CHARACTERIZATION AND REPAIR OF HYDROLYTICALLY-INDUCED DNA DAMAGE IN THE MTDNA CONTROL REGION VIA MPS ANALYSIS

Sidney A. Gaston Sanchez B.S., Charity A. Holland M.P.H., Mitchell M. Holland Ph.D.
Forensic Science Program, The Pennsylvania State University

DNA damage can impact the interpretation of sequence-based mitochondrial (mt) DNA profiles. Hydrolytic damage can occur through hydrolyisis of functional groups on nitrogenous bases resulting in a base change or through cleavage of the glycosidic bond between the base and the sugar moiety, creating an abasic site. These events are typically described as deamination and depurination, respectively. In this study, DNA extracts of donors with known haplotypes were hydrolytically damaged by incubating them in water at varying time periods and temperatures. The extracts were then repaired with the NEBNext® FFPE (formalin-fixed paraffin-embedded) DNA Repair Mix and the mitochondrial control region was sequenced through massively parallel sequencing (MPS). Samples incubated at 37°C resulted in extensive degradation, a product of severe damage, while those incubated at room temperature exhibited random deamination-like and depurination-like events. Degradation increased in samples that were incubated for longer time periods. In every experiment, non-repaired portions of extracts had more damage sites than repaired portions of extracts. Furthermore, the rate of damage sites per 100 nucleotides (nt) was higher in the non-repaired samples when compared to the repaired samples (0.31-0.52 vs 0.01-0.29 damage sites/100 nt, respectively). While the repair cocktail decreased the amount of existing damage sites, it did not completely eliminate them. However, the repaired samples were overall less damaged than the non-repaired samples. Damage variants can cause complications in the interpretation process because they can appear to be true heteroplasmy. Heteroplasmy can be used to support an identification, and therefore it is important to identify damage as a potentially confounding variable. Based on these findings, the additional step of repairing DNA extracts before MPS mtDNA sequencing could prove worthwhile when examining challenged samples that are often encountered in forensic, ancient DNA, and even clinical applications.