B178 Assessing the Impact of DNA Damage on the Interpretation of Low-Level Mitochondrial DNA (mtDNA) Heteroplasmy

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After attending this presentation, attendees will understand the importance of considering DNA damage effects on Next Generation Sequencing (NGS) sequence data. Attendees will also be more aware of how these effects will be important when developing reliable guidelines for interpreting and reporting mtDNA heteroplasmy or other types of low-level Single Nucleotide Polymorphisms (SNPs) in future forensic casework. This presentation will include a discussion on extract storage conditions, characteristics and identification of false low-level variant positions, and interpretation threshold recommendations.

This presentation will impact the forensic science community by elucidating characteristics of DNA damage in the mtDNA control region as it relates to the interpretation of heteroplasmy when using the NGS technology from Illumina®, the MiSeq®. With the introduction of NGS to forensic laboratories in the near future, the effect of DNA damage on any NGS sequence data should be characterized in order to prevent reporting of false positive sequence data. Given that low-level variants have the potential to make mtDNA more discriminating by offering the ability to better distinguish between unrelated individuals and maternal family members and by offering a more statistically significant likelihood ratio, it becomes inherently important to understand how damage affects these interpretations when made in modern forensic investigations and identifications. In addition, this information will contribute to understanding how damage may impact the analysis of nuclear DNA SNPs. There is confidence that this study of the impact of DNA damage on mtDNA heteroplasmy observations will help lead to recommendations of best practices for NGS forensic applications.

Forensic mtDNA analysis is a robust technique that is advantageous for challenging samples, but the identification through maternal haplotypes limits the discrimination potential compared to Short Tandem Repeat (STR) analysis. Heteroplasmic sequence variants can potentially provide distinction between maternal relatives and significantly increase likelihood ratios associated with matching mtDNA profiles; however, the nature of heteroplasmy as mixtures means that other sources of mixtures, such as DNA damage, must be eliminated as the cause of an apparent variant.[1] DNA damage is frequently encountered in forensic mtDNA analysis, so it is important to understand its effect on the interpretation of heteroplasmy. NGS of DNA now offers higher sensitivity than the Sanger method, allowing for detection of low-level heteroplasmy with a 1% minor variant.[2,3] Given that damaged sites can be observed with the Sanger method of sequencing, it was anticipated that damage will impact heteroplasmy interpretation using an NGS approach. Considering that mtDNA heteroplasmy may someday be more widely reported in forensic casework, a clear understanding of how damage-related anomalies affect NGS data will be important to the forensic community.

The goal of this study was to characterize the impact of DNA damage on the interpretation of mtDNA heteroplasmy, mainly in regard to low-level variants. The conditions that encourage damage mechanisms, such as deamination, which may arise from storage of DNA extracts or postmortem exposure to the element, were modeled.[4,5] Samples were run on the Illumina® MiSeq® following Nextera® XT library preparation. The sequencing data showed concordance with the Sanger method, providing consistent haplotypes across all conditions and maintaining the reliability of common DNA extract storage practices; however, it was discovered that when using an interpretation threshold of 1% for low-level heteroplasmy, as damage conditions become more intense, the number of apparent heteroplasmic positions increased. A decrease in the expected transition/transversion ratio was observed, suggesting that the sites of damage were random in relation to normal mutation mechanisms. Also, most of the false heteroplasmic positions showed a 1%-2% minor variant, with certain positions more likely to show false heteroplasmy. This may be useful for separating out and reporting true variants as interpretation criteria are developed.

Altogether, the results of this study of DNA damage advocate for careful consideration of long-term extract storage methods. NGS data produced from challenging evidence samples such as these will impact threshold considerations for reporting low-level heteroplasmy in future mtDNA casework. This assessment will also help contribute to the recommendations of best practices for forensic NGS-reporting guidelines as this technology is incorporated into crime laboratories.

Reference(s):

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MiSeq®, MtDNA Heteroplasmy, DNA Damage