DEMONSTRATION OF A MITOCHONDRIAL DNA-COMPATIBLE WORKFLOW FOR GENETICALLY VARIANT PEPTIDE IDENTIFICATION FROM HUMAN HAIR SAMPLES

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Hair is an evidentiary sample that typically does not provide sufficient nuclear DNA for forensic analysis. Therefore, state-of-the-art forensic analyses for hair samples include subjective microscopic evaluation and, more recently, mitochondrial DNA (mtDNA) analysis. Since many cases involve limited sample amounts (approximately 2 cm or less), any additional destructive analyses (besides mtDNA) would be excluded. However, current research suggests protein sequence variation in hair can be exploited for human identification purposes through imputation of non-synonymous single nucleotide polymorphisms (nsSNPs). These nsSNPs are inferred through proteomic-based identification of genetically variant peptides (GVPs). Thus, if a mtDNA-compatible protein extraction workflow could be developed, GVPs would provide additional forensic value without sacrificing any portion of the original hair sample. Here, we demonstrate an optimized method that can be used to obtain both whole genome mtDNA and putative GVP profiles from a single limited hair sample. The method involves urea-based extraction of proteins from hair, followed by buffer exchange and protease digestion. Peptides are eluted through a 30 kDa membrane and analyzed using traditional proteomic techniques. DNA is subsequently extracted from the filter and analyzed using whole mt-genome analysis. The method was verified with a range of hair sample types (head, pubic, and arm hair) from a diverse cohort of individuals. Further, the utility of the method was verified across two different laboratories. The method is applicable for proteomic-based GVP analysis and mt-genome analysis for forensic research applications.