B46 The Development of a Mitochondrial DNA (mtDNA) Assay for Forensic Human Inclusion/Exclusion Screening Using Real-Time Polymerase Chain Reaction High-Resolution Melt (PCR HRM) Analysis

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After attending this presentation, attendees will understand how PCR coupled with HRM can be used as a presumptive mtDNA screening tool for degraded DNA samples in forensic casework.

This presentation will impact the forensic science community by demonstrating a new method of screening mtDNA evidence and comparing it to reference samples to determine if further testing is required or to sort preliminary groupings. It is the intention that this process could be used as a presumptive step prior to standard DNA sequencing in forensic laboratories. In addition, equipment required for this additional screening is readily available to forensic DNA scientists in major crime laboratories.

Forensic scientists currently use mtDNA to identify missing persons and remains recovered from mass disaster cases. It is often valuable in these situations due to its higher copy number compared to nuclear DNA, which can become highly degraded and thus compromised. By comparing the recovered mtDNA to maternal lines, a potential identification can be made. While less discriminating than nuclear DNA, mtDNA is an excellent tool for screening and has been shown to differentiate human populations. It is highly advantageous for crime scene laboratories to be able to exclude or include a possible suspect(s) before focusing additional time and resources into further investigation.

Within the mitochondrial genome, there have been reported to be hypervariable regions known as one, two, and, more recently, a third has been discussed (HVI, HVII, and HVIII). Mutation rates in these areas are known to be five to ten times higher than nuclear genes due to low fidelity of mtDNA polymerase and the lack of repair mechanisms within the mtDNA overall. These regions are located within the control region of the mtDNA and code for no known medically or phenotypically significant genes. Additionally, other highly variable polymorphisms are located within and around these three hypervariable regions. Through the culmination of data from mtDNA sequencing over multiple populations throughout the world, databases such as MITOMAP have compiled known areas of variation designated as Single Nucleotide Polymorphisms (SNPs).

Many current techniques for SNP assays involve utilizing complex primer set-ups like SnaPShot® or fluorescently labeled Dideoxynucleotide triphosphates (ddNTPs), which ultimately conclude with capillary electrophoresis for visualization of the amplicons. As an alternative to costly sequencing or Short Tandem Repeat (STR) -like methods, real-time PCR HRM has been proposed as a more cost effective and faster approach to differentiate between mtDNA SNPs. In addition to forensics, research using HRM has also been applied to coding regions of mtDNA related to mutation-causing human diseases, such as MELAS and LHON, and evaluating polymorphisms within the control region of common carp.

In this study, primers have been constructed to amplify the variable regions of interest in the human mitochondrial genome (HVI, HVII, and HVIII), as well as SNPs of interest specifically modified with GC-tail primers to increase melt temperatures of specific polymorphisms and thus better differentiate identifying peak heights resulting from HRM. DNA Standards including 9947A, 2800M, and K562, as well as IRB-approved volunteer buccal samples were evaluated via real-time PCR-HRM and agarose gel electrophoresis. A known T477C transition within the HVIII of 2800M showed a distinguishable temperature shift in comparison to 9947A and K562. More specific primers were designed to highlight SNPs of interest, such as the mtDNA 16519 (A/G) variant which was probed with three sets of primers. The addition of the GC-tail to the G variant allowed for a visibly increased melt temperature in comparison to the A variant on the resulting melt curve and thus facilitated differentiation.

Mitochondrial DNA, Real-Time PCR, High-Resolution Melt

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