PREPARATION OF HIGH QUALITY, PCR-READY DNA FROM SEMEN AND MOCK SEXUAL ASSAULT SAMPLES USING AN ENZYMATIC PREPARATION METHOD

Jenny A. Lounsbury, MSFS[1], Jessica L. M. Mackness[1], Paul Kinnon[2], David J. Saul, PhD[2], and James P. Landers, PhD[1,3,4]
[1]Department of Chemistry, University of Virginia Charlottesville, VA 22904
[2]ZyGEM Corporation, Hamilton, New Zealand
[3]Department of Pathology, University of Virginia, Charlottesville, VA 22904
[4]Department of Mechanical Engineering, University of Virginia, Charlottesville, VA 22904

DNA analysis of mixed biological samples is especially difficult; however, in the special case of sexual assault samples, the differences between sperm and epithelial cells can be exploited to separate the victim's and perpetrator's DNA. Differential extraction (DE) is the most commonly used method for the analysis of these types of samples, utilizing an anionic detergent and proteinase K to selectively lyse the epithelial cells. The sperm are then centrifuged, the pellet washed to remove excess female DNA, and the sperm lysed through the addition of a reducing agent, such as DTT (dithiotheritol). Both lysed samples (female and male DNA) must still undergo solid phase extraction to purify the DNA from protein, nucleases, and other compounds that can inhibit the polymerase chain reaction (PCR). All of the steps involved in this process increase analysis time and reduce the capacity for automation.

Typically, the samples are purified using a silica-based solid phase extraction (SPE) method, where DNA binding is achieved under high salt conditions while impurities are washed away. These extraction methods can have a number of drawbacks including multiple sample transfer steps, which may result in DNA template loss and reduce the probability of obtaining a full STR profile, and a lengthy analysis time of up to 4 hours to extract DNA from semen[1]. Finally, the chaotropic reagents used to load the DNA on the solid phase and the organic reagents used to wash away the impurities are known PCR inhibitors[2].

These issues that burden SPE methods can be nearly eliminated by transitioning to a method that requires no solid phase. Liquid DNA preparation has been used in the past in the form of a phenol-chloroform extraction, where proteins and lipids move to the organic phase and DNA to the aqueous phase; however, this method is time-consuming and hazardous and exposes to the user to hazardous chemicals. Recently, a liquid DNA preparation procedure has been developed that uses a thermally-stable neutral protease to degrade cellular membranes, proteins and nucleases[3]. The enzyme used (*Bacillus* sp. EA1) has optimal activity in PCR-compatible buffers, reducing the chance for PCR inhibition. This enzyme-based DNA preparation method reduces the amount of sample transfer steps and, therefore, nearly eliminates any loss of sample that may occur, while yielding PCR-ready DNA after only 20 minutes. Commercially-available kits using this enzyme have been developed and applied to whole blood and saliva samples, but to date no kits for semen sample analysis have been shown, primarily due to the incompatibility of the enzyme with most reducing agents.

The current work focuses on the development of a procedure for the liquid preparation of DNA from sperm cells. First, sperm cells are lysed during a brief incubation, a fraction of the time required by DE, containing an alternative reducing agent. An aliquot of the lysate is then added to a solution containing buffer and enzyme and the sample is incubated to prepare DNA for PCR in just 25 minutes. Using this method, DNA yield from sperm cells is significantly increased as compared to a traditional SPE method. Amplification results of genomic DNA from purified sperm cells show a full STR profile using this method. In addition, application of the method to mock sexual assault samples[4] will be demonstrated.

Overall, this method represents a ~ 3-fold reduction in average DNA preparation time, from 1.5 hours to 25 minutes as compared to conventional SPE methodologies.

References:

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