# ASSESSING THE IMPACT OF DNA DAMAGE ON THE INTERPRETATION OF LOW-LEVEL MTDNA HETEROPLASMY

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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 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. Given that DNA damage is frequently encountered in forensic mtDNA analysis, it is important to understand how such positions affect the interpretation of heteroplasmy. Moreover, NGS now offers higher sensitivity than the Sanger method, allowing for detection of low-level heteroplasmy with a 1% minor variant. Damaged sites can be observed with the Sanger method of sequencing, so we anticipated that damage would impact heteroplasmy interpretation using an NGS approach. Considering mtDNA heteroplasmy may someday be more widely reported in forensic casework, a clear understanding of how anomalies affect NGS data will be important to the forensic community. Our research aims to characterize the impact of DNA damage on the interpretation of mtDNA heteroplasmy, mainly in regard to low level variants. We have modeled conditions that encourage damage mechanisms, such as deamination, which may arise from storage of DNA extracts or post-mortem exposure to the elements. Samples were run on the Illumina MiSeq following NexteraXT library preparation. Our long-range amplification method has shown limited ability to amplify DNA from the harshest damage conditions, which in turn affects the number of original molecules contributing to apparent heteroplasmy. Consequently, we compared this to targeting shorter amplicons using common forensic mtDNA primer positions, but with additional transposase sequences adapted (Illumina) to aid in library preparation. Our initial data indicates consistent haplotypes across all conditions, further validating NGS results and common DNA extract storage practices. However, the number of false heteroplasmic positions increases as damage conditions worsens, and most of those positions present with a 1-2% minor variant. So far, we believe our study has highlighted the requisite need for modifications to reporting thresholds of low-level variants as we continue to evaluate NGS and mtDNA heteroplasmy in the forensic context.