A161 Co-Amplification of NUMTs in Low DNA Quantity Specimens

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After attending this presentation, attendees will be informed regarding the incidence and management of co-amplified Nuclear Mitochondrial DNA (NUMT) with authentic mitochondrial DNA while producing a database of more than 550 entire mitochondrial genomes via Sanger sequencing.

This presentation will impact the forensic science community by contributing to the ongoing conversation regarding mitochondrial DNA recovery and data quality. With the increasing sensitivity of mitochondrial genome typing, especially through the emerging use of next generation sequencing chemistries, the potential for co-amplification of mitochondrial pseudogenes will require greater consideration than has been required in the past.

Forensic mitochondrial DNA (mtDNA) testing requires representative high-quality population databases for estimating the rarity of questioned haplotypes. However, currently available forensic reference population data only include information from the mtDNA control region. To address this deficiency, the Armed Forces DNA Identification Laboratory (AFDIL) undertook a National Institute of Justice-funded, large-scale databasing effort to sequence complete mitochondrial genomes (mtGenomes) spanning three U.S. population groups. Amplification of the complete mtGenome was achieved via eight overlapping fragments and each mtGenome was sequenced in 135 reactions, providing redundant and overlapping forward and reverse sequence coverage across the entire molecule.[1] To assure the generation of the highest quality profiles, nearly all pipetting steps were performed robotically and a rigorous data review process was employed.

The samples used for this databasing effort were anonymized blood serum specimens from the Department of Defense Serum Repository.[2] Only a small amount of cell-free DNA is typically present in blood serum, as the DNA-containing blood components have been removed by centrifugation. Thus, the samples to which the 8-amplicon Polymerase Chain Reaction (PCR) strategy was applied contained DNA that was generally of high quality but present in very low quantity. Though the mtDNA copy number in any given blood serum extract should readily exceed the available nuclear DNA, amplification of NUMT insert remains a possibility when: (1) total DNA quantity is very low; and, (2) NUMTs both exceed the size of the target mtDNA fragment and have high similarity to modern mtDNA in primer binding regions. Careful PCR primer design can reduce, though not eliminate, this risk.

Rare instances in which NUMTs were co-amplified and sequenced with the authentic mtDNA in the course of generating more than 550 entire mtGenome profiles from low DNA quantity specimens will be presented, along with strategies for handling the samples/data. The NUMTs were distinguished from sample contamination by the appearance of many more heteroplasmic positions than would be expected from a mixture of distantly related modern mtDNA haplotypes, and the minor profiles matched a known mitochondrial pseudogene. Characterization of the presentation and frequency of co-amplified NUMTs will be increasingly important as the sensitivity of whole mtGenome typing improves — such as with the implementation of next generation sequencing technologies in forensic laboratories — and these highly sensitive methods are applied to extremely low template samples.

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References:

[1]Lyons *et al.*, Poster presentation, AAFS 2011, Chicago, IL.

1. Serum specimens from the Department of Defense Serum Repository: The Armed Forces Health Surveillance Center, U.S. Department of Defense, Silver Spring, MD, November 8, 2010.

Mitochondrial DNA, Sequencing, NUMT

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