

## **RECOVERY OF MTDNA FROM AMMUNITION PROJECTILES AND CASINGS WITH AN ASSESSMENT OF DNA DAMAGE THROUGH MPS ANALYSIS**

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Unfired ammunition and casings from fired ammunition are familiar items found in forensic investigations involving gun crime. In larger jurisdictions, firearm cases represent a relatively high percentage of the overall caseload worked by a law enforcement agency. Nonetheless, few publications have reported on the successful analysis of DNA markers from fired or unfired ammunition, including when performing mitochondrial (mt) DNA sequence analysis. Much of the reason for this relates to the challenges of recovering sufficient amounts of intact DNA from the surface of metallic ammunition, especially those containing copper. Copper ions facilitate oxidative damage to nitrogenous bases and the backbone of DNA, and without an active repair process, the DNA will degrade beyond the point when markers associated with traditional forensic PCR typing systems are available for amplification.

An alternative to traditional DNA typing is massively parallel sequencing (MPS), which has become an accepted approach to performing mtDNA analysis in forensic casework. As MPS-based testing of mtDNA expands to sample types such as touch evidence on ammunition, it will be important to understand how best to recover DNA from metallic surfaces, and how DNA damage may impact the interpretation of low-level heteroplasmy.

The findings presented here include an evaluation of two methods for collection of DNA deposited on the surface of copper, brass, and aluminum ammunition. The recovery of mtDNA was measured using a custom quantitative PCR assay that allows for an assessment of mtDNA degradation. Recovered DNA was also analyzed using a deep coverage MPS (DCMPS) strategy for evaluation of low-level minor sequence variants across the mtDNA control region in an effort to identify sites of DNA damage and their potential impact on interpretation of heteroplasmy. Ammunition components with touch DNA were more likely to give results, especially when collection of the DNA was performed with 0.5M EDTA. As expected, results were limited for copper and brass components when the DNA was deposited in liquid form, as DNA degradation was accelerated when DNA comes in contact with copper or brass surfaces in an aqueous environment. Our findings suggest that forensic practitioners should consider collecting DNA from metallic surfaces with 0.5M EDTA in all cases, as this will maximize yield and mitigate degradation. To our knowledge, this is the first study addressing improved recovery of mtDNA from the surface of ammunition components using a quantitative and DCMPS approach.