

## **ASSESSMENT OF OXIDATIVE mtDNA DAMAGE ON BULLET CARTRIDGE CASES: A MODELED APPROACH**

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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. For this study we compared the modeling of oxidative damage in the control region (CR) of the mitochondrial (mt) DNA genome to the pattern of damage observed when pristine DNA was exposed to the surface of different types of metallic bullet cartridge cases. Active damage 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. A massively parallel sequencing (MPS) approach was used to assess the damage and characterize the lesions.

Previous studies have explored the ability to recover DNA from fired cartridge cases using 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 our laboratory, we analyzed three types of cartridge cases composed of different metals: copper, brass (copper and zinc), and aluminum. Buccal DNA was deposited on the casings through liquid extracts and touch DNA through handling. Donor haplotypes and heteroplasmy status were previously determined. The DNA was collected with the double swab method using either molecular grade water or 0.5M EDTA, and DNA extraction was performed using a low copy number approach. Following PCR amplification and library preparation with the Promega PowerSeq™ Mito Control Region Nested Kit, MPS was performed on the Illumina MiSeqFGx benchtop sequencer.

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