B47 Dual Separation of DNA and Peptides From Semen

Ashleigh K. Matzoll, University of California, Davis, Davis, CA 95616; Victoria L. Montgomery, BSc, Davis, CA 95616; Zachary C. Goecker, MPS, Visalia, CA; Glendon Parker, PhD, University of California, Davis, Davis, CA 95616*

Learning Overview: After attending this presentation, attendees will be aware of the possibility of using both DNA and proteomics in the analysis of sexual assault kits by separating DNA from trypsin-digested peptides with filtration.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating a potential new tool in the analysis of Sexual Assault Kits (SAKs) if a proteomic compatible SAK workflow is shown to be feasible.

Sexual assault is a significant issue in the United States. Many sexual assault kits collected do not contain quality autosomal DNA profiles sufficient for Combined DNA Index System (CODIS) submission. If genetically variant peptides (GVPs) are found in semen, the information inferred from them in the form of autosomal single nucleotide polymorphisms (SNPs) could be used in conjunction with short tandem repeats on the Y-chromosome (Y-STR) and/or partial autosomal short tandem repeat (STR) profiles incompatible with CODIS to increase the power of discrimination and therefore help identify the perpetrator of a sexual assault. For this process to be useful, the limited amount of sample provided in SAKs needs to be tested for both DNA and peptides, meaning DNA and peptides must be separated from the original sample, instead of sacrificing one for the other, and then analyzed with dedicated workflows for STR-typing (DNA) and tandem mass spectrometry (peptides). Selection of a compatible reagent is imperative, as reagents for DNA and peptide processing may not be compatible with other workflows. One potential approach is to exploit the size difference of digested peptides and intact DNA. For example, mitochondrial DNA has a molecular weight of approximately 10,000,000 daltons, while peptides have a molecular weight of >2,000 daltons. When a peptide mixture is digested with trypsin and then filtered through a molecular weight size-selective filter, DNA can be separated from peptides.[1] Trypsin was used because of its well-defined specificity. In this study, DNA and peptides were separated in one workflow and then mitochondrial DNA was quantified. Tandem mass spectrometry analysis of the peptides determined that a single digestion was sufficient to separate DNA and peptides in semen samples with the fractions suitable for subsequent analyses.

Semen samples from three individuals were incubated in 20 µl of digestion buffer (50mM ammonium bicarbonate, 30 mM dithiothreitol, and dH_2O) at 56°C for 20 minutes. Incubation continued at 37°C for 3 hours after adding 20 µg Sequencing Grade Modified Trypsin (Thermo Pierce) and 0.01% (w/w) ProteaseMAX™ (Promega). Digests were filtered using Amicon® membrane units (Millipore); 100K MWCO. The flow-through (peptide fraction) was incubated with 60 mM iodoacetamide for 60 minutes in the dark at room temperature. For the DNA fraction, 20 µL of dH_2O were added, the membrane unit inverted, and DNA recovered by spinning 3 minutes at 1000 rcf. The final volume was brought up to 200 µL with buffer and dH_2O. Mitochondrial DNA measurements were relative and not conducted using a known standard concentration. Results showed that 22% ± 31% (average ± SD) of DNA was retained and 0.4% ± 0.2% (average ± SD) of the DNA flowed through. Most peptides flowed through, with 25% ± 0.2% (average ± SD) of the peptides retained. Peptides in the filtrate exceeded the peptides found in the original samples, likely due to an inhibitor in the original samples that suppressed fluorescence, but which was removed during processing. In conclusion, the hypothesis that filters can separate out proteomic information from DNA information is viable. It is also of note that very little DNA was able to flow through the filters. However, sample loss is an issue; it is suspected that DNA was embedded in the membrane. Also, better consistency in readings is needed. Current work to resolve these issues include utilizing different membranes, adding salmon sperm DNA for less nonspecific binding, and performing a wash while the filter is upside down to extract embedded DNA. Current work also quantifies DNA using a TaqMan™ assay (Thermo Pierce) along with a standard rather than measuring relative mitochondrial DNA quantities.

Reference(s):

1. Kranes S, Sterling SA, Mason K, Anex D, Hart B, Parker G, Prinz M. Simultaneous DNA and Protein Extraction using Trypsin. *Forensic Science International*: Genetics Supplement Series. 2017;6:e203-e204. Doi: https://doi.org/10.1016/j.fsigss.2017.09.081.

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