

SPERM DNA CAPTURE: A NOVEL APPROACH FOR SEPARATION OF NUCLEIC ACIDS IN MIXED SEXUAL ASSAULT SAMPLES

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Analysis of DNA from sexual assault samples presents unique challenges due to the presence of victim DNA, sample degradation, and trace DNA from the assailant. Conventional analysis of sexual assault samples relies on Differential Extraction that is both laborious and requires intact sperm heads, significantly limiting the time-window in which samples can be collected. Methods to date require intact cells to allow separation of the two DNA fractions. We have developed a novel approach for isolation of sperm DNA from mixed samples based on affinity capture of proteins uniquely associated with sperm DNA. Chromatin-based sperm DNA capture takes advantage of the unique form of chromatin found exclusively in sperm cells. The DNA binding proteins protamine 1 (PRM1) and protamine 2 (PRM2) replace a majority of histones during the haploid phase of spermatogenesis. The PRM1:PRM2:DNA complex is incredibly stable in cell lysates. We have modified techniques used in traditional chromatin immunoprecipitation (ChIP) to target the DNA/protamine complex from solutions derived from cell mixtures. As this process is dependent on a highly specific antibody, we undertook a screening of 10 antibodies using a Biacore based assay to determine binding kinetics for the antibody to DNA:protamine complexes and have down-selected candidate antibodies that show the most promising results. The sensitivity of the capture was routinely demonstrated on as little as 100 input sperm, though the lower limit has yet to be completely defined. Preliminary results demonstrate the feasibility of our method which incorporates rapid sample lysis, novel antibody incubation conditions, and simplified sample purification in a workflow compatible with STR analysis. Adapting conventional ChIP methods for rapid protamine-based sperm chromatin has enabled the streamlining of our method. Chromatin-based sperm DNA capture has the potential to enable successful processing of sexual assault samples that are classically refractive to analysis, such as aged/lysed samples. Sperm DNA Capture has eliminated many of the manual steps inherent in Differential Extraction, and can be fully automated. Current experiments are focused on recovery of sperm DNA from post-coital swabs of delayed collection time points ranging from immediately to nine days post-coitus. Results suggest Sperm DNA Capture is a more highly sensitive and specific method for separation of sperm DNA from a mixture than Differential Extraction. Method development is ongoing under NIJ award 2014-DN-BX-K017.