B29 The Use of a Mitochondrial DNA-Specific qPCR Assay to Assess Degradation and Inhibition

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The goal of this poster presentation is to describe the utility of a mitochondrial DNA-specific quantitative PCR (mito-qPCR) assay in the forensic setting. This presentation will demonstrate how this assay was utilized at the Armed Forces DNA Identification Laboratory's (AFDIL) Research Section to quantitate mitochondrial DNA. The attendee will learn about qPCR as it relates to sample inhibition and/or degradation as well as how information from the assay is used for downstream assays other than traditional STR analyses.

This presentation will impact the forensic community and/or humanity by informing the forensic community of a variety of different uses for a mitochondrial qPCR assay as well as how laboratories could make use of the changes observed in the assay's internal PCR control cycle threshold (IPCCt) to assess the inhibitors present in a sample and different amplicon sizes to detect sample degradation.

Forensic DNA typing systems, including mitochondrial DNA analysis, allow the routine processing of many different samples types. Downstream assays such as short tandem repeat (STR) analyses require precise quantities of input DNA (0.5 to 2ng) in order to achieve optimal results, or profiles. Traditionally, slot-blot hybridization assays have been used to quantitate the amount of nuclear DNA in forensic samples. This method, though sensitive and human-specific, is time-consuming, labor-intensive, and not readily amenable to automation. More recently, quantitative PCR (qPCR), in which the amplification of specific target sequences is measured in real-time, has become a popular choice for forensic DNA quantititation. The production of a commercial kit containing all of the components necessary to run the assay has made it readily available to forensic labs requiring extensive validation of such protocols. Additional assays recently published in the forensic literature have also been developed that target human nuclear DNA, the human Y chromosome, and the human mitochondrial genome.

Although the downstream analysis of mitochondrial DNA, i.e. cycle sequencing, does not have the same requirements as STR assays regarding input DNA quantity, other information provided by a qPCR assay can be useful for analysis. For example, some qPCR assays include an IPCCt to assess inhibition in the extracts tested. Variation in the IPCCt of a sample in comparison to that of the non-template controls (NTC) would indicate a potential inhibitor present in that sample. Since the quantitation is performed using the same mechanism as the downstream assay, that is, the polymerase chain reaction, the results of the qPCR assay mimic the extract's amplification behavior in the downstream assay. Therefore, if inhibition is detected during quantitation, additional measures such as further purification and/or dilution can be employed to allow successful subsequent PCR amplification and sequencing. Similarly, the successful quantification of target amplicons of different sizes can be used to assess a sample's state of degradation and to determine whether mini-primer sets should be employed for amplification.

The AFDIL Research Section is continuously evaluating new extraction protocols as well as producing large mitochondrial control region databases. A mito-qPCR assay would be useful in evaluating the success of these new extraction procedures as well as predicting the success of the sequencing of database samples. The authors have utilized an in-house mito-qPCR assay developed by the Institute of Legal Medicine (Innsbruck, Austria), which was based upon the duplex nuclear-mito qPCR assay described by Andreasson, et al. (2002). This assay employs three different target amplicon sizes to allow for the assessment of the level of degradation of a sample, as well as an IPC to assess any potential inhibitors present in the extract. In this poster the authors will present a variety of uses for this mitochondrial qPCR assay, the changes observed in the assay's IPC Ct as it relates to the inhibitors present in a sample, and the detection of sample degradation. These parameters will be related to the samples' performance in the selected downstream application, in this case, PCR amplification, and cycle sequencing.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

Reference:

Andreasson H, Gyllensten U, Allen M. Real-time DNA quantification of nuclear and mitochondrial DNA in forensic analysis. Biotechniques. 2002 Aug;33(2):402-4, 407-11.

Quantitative PCR, Mitochondrial DNA, Inhibition

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