



B132 Development of a Multiplex Quantitative PCR (qPCR) Assay for Simultaneous Quantification of Human Nuclear and Mitochondrial DNA From Forensically Relevant Samples

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After attending this presentation, attendees will have a better understanding of ongoing efforts to develop a multiplex quantitative Polymerase Chain Reaction (qPCR) assay that enables simultaneous quantification of human nuclear and mitochondrial DNA (mtDNA). Additionally, principles of droplet digital PCR (ddPCR), a newly emerging quantitative technique, will be presented.

This presentation will impact the forensic science community by providing a method for comprehensive quantitative analysis of DNA in forensically relevant samples.

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, mtDNA 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, a powerful multiplex 5' nuclease real-time PCR assay is described 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 275bp 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 105bp 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 several forensic laboratories.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 was characterized using ddPCR for use as a qPCR standard. This absolute quantitative technique enables accurate and precise quantitation of nucleic acids. Briefly, a 20 μ L sample containing TaqMan[®] PCR reagents and sample DNA extract is partitioned into approximately 20,000nL-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 mtDNA 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 10ng/ μ L to 1fg/ μ L of nuclear DNA and 400,000 copies/ μ L to 0.04 copies/ μ L 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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References:

1. Xing J, Salem AH, Hedges DJ, Kilroy GE, Watkins WS, Schienman JE, Stewart CB, Jurka J, Jorde LB, Batzer MA. Comprehensive analysis of two Alu Yd subfamilies. *J Mol Evol* 2003;57:S76-S89.
 2. Walker JA, Kilroy GE, Xing J, Shewale J, Sinha SK, Batzer MA. Human DNA quantitation using Alu element-based polymerase chain reaction. *Anal Biochem* 2003; 315:122-28.
 3. Hansen HM, Wiemels JL, Wrensch M, Wiencke JK. DNA quantification of whole genome amplified samples for genotyping on a multiplexed bead array platform. *Cancer Epidemiol Biomarkers Prev* 2007; 16:1686-90.
 4. Kavlick MF, Lawrence HS, Merritt RT, Fisher C, Isenberg A, Robertson JM, Budowle, B. *Quantification of human mitochondrial DNA using synthesized DNA standards*. 2011; 56:1457-63.
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Quantitative PCR, Droplet Digital PCR, Multiplex