to a 105 bp segment of the NADH dehydrogenase subunit 5 gene, and is described by Kavlick et. al. for use in an assay that is utilized routinely in our laboratory.[4] Both targets have been shown to exhibit little to no cross-reactivity with non-human sources.[2,4] In addition to these primary targets, an internal positive control (IPC) has also been included for assessment of possible PCR inhibition.

Initially, 2800M human control DNA (Promega, Madison, WI) was characterized using droplet digital PCR (ddPCR) for use as a qPCR standard. This absolute quantitative technique enables accurate and precise quantitation of nucleic acids. Briefly, a 20 µL sample containing 5' nuclease PCR reagents and sample DNA extract is partitioned into approximately 20,000 nL sized droplets. The droplets then act as independent microreactors, where fluorescent reporter dyes are liberated from target specific probes during PCR. Following PCR, the droplets are counted as target positive or negative depending on the presence or absence of fluorescence detected by a droplet reader. Target concentrations are estimated by software using a Poisson model. Human nuclear and mitochondrial DNA in control sample 2800M were separately quantified in singleplex ddPCR reactions. This data was then used to prepare a single standard dilution series of 2800M DNA with quantitative ranges of 10 ng/µL – 1 fg/µL of nuclear DNA, and 400,000 copies/µL – 0.04 copies/µL of mtDNA. Each qPCR quantitation was performed both as a duplex assay (primary target and IPC together), and as a multiplex assay (both primary targets and IPC together). Resulting PCR efficiencies, and sample quantitations were compared. Preliminary data suggests that multiplexing has a minimal derogatory effect on the efficiency of each assay, and as a result sample quantitations are similar when using singleplex or multiplex qPCR.

The assay described herein offers a high degree of specificity and sensitivity, and facilitates preservation of limited samples while allowing the analyst to determine the

optimum analytical approach for each sample. In the future, a larger target of the mtGenome will also be included in the multiplex assay to enable assessment of DNA degradation.

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