

SINGLE CELL
ANALYSIS

Low-volume on-chip single sperm cell analysis

I. E. Pickrahn^{1,2}, G. Schmidt-Gann¹, T. Kroneis^{1,3,4}¹Institute of Forensic Medicine, Medical University, Graz, Austria²University of Salzburg, Salzburg, Austria³Institute of Cell Biology, Histology & Embryology, Medical University, Graz, Austria⁴Research Unit for Single Cell Analysis, Medical University, Graz, Austria

Differential extraction is the preferential method to isolate sperm cell DNA from vaginal swabs for subsequent DNA typing in sexual assault investigation to generate the perpetrator's DNA profile. However, the use of DNA isolated via differential extraction for DNA typing shows two major limitations. First, DNA typing results in mixed DNA profiles as sperm cell DNA fractions are contaminated by DNA derived from the victim, and hence, cause a decrease in significance. Second, the total number of sperm cells decreases with time since intercourse which might cause the perpetrator's DNA to drop far below the contaminating victims DNA, thereby escaping from being detected.

To overcome the limitations resulting from mixed DNA samples and low numbers of sperm cells we applied low-volume on-chip DNA typing to single sperm cells and compared the data to the current gold standard technique of differential extraction.

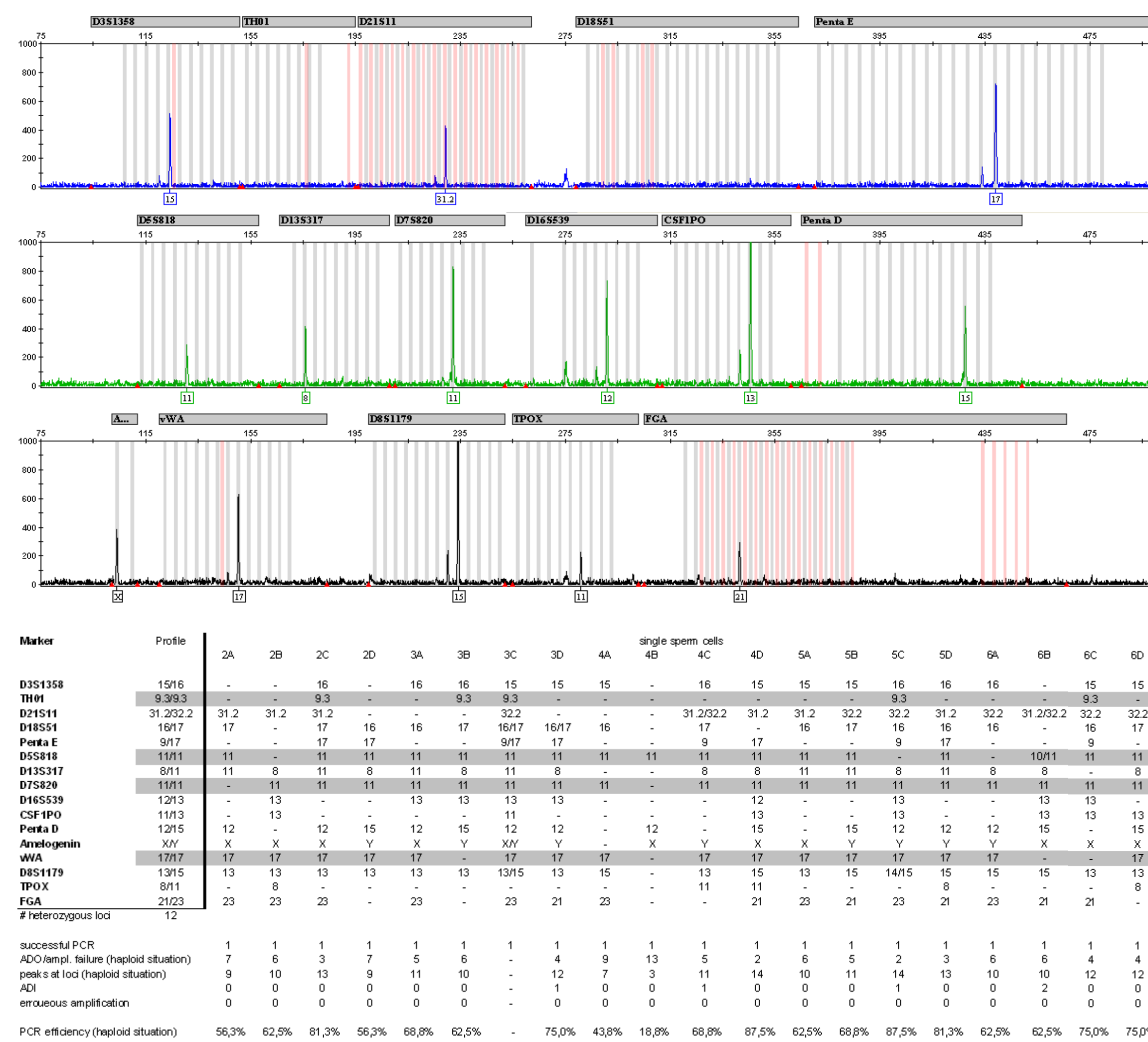
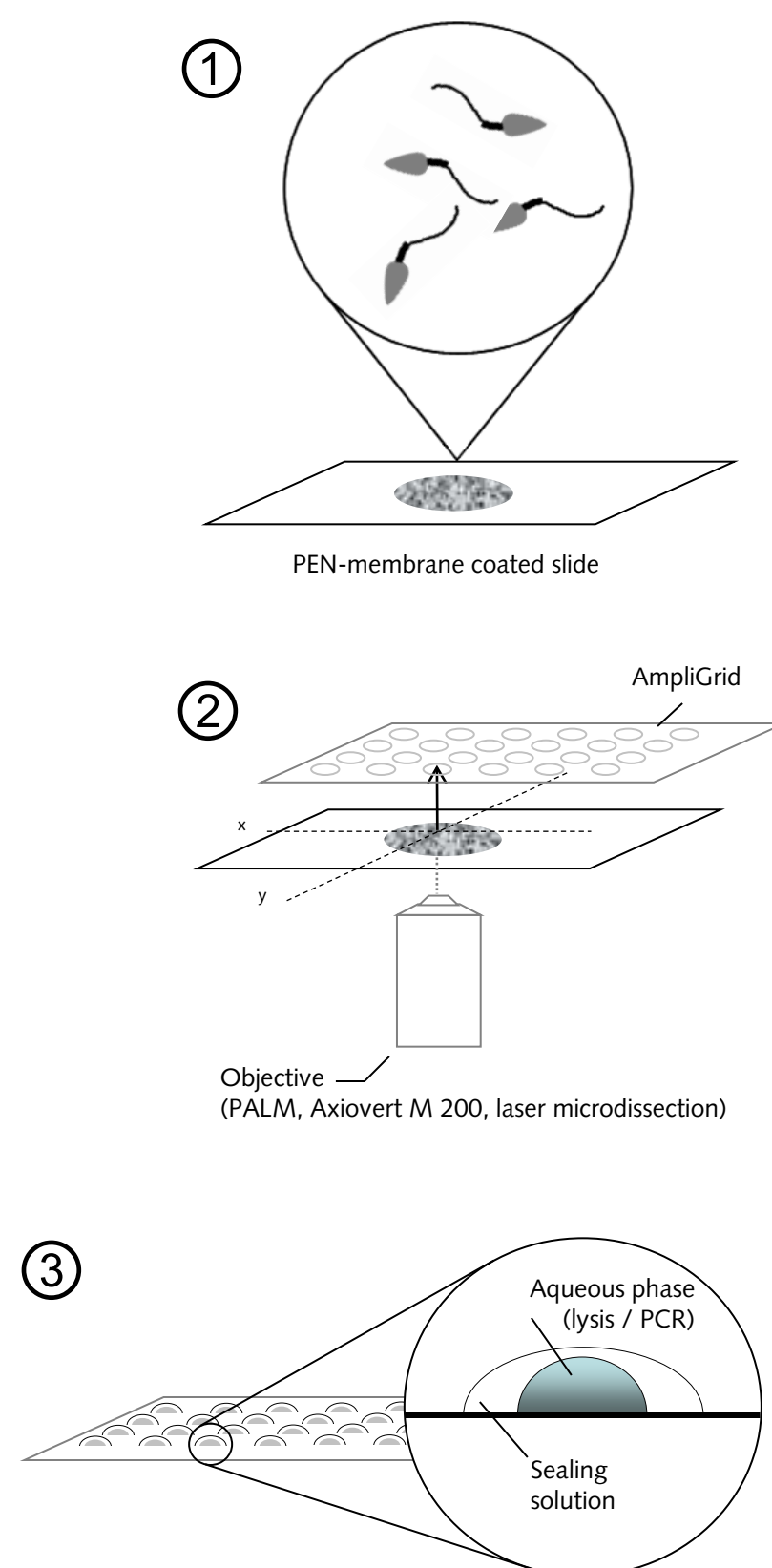
Single sperm cell analysis

(1) Sperm cell suspension obtained from healthy individuals were cytocentrifuged onto PEN-membrane coated slides and allowed to air-dry.

(2) Individual sperm cells were laser micro-dissected onto chips (2) capable of ultra low volume amplification (AmpliGrid AG480F, Advalytix).

(3) Single sperm cells lysis was performed in a volume of 0.75 µl (Cell Extraction Kit, Advalytix, containing 5 mM DTT) at 65°C for 2 h followed by an inactivation step at 95°C for 10 min. Lysis solutions were covered with 5 µl of sealing solution to avoid sample evaporation. For multiplex PCR 0.75 µl of a 2x PowerPlex 16 master mix (Promega) was added to the single sperm cells. Low-volume on-chip PCR was performed on a slide cycler (AmpliSpeed, Advalytix) according to the manufacturer's recommendations.

Amplification products were purified (Wizard, Promega) and run on an ABI 3730 DNA analyzer.

SINGLE CELL
ANALYSIS

Results

Single sperm cell analysis

Analysis of single sperm cells was successful in 39 of 40 (97.5%) samples with DNA profiles (plot) showing PCR products at minimum 3 to maximum 14 of 16 loci.

Mean PCR efficiency calculated from single sperm cell profiles (table) was 62.8% (18.8 - 87.5%, median: 62.5%).

Allele drop-out and allele drop-in (table, 4C) was detected in 37.2% and 2.2%, respectively.

One sample (table, 3C) was detected to consist of at least two cells due to 4 loci yielding heterozygous signals.

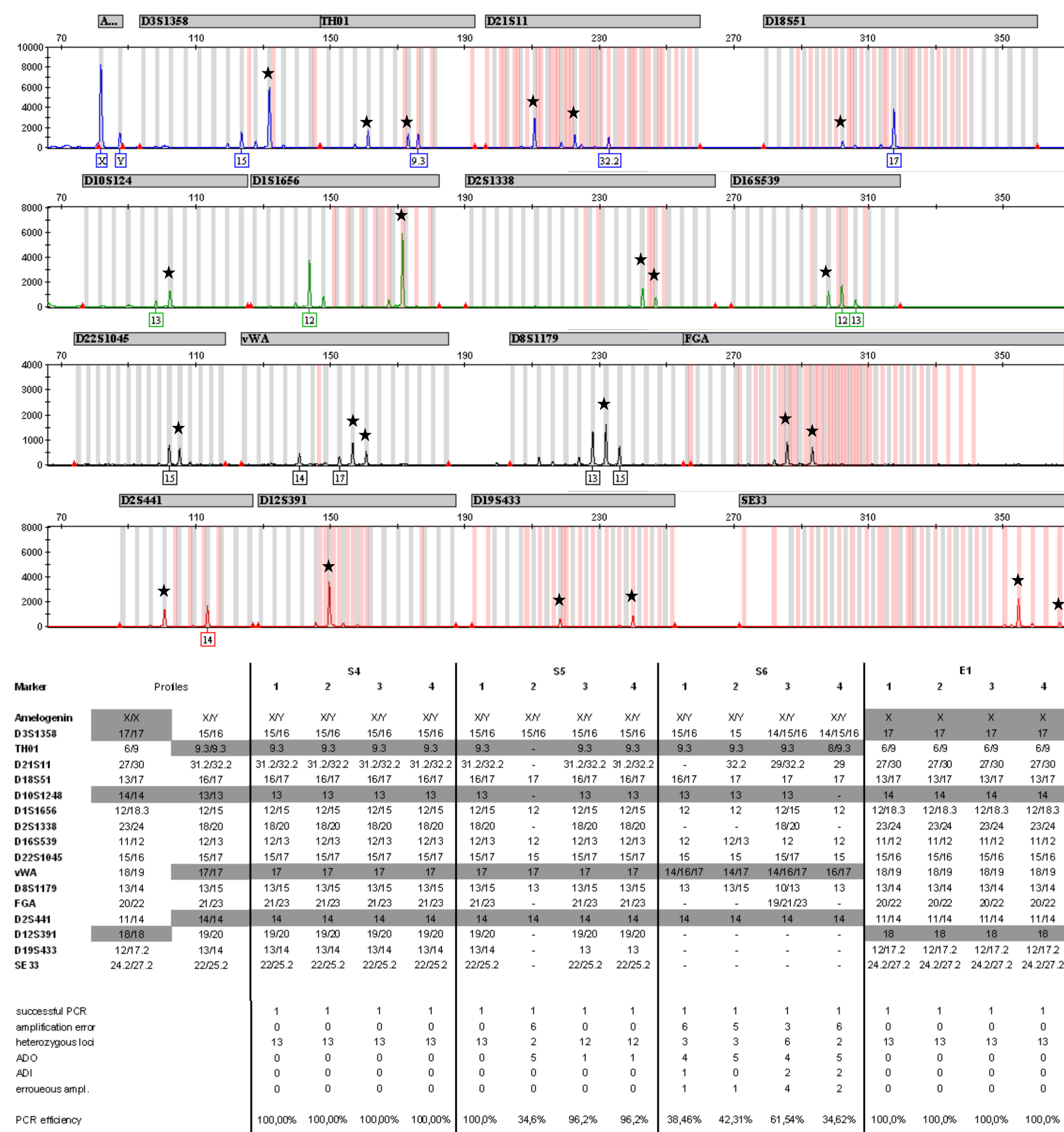
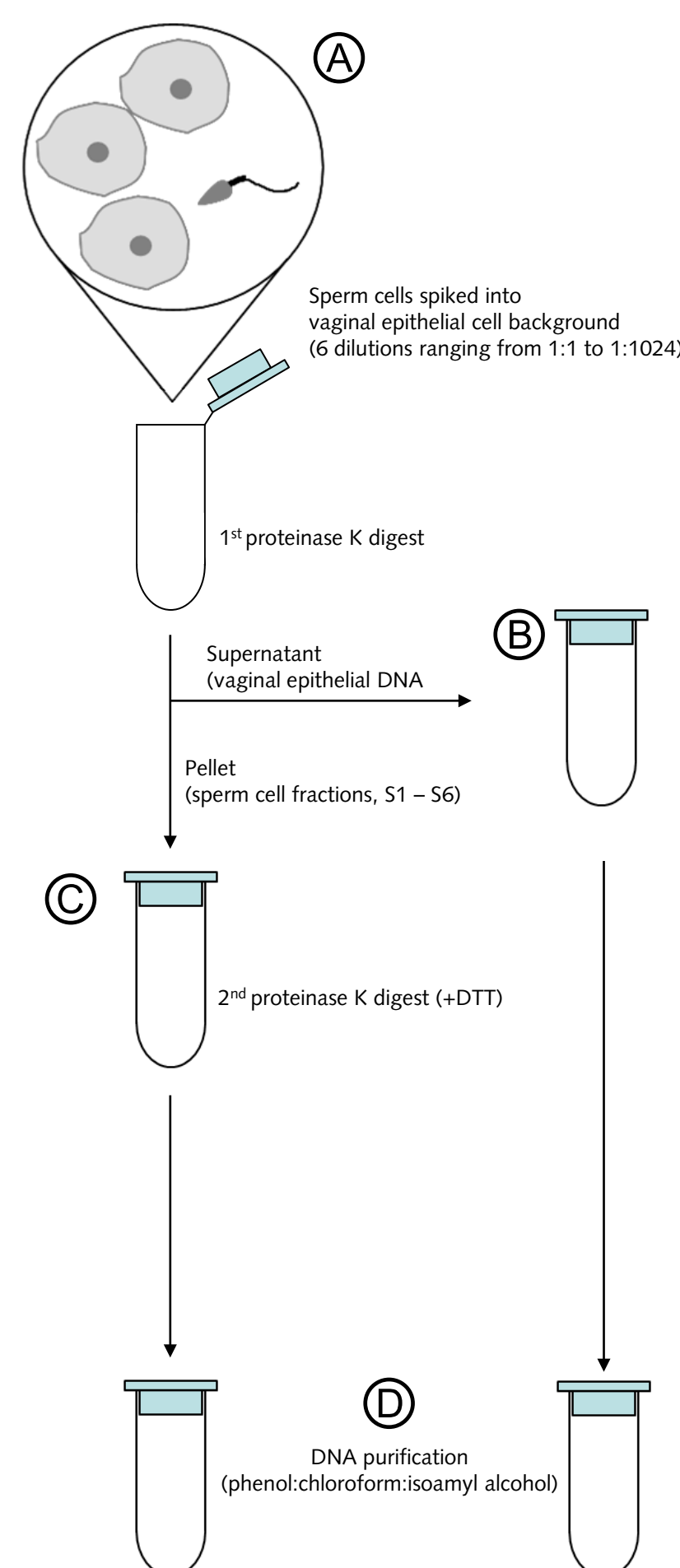
Sperm cell DNA fraction analysis

(A) Dilution series of sperm cells were prepared in the background of 5·10⁵ vaginal epithelial cells at ratios of 1:1, 1:4, 1:16, 1:256, and 1:1024.

Sperm cell DNA was obtained from the mixed samples according to the standard protocol of differential extraction: In short, the samples were subjected to two rounds of lysis. In the first round proteinase K caused the vaginal epithelial cells but not the sperm cells to lyse. After centrifugation DNA from vaginal epithelial cells (E1 – E6) was removed with the supernatant (B) whereas the pelleted sperm cell fractions (S1 – S6) were subjected to a second round of lysis containing proteinase K/DTT (C).

(D) DNA from vaginal epithelial cell preparations (supernatant) and sperm cell fractions was isolated by standard phenol-chloroform extraction.

Multiplex PCR using PowerPlex 17 ESX (Promega) was carried out in-tube in a volume of 10 µl according to the manufacturer's recommendations. Amplification products were directly forwarded to analysis on an ABI 3730 DNA analyzer.

SINGLE CELL
ANALYSIS

Results

Sperm cell DNA fraction analysis

DNA profiles of sperm cell and vaginal epithelial cell fractions were obtained from all 48 samples (quadruplicates of 6 dilution series).

100% PCR efficiency was obtained from all vaginal epithelial cell fractions (E1¹⁻⁴ – E6¹⁻⁴) and from sperm cell fractions S1¹⁻⁴ (1:1) to S4¹⁻⁴ (1:56) and S5¹ (1:256).

Fraction S5 (~2000 sperm cells, table) and S6 (~500 sperm cells, plot and table) showed elevated levels of contamination by DNA from vaginal epithelial cells (asterisked in plot). In the corrected DNA profile the mean “PCR efficiency” of S5 and S6 drops to 81.7 (43.6% – 100%) and 44.2% (34.6% - 61.1%), respectively.

Summary

We successfully applied low-volume on-chip DNA typing to single sperm cells yielding a PCR success in 97.5% of all analyzed single sperm cell samples with a mean PCR efficiency of 62.8%. Furthermore, we were able to identify a sample containing more than one cell allowing to exclude “contaminated” samples from further analysis.

As shown, differential extraction - a standard technique widely accepted in forensics for isolating sperm cell DNA from mixed samples - encounters application limitations already at a total number of approximately 500 sperm cells isolated from a mixed sample containing 5·10⁵ vaginal epithelial cells (1:1024). We therefore suggest to further investigate the use of single cell DNA typing especially in mixed samples containing only a few target cells in an overwhelming background of non-target cells as this is true for a number of samples in forensic casework.

This work was supported by the Country of Styria

Das Land
Steiermark

→ Wissenschaft