A163 Detection of Low-Level DNA Sequences Associated With Nuclear Mitochondrial Pseudogenes (NumtS) From Human Mitochondrial Control Region Amplicons Using Massively Parallel 454 Pyrosequencing

Brittania J. Bintz, MSc, Groves B. Dixon, BS, and Erin S. Burnside, MSc, Western Carolina Univ, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723; and Mark R. Wilson, PhD, Western Carolina Univ, Dept of Chemistry/Physics, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723*

After attending this presentation, attendees will have a better understanding of next-generation sequencing (NGS) as applied to mitochondrial DNA control region analysis. Approaches to quantify minor variant detection thresholds of the Roche GS Junior 454 pyrosequencing instrument will be described. Additionally, the detection of nuclear pseudogenes during mixture study deep sequencing runs, and steps taken to confirm the presence of these pseudogenes in our donor population, will be discussed. Finally, detection of inconsistent, non-NumtS affiliated minor variants in hair shaft tissues will be discussed, and research approaches taken to elucidate causes of these variants.

This presentation will impact the forensic science community by outlining foundational research being performed in the area of Next-Generation Sequencing (NGS) of the human mtDNA control region. Preliminary suggestions for streamlined NGS sample preparation, use of Roche Amplicon Variant Analyzer (AVA) software, and data analysis considerations will be offered. Reasonable expectations for instrument detection threshold, pyrosequencing-specific sequencing errors, and tissue dependent differences in sequence data will be described. A per-run cost analysis using the Roche GS Junior platform will be presented.

Massively parallel pyrosequencing on the Roche GS Junior provides thousands of independent reads per sequencing run, and thus has the potential to detect and quantify minor variants with vastly greater sensitivity and precision than traditional Sanger sequencing. The minor variant detection threshold was determined for the Roche GS Junior instrument using mixtures of mtDNA hypervariable (HV) region amplicons with known sequences. In addition to the expected variants originally obtained using dideoxy terminator sequencing, a set of nineteen unexpected variants in HV1b reads (corresponding to base pairs 16159 – 16391 in the mitochondrial control region) at a level of approximately 1% were detected. These variants are reproducible and are always detected as a set within individual reads of HV1b amplicons. The total depth of coverage did not appear to affect the level at which the unexpected variants were detected. A standard nucleotide BLAST search of the variant sequence was performed which showed 100% sequence similarity to a segment of a 611bp nuclear mitochondrial pseudogene (NumtS) originally reported in 1995 by Zischler *et al*.[1] This NumtS is an insertion of the mitochondrial control region (bases 16,089 – 59) on the short arm of chromosome 11, spanning the primer binding sites of the targeted HV1b region.[2] Nuclear DNA specific primers flanking the insertion were used to amplify the pseudogene from buccal extracts without amplifying DNA from the mitochondrial control region.[3] This amplification strategy confirmed the presence of the NumtS in 19 out of 20 donors, with one donor being homozygous-negative for the insertion. Dideoxy terminator sequencing was used to successfully confirm the presence of the variant sequence in the amplified NumtS from donors positive for the insertion. This identification furthers the understanding of human mtDNA variants and is expected to have a positive effect on the interpretation of mtDNA profiles using deep sequencing methods in forensic casework. This work will ultimately allow expansion in the analysis of mtDNA beyond the control region using a whole genome amplification (WGA) technique to increase starting concentrations of extract from compromised samples.

References:

1. Zischler H, Geisert H, von Haeseler A, *et al.* A nuclear “fossil” of the mitochondrial D-loop and the origin of modern humans. Nature 1995;378:489-492.
2. Lang M, Sazzini M, Calabrese FM, Simone D, Boattini A., Romeo G, Luiselli D, Attimonelli M, Gasparre G. Polymorphic NumtS trace human population relationships. Hum Genet 2012;131(5):757-771.
3. Thomas R, Zischler H, Pääbo S, Stoneking M. Novel mitochondrial DNA insertion polymorphism and its usefulness for human population studies. Hum Biol 1996;68:847-854.

Mitochondrial DNA, Pyrosequencing, Variants

 ** Presenting Author*