DETECTION OF LOW-LEVEL DNA SEQUENCES ASSOCIATED WITH NUCLEAR MITOCHONDRIAL PSEUDOGENES (NUMTS) FROM HUMAN MITOCHONDRIAL CONTROL REGION AMPLICONS USING MASSIVELY PARALLEL 454 PYROSEQUENCING

Brittania Bintz, M.S.
Western Carolina University, Chemistry & Physics, 111 Memorial Drive, Cullowhee, NC 28723, USA

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. We have determined the minor variant detection threshold of 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, we also detected a set of nineteen unexpected variants in HV1b reads at a level of approximately 1%. 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 had 99% sequence similarity to a 611 bp nuclear mitochondrial pseudogene (NumtS) originally reported in 1995 by Zischler et al[3]. 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. Nuclear DNA specific primers[1,2] flanking the insertion were used to amplify the pseudogene from buccal extracts without amplifying DNA from the mitochondrial control region. This amplification strategy confirmed the presence of the NumtS in nineteen out of twenty 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 the donors positive for the insertion. This identification furthers our 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.

References:

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