The Development and Optimization of a Direct Lysis Differential Extraction Process

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In the first published article on the forensic application of deoxyribonucleic acid (DNA) analysis, the authors demonstrated the ability to process sexual assault samples—samples that oftentimes contain a mixture of sperm and epithelial cells (E-cells). This method, commonly referred to as the Gill method or differential extraction, preferentially lyses E-cells while leaving sperm DNA out of solution. The sample is then centrifuged to pellet the sperm cells and the E-cell DNA is removed. The sperm pellet is subsequently washed several times to minimize residual E-cell DNA. These steps are followed by sperm lysis. Since its introduction, this method has been extensively used in the processing of sexual assault samples, relatively unmodified.

While DNA extraction and analysis of forensic samples have been improved significantly throughout the years, the cell separation portion of the Gill method remains a labor intensive process. Additionally, the use of detergents (usually SDS) and Proteinase K (PK) to digest cellular proteins and cause cell lysis requires a subsequent DNA purification step. The purification step is associated with varying degrees of DNA loss and additional processing time, but provides a product that is largely free of inhibitors and is amenable to subsequent PCR processing steps.

Various direct lysis methods have been developed that allow for the processing of DNA without a subsequent purification step. To facilitate the use of direct lysis in differential extraction, we have combined the use of the thermophilic EA1 protease and Acrosolv (both from MicroGEM), and a non-specific endonuclease to create a multi-enzyme approach for differential extraction which requires only a single centrifugation and no subsequent wash steps. After the single centrifugation step, all remaining steps for both the E-cell and sperm cell fractions occur in a thermal cycler. The process produces an epithelial cell lysate and a sperm cell lysate both of which require no downstream DNA purification and can, in most cases, be taken directly to quantification and STR amplification. Data showing results from design experiments and mock sexual assault samples will be presented.