WHOLE MITOCHONDRIAL GENOME COVERAGE OF TOUCH DNA RECOVERED FROM SPENT CARTRIDGE CASINGS USING DOUBLE SWAB METHOD WITH COTTON AND FLOCKED SWABS

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DNA recovered from spent cartridge casings is traditionally collected with the double swab method using cotton swabs. However, epithelial cells may be trapped in the matrix of the swab, affecting DNA yield during extraction. Therefore, a comparison of conventional and alternative touch DNA collection methods on spent cartridge casings is needed.

In this study, we compared four touch DNA collection methods: double swab method with cotton swabs, double swab methods with flocked swabs, FTA™ cards, and the soaking method. To simulate touch DNA recovered from spent cartridge casings, each of the five law enforcement volunteers loaded 40 pre-cleaned 9mm Smith and Wesson cartridges and fired. A total of 197 spent cartridge casings were collected and packaged at the scene. All touch DNA samples were extracted and then quantified using a qPCR assay.

Seventy-eight samples yielded detectable nuclear DNA, and all samples yielded mitochondrial DNA (mtDNA) ranging from ~12 copies to ~4.66×10^4 copies. Flocked swabs yielded significantly higher quantities than FTA™ cards and the soaking method. Cotton and flocked swabs yielded the highest mtDNA quantities. Based on nDNA qPCR results, standard STR analysis using capillary electrophoresis would most likely fail. Also, since the DNA is expected to be degraded due to high heat, PCR based methods used for mtDNA sequencing may fail.

To increase chance of sequencing success, we chose flocked and cotton swab samples that yielded >5,000 mtDNA copies and used our custom probe capture assay to capture and sequence the mitochondrial genome. Touch DNA collected using cotton swabs exhibited an average of 93.7% mitochondrial genome coverage with an average coverage of ~5,000 reads per base. Touch DNA collected using flocked swabs exhibited 100% coverage with an average coverage of ~11,000 reads per base.

A subset of the touch DNA samples collected using flocked swabs exhibited 100% coverage of the mitochondrial genome (>500x read depth) with an average coverage ranging from ~16,000 – 64,000 reads. For these samples, the major variants were consistent with each other and the subject's major mtDNA variant sequences. The mtDNA haplogroup was determined to be H7 for all of the major mtDNA sequences for the four touch DNA samples and the reference sample. Based on these preliminary results, we have shown that flocked swabs may be a better alternative to cotton swabs for collecting touch DNA on spent cartridge casings. In addition, this probe capture NGS method can be used to recover and sequence the mitochondrial genome from touch DNA samples collected from spent cartridge casings.