



Research paper

DNA profiling of sperm cells by using micromanipulation and whole genome amplification

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ABSTRACT

The differential extraction method is based on separate lysis of vaginal cells and spermatozoa and was designed to prevent mixed DNA profiles from intimate swabs. However, DNA from the victim can still be present in the sperm fraction, and the suspect's DNA cannot be identified when only minute amounts of spermatozoa are present. Moreover, differential extraction is not effective when swabs contain sperms from more than one individual. Mixed profiles could ideally be overcome by analysing single spermatozoa. However, current multiplex STR kits are not yet sensitive enough to generate DNA profiles from single cells.

The aim of this study was to develop a method that enables DNA profiling of up to a single sperm cell. Spermatozoa were isolated through micromanipulation. Spermatozoa were lysed and their DNA was pre-amplified by whole genome amplification (WGA) to generate sufficient template for PCR. To these ends, several WGA methods were first tested on different amounts of genomic DNA (gDNA) and assessed for allele recovery, allele drop-out (ADO) and allele drop-in (ADI). The best WGA method was selected for use on cell material.

The REPLI-g method turned out as the only protocol increasing the sensitivity of DNA profiling. Results of WGA performance on gDNA as well as multiple and single cells will be presented.

1. Introduction

Mixed DNA profiles often hamper the clarification of sexual assault cases. Such mixtures could ideally be overcome by analysing single spermatozoa. However, the DNA quantity of single cells is insufficient for conventional STR analysis. Whole genome amplification (WGA) protocols represent promising methods to pre-amplify the extracted DNA to generate sufficient template for a forensic multiplex PCR.

The performance of different WGA methods in forensic contexts has been reported by several studies [1]. WGA has been performed on both gDNA and single cells [2]. Though, no study so far reported the application of WGA on single sperm cells in combination with forensic multiplex STR kits. Moreover, the performance of recently commercialized, highly sensitive WGA kits dedicated for single cell analysis has not yet been compared with previous protocols.

The aim of this study was to develop a protocol for forensic STR profiling of single sperm cells based on WGA. We first assessed various WGA methods for allele recovery, ADO and ADI. The best WGA protocol was then tested on isolated cells.

2. Materials and methods

2.1. DNA and cell material

Purified gDNA from HeLa cells was purchased from NEB (Germany). Buccal cells were donated by a mouth swab from one person who gave his consent. To release buccal cells for micromanipulation, fresh mouth swabs were incubated in 500 µl DNA-free water at 37 °C for 30 min. Sperm cells were donated by the same person. In forensic samples spermatozoa are often present without their tail. Therefore, fresh semen was dried on a mouth swab for 48 h for the spermatozoa to shed their tails. Swabs with dried semen were incubated in the same way as the swabs with buccal cells to release the spermatozoa.

2.2. WGA

WGA methods were tested with 1 ng, 100 pg, or 30 pg of gDNA. The modified improved primer extension pre-amplification (mi-PEP) was performed as described [1]. TruePrime single cell WGA kit (Sygnis GmbH, Germany), MALBAC single cell WGA kit (Yikon Genomics,

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China), illustra Single Cell GenomiPhi DNA Amplification Kit (GE Healthcare, UK), GenomePlex® Single Cell Whole Genome Amplification Kit (Sigma-Aldrich, USA) and REPLI-g single cell WGA kit (Qiagen, Germany) where used following the manufacturers' protocols. WGA products were cleaned up with innuPREP PCRpure Kit (Analytik Jena, Germany).

2.3. Micromanipulation of cells

Buccal cells were isolated using glass micropipettes (BioMedical Instruments, Germany) with an opening diameter of 63 µm, Transferman micromanipulation system and CellTram™ Vario microinjector (Eppendorf, Germany). Capillary and microinjection tube were filled with liquid paraffin oil (Sigma-Aldrich, USA). To identify spermatozoa without tails, cells were stained with the SPERM HY-LITER™ PI kit (Independent Forensics, USA) and analysed using an Axiovert S100 inverted fluorescent microscope (Zeiss, Germany). For isolation of single spermatozoa, glass micropipettes with an opening diameter of 6 µm were used. Isolated cells were transferred to 200 µl PCR tubes and lysed according to the REPLI-g protocol for single cells.

2.4. Amplification of STRs

16 autosomal STRs and Amelogenin were amplified using MPX-5 ESS Multiplex PCR kit (Serac GmbH, Germany) or PowerPlex ESX 17 (Promega, Germany). 1 µl of purified WGA product (diluted 1:50) was used as template. Reactions were carried out in 5 µl reaction volumes using a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, USA).

2.5. Fragment analysis

1 µl PCR product was analyzed by capillary electrophoresis on an

ABI PRISM® 310 Genetic Analyzer using GeneMapper® v3.1 software (Applied Biosystems, USA).

3. Results

With the highest allele recovery and no ADI, REPLI-g turned out as the best performing WGA method and markedly improved allele recovery over non-pre-amplified DNA ("No WGA") (Table 1).

As shown in Table 2, Repli-g performed better on buccal than on sperm cells, respectively. From single spermatozoa, allele recovery was unsatisfactory, and in one sperm an ADI was observed. In buccal cells, low peak-height ratios within individual STR loci were observed.

4. Discussion

Apart from Repli-g, all WGA methods tested were inferior to non-pre-amplified DNA. The ADIs and stutter peaks observed after MALBAC might be due to the *Bst* polymerase which is prone to allele slippage [3].

Surprisingly, REPLI-g performed better on 3 buccal cells than on 6 sperm cells (containing the equivalent amount of gDNA), pointing towards a less efficient DNA extraction from the latter cell type and subsequent stochastic effects. ADOs might in addition result from amplification bias which might also explain the low peak height ratios in the buccal cell-derived DNA.

5. Conclusion

The REPLI-g WGA method increases the sensitivity of forensic DNA profiling. However, DNA extraction and WGA still require optimization to generate a more uniform amplification and improved sensitivity to the level of a single sperm cell.

Table 1
Performance of WGA methods on low template DNA.

WGA method	Template DNA (pg)	Allele recovery (%)	ADO (Number)	ADI (Number)
mi-PEP	1000	100	0	0
	100	N/A	N/A	N/A
	30	21	27	4
TruePrime	1000	88	4	0
	100	N/A	N/A	N/A
	30	0	34	0
MALBAC	1000	53	16	4
	100	N/A	N/A	N/A
	30	N/A	N/A	N/A
GenomiPhi	1000	93	3	0
	100	93	3	0
	30	30	24	0
GenomePlex	1000	89	4	0
	100	65	12	3
	30	24	26	0
REPLI-g	1000	100	0	0
	100	100	0	0
	30	59	14	0
No WGA	1000	100	0	0
	100	100	0	0
	30	38	12	0

Table 2
Performance of REPLI-g on isolated cells.

Sample (3 independent experiments each)	Allele recovery (± SD)	Peak height ratio (± SD)	Allele Drop Ins (± SD)
3 buccal cells	85% (± 24%)	39% (± 5%)	0
6 sperm cell	52% (± 26%)	N/A	0
1 sperm cell	10% (± 15%)	N/A	0,33 (± 0,58)

Conflict of interest

None.

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