A93 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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After attending this presentation, attendees will have learned the basics of how the Roche GS Junior works, the benefits of such an instrument, and how it may be incorporated into forensic casework.

This presentation will impact the forensic science community by demonstrating the applicability of this new and promising technology for forensic casework.

When forensic samples contain limited or degraded nuclear DNA, mitochondrial DNA (mtDNA) analysis is a valuable substitute for short tandem repeat (STR) genotyping.[1] 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.[2] Heteroplasmy describes the presence of two or more unique mtDNA types within a single individual, tissue, cell, or mitochondrion.[3] 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.[4] 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.[1,5,6]

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.[7] 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.[8] 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.[2]

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 and expanding the breadth of the mitochondrial molecule that is examined.[9-12] 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, the utility of the 454 Roche GS Junior Titanium MPS platform for forensic applications was assessed. The objectives included: (1) to optimize a single amplification protocol that enables forensic crime laboratories to analyze mtDNA using both Roche 454 MPS and Sanger methods: and (2) to assess the ability of the instrument to detect low level variants in mixtures of mtDNA.

The DNA from hair, blood, and buccal samples from twenty individuals was extracted. From these, modified amplicon libraries for use on the Roche GS Junior were generated. Reference sequences for mtDNA hypervariable (HV) regions were obtained for each donor with Sanger sequencing using these modified libraries. Mixtures of mtDNA HV amplicons were prepared in ratios of 95% / 5%, 98% / 2%, and 99% / 1%. These mixtures were sequenced using 454 pyrosequencing technologies to assess the ability of the Roche GS Junior instrument to accurately detect minor variants of mixed mtDNA.

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. Low level variants at mixture ratios of 99% / 1% were detected using the Roche GS Junior. Additionally, an optimized a protocol that allows seamless inclusion of the technology into forensic crime laboratories using current mtDNA testing methodology was developed.

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