B12 Oxidative Mitochondrial DNA (mtDNA) Damage and Repair: A Modeling Approach Compared to DNA Recovered From Bullet Cartridge Cases

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After attending this presentation, attendees will understand the practice in identifying, characterizing, and repairing oxidative DNA damage lesions and will be able to observe oxidative mtDNA damage in both modeled samples and forensic-type samples.

This presentation will impact the forensic science community by helping in the development of best practices for collection of DNA evidence from cartridge case evidence, as well as accurately identifying, characterizing, and repairing oxidative damage lesions when performing mtDNA sequence analysis in forensic casework.

DNA damage involves a change in the chemical structure of DNA through the introduction of strand breaks and lesions and is a well-known characteristic of forensic evidence and ancient samples. The type of damage observed will depend on the conditions under which the evidence is exposed, the length of exposure, and the type of evidence that is collected. There are several categories of DNA damage; for example, hydrolytic and oxidative. Damage can result in deamination, depurination, and oxidation of nitrogenous bases.

The first part of this study compared the modeling of oxidative damage in the Control Region (CR) of the mtDNA genome to the pattern of damage observed when pristine DNA was exposed to the surface of different types of metallic bullet cartridge cases. Previous studies have explored the ability to recover DNA from fired cartridge cases using Short Tandem Repeat (STR) analysis. A challenge has been the ability to effectively recover enough DNA for a partial or complete STR profile. In particular, there has been little success when attempting to recover DNA from copper and brass cartridge cases. One hypothesis is that the DNA is highly damaged due to the oxidative properties of copper. In the laboratory, three types of cartridge cases composed of different metals were analyzed: copper, brass (copper and zinc), and aluminum. Buccal DNA was deposited on the casings through liquid extracts and touch DNA through handling, was recovered with swabs moistened in molecular grade water or 0.5 M EDTA, and DNA extraction was performed using a low copy number approach. Damage to pristine DNA was accomplished through a Fenton reaction (an iron catalyst reacting with hydrogen peroxide to create hydroxyl radicals that inflict damage lesions on the DNA to effectively model oxidative damage). The second part of the study utilized NEBNext® Formalin-Fixed, Paraffin-Embedded (FFPE) DNA Repair Mix to repair the damaged DNA. Following Polymerase Chain Reaction (PCR) amplification and library preparation with the Promega® PowerSeq™ Mito Control Region Nested Kit, Massively Parallel Sequencing (MPS) on the Illumina® MiSeq® FGx benchtop sequencer was used to assess the damage, characterize the lesions, and observe the effects of the DNA repair mix. Donor haplotypes and heteroplasmy status were previously determined for comparison purposes.

A preferred approach was identified for lifting DNA from cartridge cases containing copper. Oxidative damage was characterized through active damage, compared to results for DNA samples recovered from bullet casings and repaired using FFPE DNA Repair Mix. The findings presented should aid the practitioner in developing best practices for collecting DNA evidence from cartridge case evidence, accurately identifying, characterizing, and repairing oxidative damage lesions, and applying the findings when performing mtDNA sequence analysis in forensic casework.

Mitochondrial DNA (mtDNA), DNA Damage/Repair, Massively Parallel Sequencing

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