

DEVELOPMENT OF A SINGLE MITOCHONDRIAL DNA AMPLIFICATION STRATEGY FOR TWO PLATFORMS: NEXT GENERATION AND SANGER SEQUENCING FROM THE SAME AMPLICON LIBRARY

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When forensic samples contain limited or degraded nuclear DNA, mitochondrial DNA (mtDNA) analysis is a valuable substitute for short tandem repeat (STR) genotyping (Salas *et al.* 2001). Drawbacks to mtDNA analysis include inferior statistical strength compared with STR genotyping and interpretational challenges associated with rapid mutation rate and tendency toward heteroplasmy of the mitochondrial genome (Wilson *et al.* 2011 proposal). Heteroplasmy describes the presence of two or more unique mtDNA types within a single individual, tissue, cell, or mitochondrion (Paneto *et al.* 2007). Researchers now consider both length heteroplasmy (mixtures of mtDNA types that differ by indel mutations) and sequence heteroplasmy (mixtures that differ by substitutions) as expectations, rather than exceptions in mtDNA analyses (Li *et al.* 2010). The pattern of variation of heteroplasmy in the mitochondrial genome is still a matter of debate, making interpretation of similar but distinct mtDNA sequence data of particular difficulty to forensic technicians (Naue *et al.* 2011, Salas 2001, Budowle *et al.* 2003).

Massively parallel sequencing (MPS) (also called Next Generation Sequencing or NGS) technologies have the potential to generate orders of magnitude more sequence data than traditional Sanger sequencing at a competitive cost per base pair sequenced (Ronaghi 2001). MPS platforms make use of spatially separated independent sequencing reactions of clonally amplified single molecules. This allows for the generation of thousands of independent sequence reads (Voelkerding *et al.* 2009). Thus, MPS allows greater breadth (the proportion of the genome that is sequenced) and depth (the number of independent sequencing reads taken of each nucleotide position in a region of interest) of sequence data (Wilson *et al.* 2011 proposal).

To improve the statistical strength and interpretational issues of mtDNA analysis, researchers recommend establishing a profile of the frequencies with which point heteroplasmy occurs at each nucleotide position (Santos *et al.* 2008, Paneto 2010, Irwin *et al.* 2009), and expanding the breadth of the mitochondrial molecule that is examined (Salas *et al.* 2007). Because they enable significantly higher throughput and sensitivity than traditional Sanger sequencing methods, MPS technologies hold great promise for each of these recommendations.

In this study, we assess the utility of the 454 Roche GS Junior Titanium MPS platform for forensic applications. We have optimized a single amplification protocol that enables forensic crime laboratories to analyze mtDNA using both Roche 454 MPS and Sanger methods. In future studies, we will assess the ability of the instrument to detect low-level variants in mixtures of mtDNA.

We extracted DNA from hair, blood and buccal samples from twenty individuals. From these, we generated modified amplicon libraries for use on the Roche GS Junior. Reference sequences for mtDNA hypervariable (HV) regions were obtained for each donor with Sanger sequencing using these Roche modified libraries.

We showed that modified amplicon libraries for an MPS platform can be sequenced using traditional Sanger sequencing. This protocol allows for selective use of a sequencing method based on the quality of the sample. Straightforward exclusions can be interpreted directly from Sanger sequence data, however in cases where Sanger sequence data provides insufficient resolution for confident interpretation, the analyst can return to the same original amplified library for MPS.

Works Cited:

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