DE-ICE: Differential Extraction Improved by a Cold adapted Enzyme (HL-dsDNase)

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Background

Case-work of sexual assaults samples is of high priority for forensic laboratories. Samples containing a mixture of sperm cells and non-sperm cells are usually processed by differential DNA extraction (DE). The aim of DE is to separate the DNA into two fractions: a sperm DNA fraction and a non-sperm DNA fraction (epithelial cell DNA fraction) before STR-typing. However, obtaining a complete DNA profile from the sperm cells often represents a significant challenge, especially for samples containing few sperm cells relative to non-sperm cells.

Most DE protocols involve repeated washing steps to physically remove contaminating non-sperm DNA from the sperm cells. As an alternative approach, Garvin *et al* (2009) have demonstrated that contaminating DNA can be removed by DNAse I digestion prior to sperm cell lysis. However, DNase I is a relatively robust enzyme and permanent inactivation of this enzyme may be challenging (Silkie *et al*, 2008). Incomplete inactivation or reactivation of the DNase I after sperm cell lysis will have dramatic negative effects on the downstream STR-typing of the sperm cells.

We here present the use of a novel DNase, HL-dsDNase™ (ArcticZymes), for removing non-sperm DNA in DE procedures. The procedure takes advantage of the unique heat labile nature of the HL-dsDNase™ (Figure 1). We have named this approach DE-ICE (Differential Extraction Improved by a Cold adapted Enzyme).

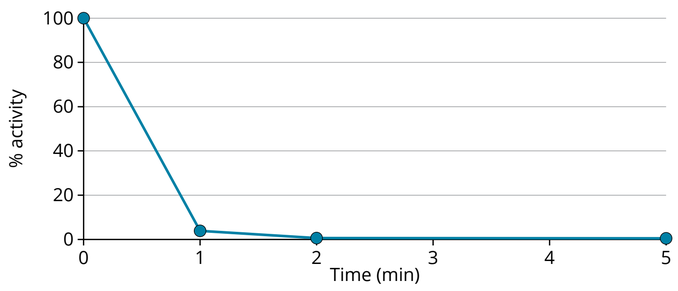


Figure 1. Inactivation of HL-dsDNase at 55°C. Then remaining activity was measured using a modified Kunitz assay. From Nilsen *et al* (2010).

Samples

Vaginal samples were collected on cotton swabs and dried. Sperm cell samples were counted in a haemocytometer and serial diluted in PBS before seeded on to cotton swabs. All samples were provided by healthy volunteers.

Methods

DNA extraction

The cotton swabs were first incubated at 56°C for 1h in 500 µl of cell lysis buffer consisting of TE-buffer w/Proteinase K and either 2 % Triton (DE-ICE), 0.5 % SDS ("wash"). Next, the samples were centrifuged in QIAshredder spin columns (Qiagen) to remove the cotton fabric and pellet the sperm cells. The principle of the DE-ICE and the "wash" method is outlined in figure 2. Finally, DNA isolation was performed on the AutoMate Express™ according to the supplier's protocol using the PrepFiler Express™ kit (Thermo Fisher Scientific) with some modifications. Of special note is that for the sperm fraction, 100 µl of sperm cells were mixed with 400 µl Prepfiler lysis buffer. DE with the Investigator kit (Qiagen) was performed according to the manufacturer's instructions and using ATL lysis buffer.

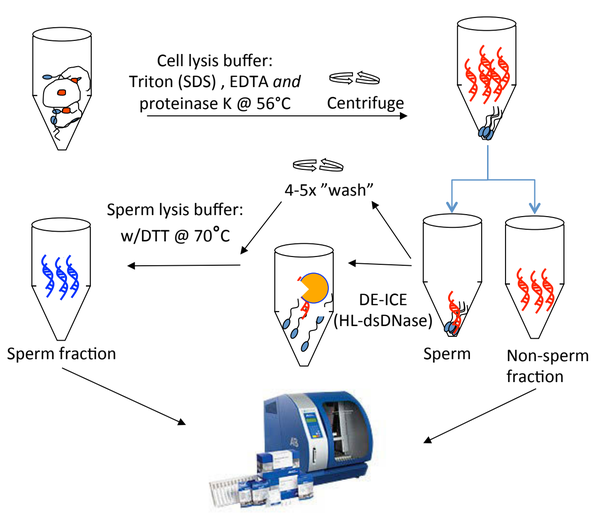


Figure 2. The principle of the DE-ICE method. Remaining DNA from non-sperm cells is digested by the HL-dsDNase. The need for repeated washing of sperm is hence circumvented.

DNA Quantification and STR-typing

DNA was quantified by the Quantifiler Duo or Quantifiler Trio kits (Thermo Fisher Scientific). STR-PCR was performed with the NGM SElect™ (Thermo Fisher Scientific) and PCR products analysed on a 3500xL Genetic Analyzer (Thermo Fisher Scientific). The resulting data were processed using GeneMapper ID-X software (Thermo Fisher Scientific) with peak amplitude thresholds set to 95 rfu (Vic), 115 rfu (Fam), 145 rfu (NED), 150 rfu (PET).

Results

To evaluate the release of sperm cells and the efficiency of DNA extraction from cotton swabs, approximately 1000 sperm cells were seeded on cotton swabs and subjected to DE with either the DE-ICE protocol, the "wash" protocol, or the Qiagen investigator protocol (Figure 3). As a reference, total DNA extraction was performed following the direct PrepFiler Express protocol. Quantification of the samples revealed no significant differences in DNA-yield from the DE protocols. For all three DE-protocols the yield of DNA was in the range of 32-40 % compared the total DNA yield.

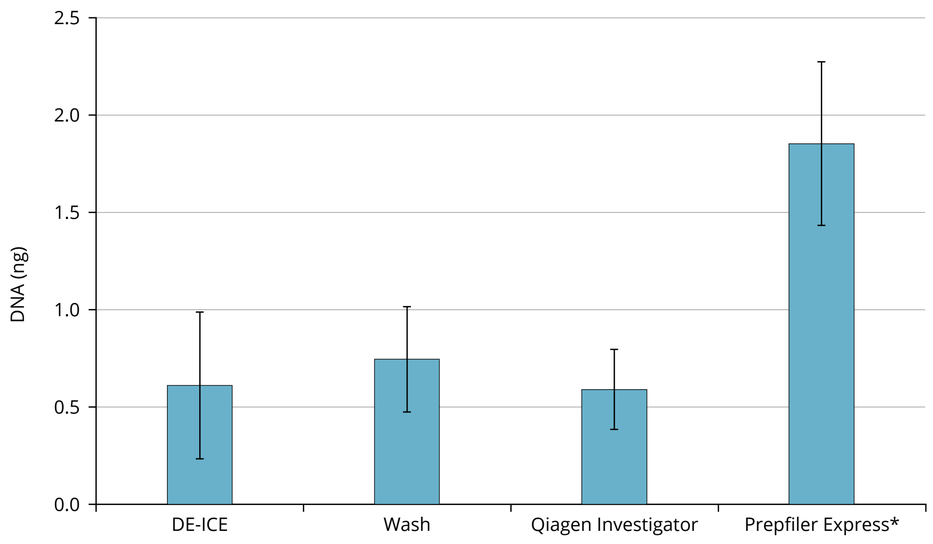


Figure 3. Total DNA yield from cotton swabs seeded with 1000 sperm cells and subjected to differential extraction by either DE-ICE, "wash" or Qiagen Investigator protocol. *For the Prepfiler Express protocol direct DNA extraction was performed (not DE).

Results...

The effect of DE-ICE treatment compared to washing was assessed on simulated sexual assault samples. Vaginal cotton samples from 4 different donors (Donors 1- 4) were seeded with approx 1000 sperm cells (same donor) and subjected to DE using the DE-ICE and a "wash" procedure respectively (Figure 4). For the samples treated with the "wash" procedure, the amount of male DNA range from 0.04-0.34 ng and the amount of total DNA ranged from 2.25-18.86 ng. In contrast DE-ICE treated samples contained significantly more male DNA (0.18-0.63 ng) and less total DNA (0.26-0.63 ng). The improved effect of the DE-ICE method became clearly evident on the STR-profiles of the same samples (Figure 5). The fraction of drop-out male alleles was 72.37% for the wash method. Only 5.26% of the male alleles were not detected for the DE-ICE method (see table insert in Figure 5). For two of the four DE-ICE samples no drop-out of sperm alleles were detected, whereas all four samples subjected to the " wash" methods showed a relatively high number of drop-out of sperm alleles. It is of note that these samples simulate sexual assault samples containing very low amounts of sperm cells relative to vaginal cells, hence representing challenging samples.

The efficiency of DE-ICE procedure has also been evaluated on post-coital samples. Figure 6 shows a representative STR-profile of a vaginal sample taken 48 h after consensual sexual intercourse. Although further validation of the DE-ICE method is warranted, the superior quality of the STR-profiles in such samples indicates that the method holds great promise for improving case-work of sexual assault samples.

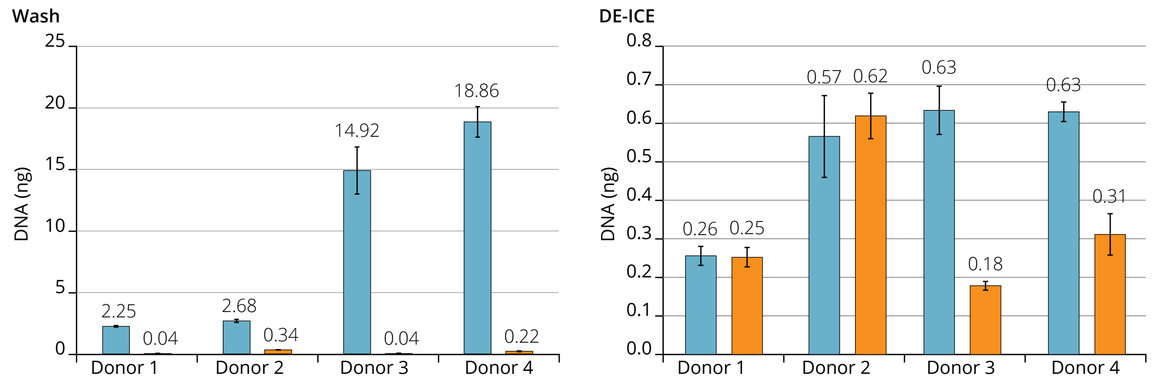
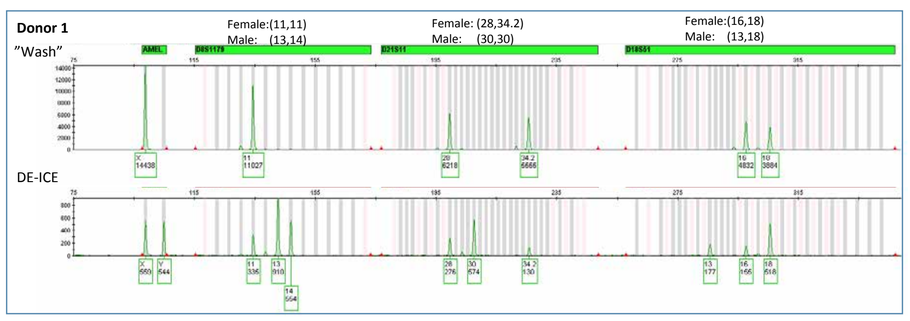
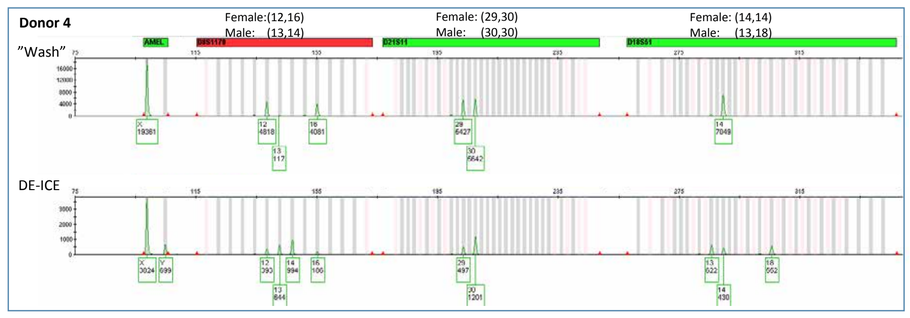


Figure 4: DNA yield from vaginal swabs from 4 donors seeded with approx 1000 sperm cells and subjected to DE by "wash" method (left panel) or the DE-ICE method (right panel). The swabs were split in two before adding sperm cells. DNA was quantified by the Quantifiler Trio kit. Total Male





	"Drop-out" of male alleles	
	Wash	DE-ICE
Donor 1	17 (20)	1 (20)
Donor 2	3 (17)	0 (17)
Donor 3	19 (20)	3 (20)
Donor 4	16 (19)	0 (19)
Total	55 (76)	4 (76)

Figure 5: STR-profiles of the samples from Figure 4 (only Donor 1 and Donor 4 shown). The table shows the number of drop-outs of male alleles relative to number of unique male alleles for each sample (parentheses).

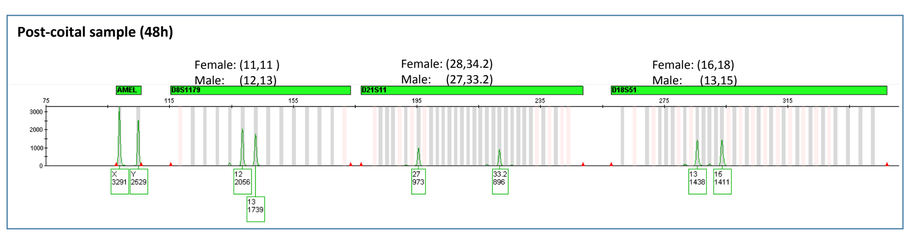


Figure 6: STR-profile of a vaginal sample taken 48 h after consensual sexual intercourse and processed by the DE-ICE protocol. Only male alleles are detected.

Conclusions

The DE-ICE protocol, using a heat labile HL-dsDNase™ (ArcticZymes), efficiently removes non-sperm DNA during the differential DNA extraction procedure. The advantage of the HL-dsDNase™ over other commercial available DNases is that is irreversibly inactivated by moderate heat treatment. Hence, HL-dsDNase™ minimizes the potential risk of degrading the sperm cell DNA.

For challenging samples containing a high fraction of non-sperm cells compared to sperm cells, the DE-ICE protocol resulted in superior STR-profiles compared to wash-based protocols.

The DE-ICE method is rapid and reduces the hands-on time for differential DNA extraction. DE-ICE is flexible with respect to choice of DNA extraction platforms and compatible with semi-automated DNA extraction systems such as the AutoMate Express™ Forensic DNA Extraction System (Thermo Fisher Scientific).

Refrences

Garvin AM *et al* (2009) DNA preparation from sexual assault cases by selective degradation of contaminating DNA from the victim. *J Forensic Sci.* 2009 Nov;54(6):1297-303.

Nilsen IW *et al* (2010) The enzyme and the cDNA sequence of a thermolabile and double-strand specific DNase from Northern shrimps (*Pandalus borealis*). *PLoS One.* 22;5(4):e10295

Silkie *et al* (2008) Reagent decontamination to eliminate false-positives in *Escherichia coli* qPCR. *J Microbiol Methods*; 72(3):275-82.

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