Purification of Sperm DNA from Vaginal Swabs using the *Erase* Sperm Isolation Kit

Christian Carson[1], Alex Garvin[1], Michelle Beckwith[1], Donna Becherer[2] and Kim Gorman[1]
[1] Paternity Testing Corporation, Columbia, MO; [2] St. Louis Metropolitan Police Department, St. Louis, MO

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Results

Major contributor profiles from DNA isolated from a mock sexual assault swab using the *Erase* protocol and amplified using PowerPlex16 are easily obtained and can be observed in Figure 1. The sperm fraction DNA profile is consistent with the male donor on the swab and the non sperm fraction profile is consistent with the female donor on the swab.

Figure 1: Sperm and Non Sperm Fraction Amplification
Sedgwick County Crime Laboratory

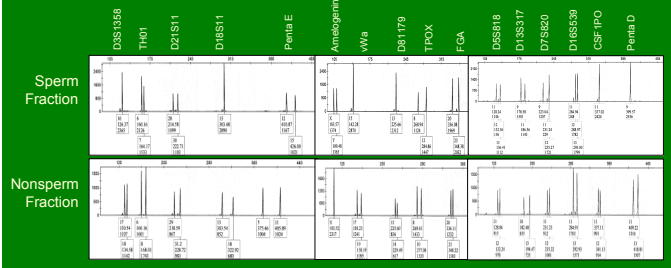
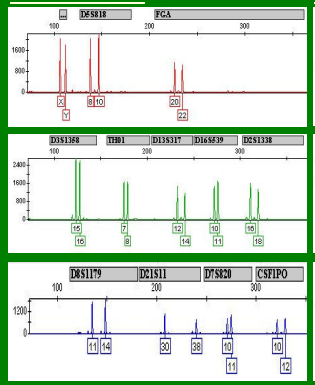


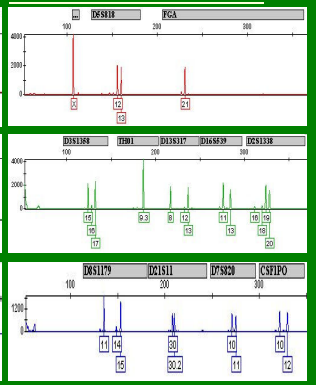
Figure 2: Erase vs. Standard Method on a Difficult Sample
St. Louis County Crime Laboratory

The sperm fraction of a sexual assault sample was processed by a crime laboratory using a standard differential extraction method. The profile obtained by the crime laboratory was female with a trace of male contributor. Subsequent attempts by the laboratory produced the same result. The sample was sent to PTC for processing with *Erase* and then the extract was returned to the crime laboratory. A full, mixture free male profile was obtained by the crime laboratory using the *Erase* extract.

Erase Profile



Standard Method Profile



DNA Purification

The nuclease differential extraction method prepares non-sperm and sperm fractions that can easily be purified using the laboratories preferred DNA purification method. Organic extraction followed by either ethanol precipitation or centrifugation filtration spin columns (microcon and vivacon), Qiagen EZ1 and Maxwell 16 are examples of methods that may be used with the *Erase* extraction kit.

Availability

The *Erase* Sperm Isolation Kit is now available. For questions or comments email erase@ptclabs.com or visit www.ptclabs.com/erase.

Acknowledgements

Special thanks to NIJ, Kathy Press (AZ DPS), Sedgwick County Crime Laboratory, St. Louis County Crime Laboratory, and all of the staff at PTC Labs.

Figure 3: Erase Differential Extraction Procedure

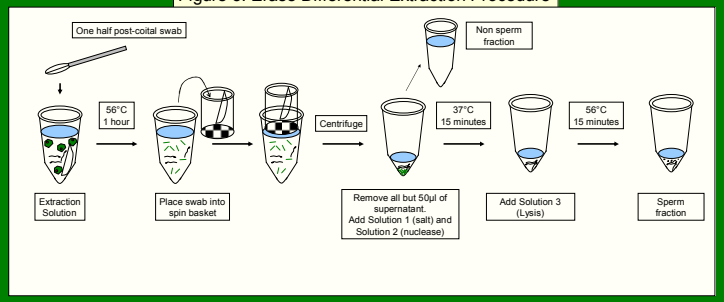
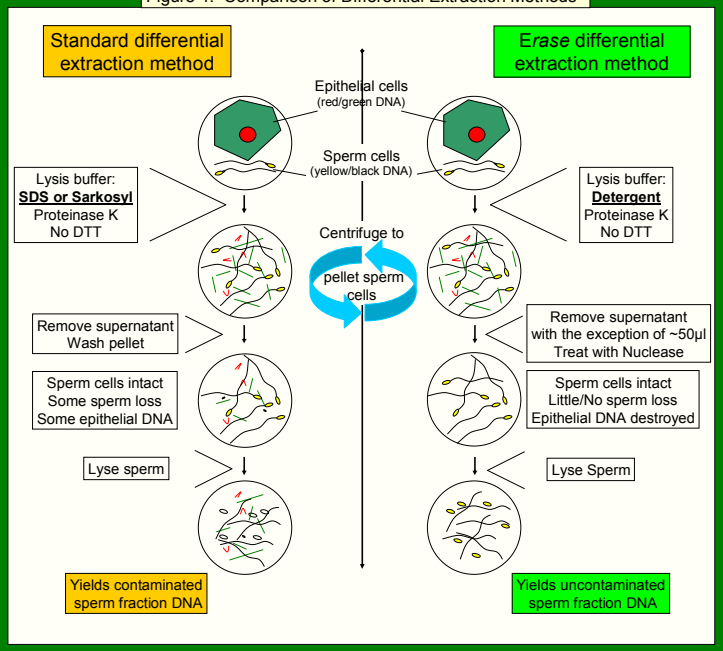


Figure 4: Comparison of Differential Extraction Methods



Automation

The *Erase* nuclease method is being validated in a 96 well automated format using the Slicprep 96 Device from Promega. An entire plate of samples can be differentially extracted with little hands on time in a few hours and ready for automated DNA purification with fewer controls to analyze.

Methodology

Time constraints and cost often limit efforts to analyze unknown subject sexual assault cases. However, with the availability of the Combined DNA Index System (CODIS) and the National DNA Index System (NDIS) to search for offender profiles, processing unknown subject cases is much more likely to result in identifying the suspect than it was previously. Automated methods of differential extraction are necessary to reduce the backlog and process unknown subject cases.

Processing differential extractions using traditional methods is time consuming and often does not achieve total separation of epithelial and sperm cells. Traditional differential extractions are very difficult or impossible to automate. This method improves upon the methodologies used for differentially separating sperm DNA from victim DNA and is amenable to automation. A nuclease is used to selectively destroy contaminating epithelial cell DNA before the sperm cells are lysed. Epithelial DNA contamination is effectively eliminated. Because the nuclease will only degrade DNA in solution, it does not destroy the DNA contained within the intact sperm cells (Figure 5). The *Erase Sperm Isolation Kit* for differential extraction utilizes selective degradation to produce single source male profile from sexual assault evidence.

Figure 5: DNA Yield of Nuclease Treated Samples

Cell Type	Nuclease Treated	DNA Yield (pg)	DNA Reduction when Treated with Nuclease
Buccal Swab Epithelial Cells	No	140,000	99.98%
	Yes	30	
12,000 Sperm Cells	No	16,800	7%*
	Yes	15,600	

*DNA reduction is due in part to loss of male epithelial cell DNA in semen.

Differential extraction using Standard Method

The Standard differential extraction method was first devised by Gill, Jeffreys and Werrett in *Nature*, 1985.

1) A sample of sexual assault evidence including sperm is added to a solution of detergent (typically SDS or Sarkosyl) and PK to lysis the epithelial cells without lysing the sperm cells.
2) The sample is centrifuged to pellet intact sperm cells and the supernatant with non-sperm DNA, is carefully removed by manual pipette and saved for the non sperm DNA fraction.
3) The pellet is subjected to additional washes using buffer to dilute excess epithelial cell DNA.
4) Lysis buffer with dithiothreitol (DTT) releases the DNA from the sperm cells to obtain the sperm DNA fraction.

Sperm loss and incomplete separation of the epithelial cells and sperm cells are often encountered using this method. Successful separation of cells types of can vary greatly based on the skills and experience of the analyst and cannot be easily automated.

Differential extraction using the *Erase* Sperm Isolation Kit

Garvin, A., Bottinelli, M., Gola, M., Conti, A., Soldati, B. Journal of Forensic Sciences; Nov 2009, Vol. 54 Issue 6, p1297-1303

1) A sample of sexual assault evidence including sperm is added to a solution of a detergent and PK to lysis the epithelial cells without lysing the sperm cells. The sample is incubated at 56°C for 1 hour.
2) The sample is centrifuged to pellet intact sperm cells and ~85% of the supernatant is removed without disturbing the sperm pellet by manual or automated pipette and saved for the non sperm DNA fraction.
3) To the remaining ~50µl of sample, 10µl of **Solution 1** ($MgCl_2$ and $CaCl_2$) is added and mixed followed by adding 10µl of **Solution 2** (Nuclease). The sample is then incubated at 37°C for 15 minutes.
4) Lastly, 10µl of **Solution 3** (EDTA and DTT) is added and incubated at 56°C for 15 minutes. EDTA stops nuclease activity and DTT releases the DNA from the sperm cells to obtain the sperm DNA fraction.

Because the epithelial DNA in the sperm fraction is destroyed rather than physically separated, it is possible to obtain a major contributor sperm profile even when the ratio of sperm cells to epithelial cells is low. Although there can still be some variation of success based on the skills of the analyst, the impact is reduced and this method can be automated.