CHARACTERIZATION OF MTDNA DEAMINATION DAMAGE USING MASSIVELY PARALLEL SEQUENCING AND EVALUATION OF THE NEBNEXT® FFPE DNA REPAIR MIX

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Through the use of massively parallel sequencing (MPS), it is now possible to detect low-level heteroplasmic variants (≥2%) in mitochondrial (mt) DNA sequence data, thereby increasing the discrimination potential of mtDNA analysis in forensic casework. Because of this, it has become increasingly important to differentiate heteroplasmy from DNA damage often observed in low quality, challenged evidence samples encountered in forensic mtDNA casework. Patterns of DNA damage across genomic sequence have been well studied, especially in the ancient DNA community. Likewise, studies on DNA damage conducted in the forensic community have typically focused on the impact of the damage on short tandem repeat (STR) analysis in nuclear DNA. However, it is clear that DNA damage is routinely observed when analyzing typical mtDNA evidence samples, such as older hair shafts and skeletal remains, using conventional Sanger sequencing. While much is known about the effects of damage on Sanger sequencing data, little is known about the impact on MPS results. And although many of the ancient DNA studies have included mtDNA, it is rarely possible to relate the results to modern reference sources. Our laboratory has been conducting such DNA damage studies under the NIJ Grant Award 2015-DN-BX-K025. One area of our research has dealt specifically with modeling and characterizing deamination of the mtDNA control region using MPS. The deamination modeling was accomplished by using the EpiTect® Fast BiSulfite Conversion Kit (Qiagen) containing bisulfite as the damaging agent in conjunction with heat exposure (60°C). Once the DNA was damaged, the amount of mtDNA was quantified using a custom mtqPCR approach. The PCR amplification and library preparation was performed using the Promega PowerSeq™ Mito Control Region Nested Kit and sequenced using the Illumina MiSeq. The resulting sequence data showed numerous deaminated damage sites across the mtDNA control region (~20-80 sites per sample). Similar results were also obtained from 7th-12th century skeletal remains. Using an enzyme repair mix (NEBNext® FFPE DNA Repair Mix, New England BioLabs, Inc.), we evaluated whether this repair system can reverse the effects of the damage. This poster will present our findings with respect to characterization and patterns observed in deamination damage of MPS mtDNA sequence data, as well as the subsequent results from the DNA repair studies.