## B106 The Characterization and Repair of Hydrolytically Induced DNA Damage in the Mitochondrial DNA (mtDNA) Control Region Through Massive Parallel Sequencing (MPS) Analysis

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**Learning Overview:** After attending this presentation, attendees will gain insight on the effects of hydrolytic DNA damage observed on mitochondrial DNA (mtDNA) sequenced through Massive Parallel Sequencing (MPS) and the potential value in repairing damaged extracts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving analysts' ability to identify DNA damage. If extracts are designated as damaged, applying a repair cocktail prior to library preparation and sequencing can reduce damage as an artifact downstream in the interpretation process.

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DNA damage can impact the interpretation of sequence-based mtDNA profiles, including when interpreting heteroplasmy. Hydrolytic DNA damage can be observed in forensic and ancient DNA samples and can result in lesions and both single- and double-stranded breaks. Two common products of hydrolytic DNA damage are deamination and depurination. Deamination can occur through hydrolysis of functional groups on nitrogenous bases resulting in a base change, while depurination occurs through cleavage of the glycosidic bond between the base and the sugar moiety, creating an abasic site.

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® Formalin-Fixed Paraffin-Embedded (FFPE) DNA Repair Mix and the mitochondrial control region was sequenced through MPS analysis. 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; 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. Therefore, damage variants should be expected when working with forensic evidence that may complicate the interpretation of heteroplasmy. To mitigate this effect, replicate amplifications are recommended, as this resulted in no duplicate sites of damage in the current study, consistent with numerous previous studies.

Future experiments will focus on a single incubation time and temperature to assess the reproducibility of damage patterns and the performance of the repair method. Based on our collective findings, we will assess the overall value of conducting the additional step of repairing DNA extracts before MPS mtDNA sequencing from a modeling perspective.

**Hydrolytic DNA Damage, Repair, Mitochondrial DNA**