B134 Hair and Calcified Tissue DNA Extracts: qPCR-Based Guidelines/Strategies for Streamlined Mitochondrial DNA (mtDNA) Amplification and Improved mtDNA Sequence Recovery

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After attending this presentation, attendees will better understand how mtDNA quantitative Polymerase Chain Reaction (qPCR) values can be used to increase sequence data recovery and minimize sample consumption.

This presentation will impact the forensic science community by providing practical, data-driven mtDNA-processing guidelines for streamlined mtDNA workflows.

Mitochondrial DNA (mtDNA) is a valuable forensic marker for samples such as hair and calcified tissue, as they routinely fail upon nuclear DNA (nDNA) analysis due to DNA degradation or insufficient template. Though mtDNA can generally be recovered from these sample types, the mtDNA content is often still quite limited and the source material is almost always limited. Under these circumstances, a mechanism for determining the most efficient and successful amplification strategy would lead to reduced sample consumption and an increase in the quantity of sequence information obtained.

The mtDNA Control Region (CR, rCRS 15998-616) is routinely targeted for analysis due to its high inter-individual variation. Historically, CR data have been recovered at the Federal Bureau of Investigation (FBI) laboratory with four amplicons (designated HV1A, HV1B, HV2A, HV2B, each ~230bp to 290bp) that sit within mtDNA Hypervariable Region 1 (HV1, rCRS 15998-16390) or Hypervariable Region 2 (HV2, rCRS 49-408). Though amplification of smaller fragments is also often performed, previous studies and two decades of casework experience have shown that larger fragments are seldom recoverable. As a result, they are rarely targeted for amplification.

With the recent implementation of new extraction procedures for calcified tissues and hairs, a dramatic improvement in the quantity/quality of mtDNA recovered from these specimen types has been observed. Studies have shown a 2- to 30-fold increase in mtDNA copy number (as determined by a custom real-time quantitative PCR (qPCR)) and established that fragments >350bp are now recoverable.[1] In addition, results show that the qPCR values, though based on a 105bp amplicon, provide an indication of CR amplification success with amplicons of varying size. Not surprisingly, extracts with greater qPCR values tend to produce amplicons of greater size.

In order to further define the mtDNA quantities required for successful amplification and develop practical guidelines for casework implementation, representative extracts were quantified by qPCR and then amplified with primer sets targeting CR fragments of various size. Amplicons ranging from ~230bp to 1,100bp (the entire CR) were tested, then extracts producing successful amplification were evaluated for their corresponding mtDNA quantities. Minimum mtDNA quantities required to produce successful amplification at a given fragment size were conservatively established based on the highest quanting extracts (75%) that led to amplification product. Based on this criterion, guidelines were established to amplify entire HV1 and HV2 region amplicons (~360bp to 390bp) from hair extracts when ≥1,500 copies/µl are detected (100% successful in studies) and entire CR with values ≥8,000 copies/µL (95% successful in studies). Similarly, for calcified tissue, the entire CR can be amplified with values ≥30,000 copies/µL (85% successful in casework and studies) and HV1 and HV2 can be targeted with as little as 400 copies/µL (100% successful in casework and studies). As a result of these guidelines, approximately 60% and 85% of calcified tissue and hair cases, respectively, have successfully yielded HVI and HVII amplicons. Moreover, the entire control region (~1100bp) has been successfully amplified from casework hair and calcified tissue specimens, whereas previously this would have not even been attempted.

The guidelines are continually being updated with casework data to maintain the most efficient and successful use of DNA extracts for current mtDNA analyses. In addition, it is expected that qPCR-based guidelines may be similarly useful with forthcoming Next Generation Sequencing (NGS) assays and applications. In fact, recent experiments have shown that 2kb to 3kb fragments spanning the entire mtGenome can be recovered from some hair samples with values of ≥30,000 copies/µL and that these amplicons can be successfully sequenced using the Nextera® XT DNA Sample Preparation Kit and the Illumina MiSeq™.

For poor quality evidentiary material containing damaged and/or low quantities of DNA, knowledge of the amplifiable mtDNA content has led to more informed casework decisions on downstream PCR. This in turn has led to decreased sample processing time and cost, more judicious use of limited sample material, and, most importantly, more successful mtDNA testing outcomes.

Reference:

1. Kavlick, M.F., Lawrence, H.S., Merritt, R.T., Fisher, C., Isenberg, A., Robertson, J.M., *and* Budowle, B.2011. Quantification of human mitochondrial DNA using synthesized DNA standards. *J Forensic Sci.*56:1457-1463.

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mtDNA, NGS, qPCR