Deconvolution of blood-blood mixtures using DEPArray™ separated single cell STR profiling

K. Anslinger, B. Bayer

The DEPArray™ Technology

Menarini, Silicon Biosystems, Bologna, Italien

⇨ enables a software based

- identification,
- independent movement and
- separation

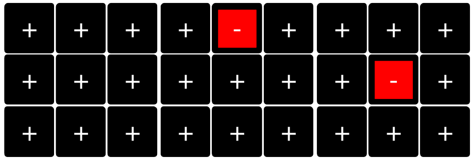
of single, immunologically fluorescent labelled cells

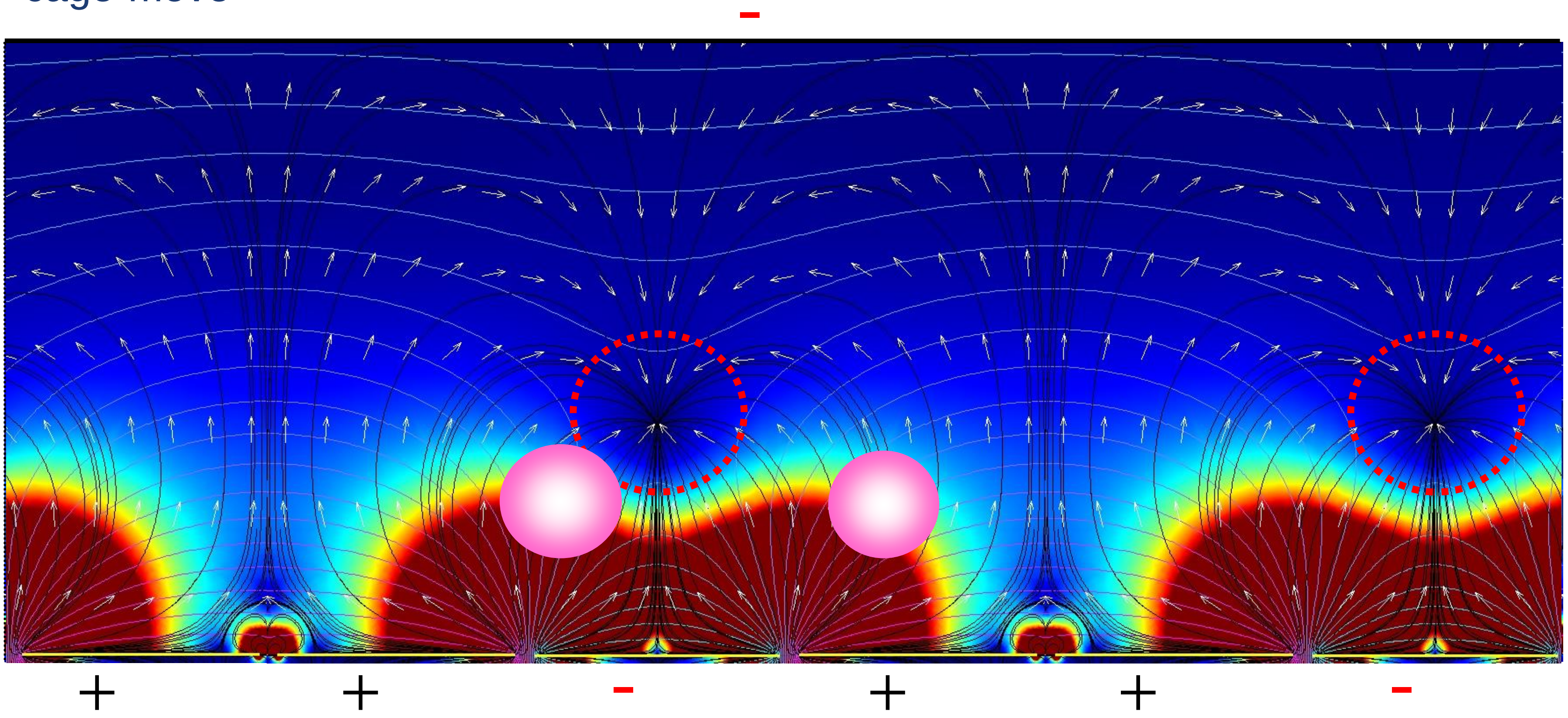
Quelle: www.siliconbiosystems.com/deparray-system

Trapping and Moving cells in «DEP Cages»

Non-uniform electric field
generated by the chip electrodes (cross section)

Cell trapping by DEP cages
cage-move

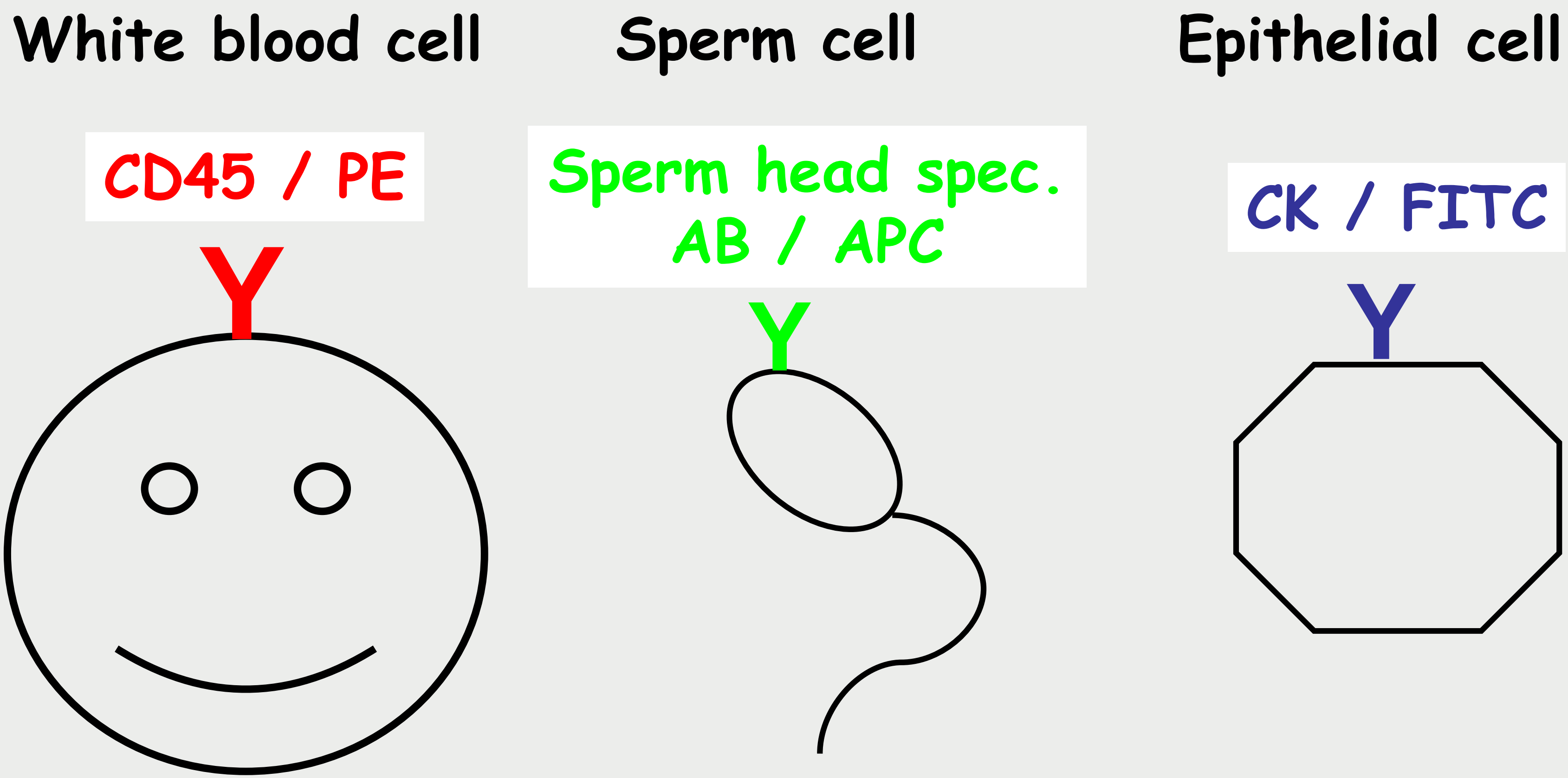




MENARINI silicon biosystems

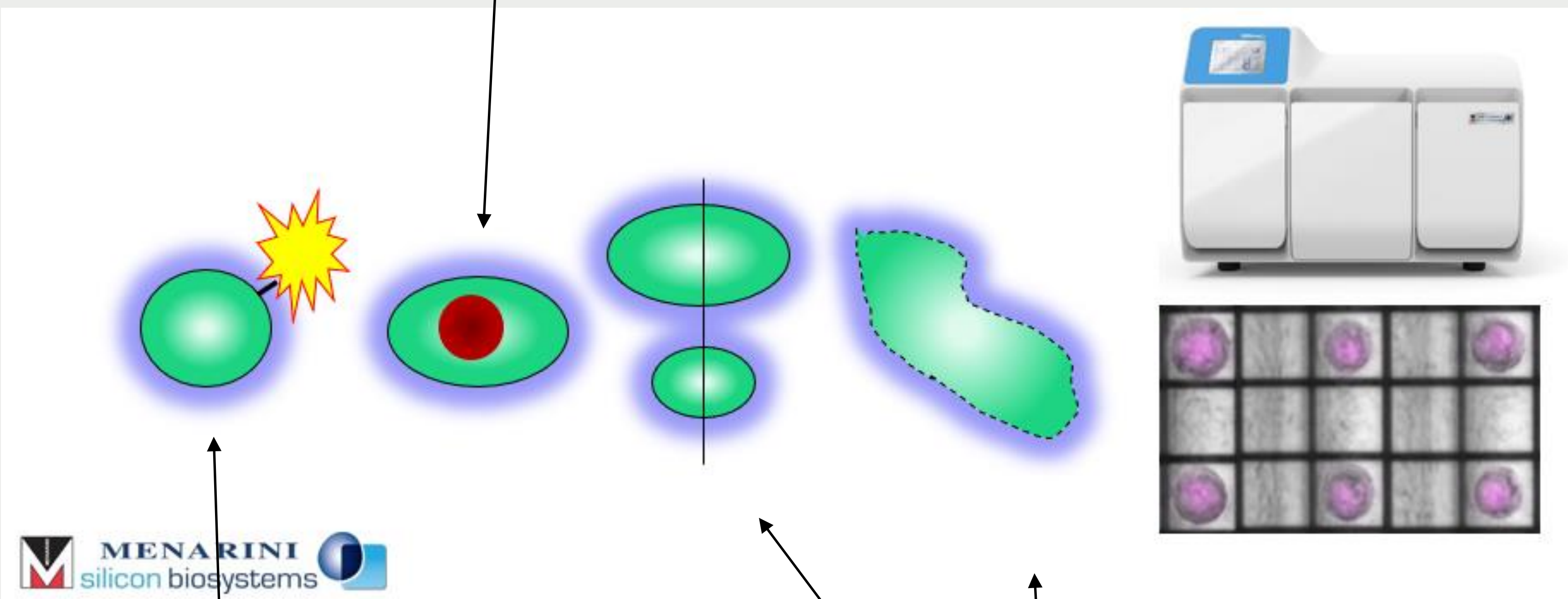
DEPArray™ Forensic Sample Prep Kit

Menarini, Silicon Biosystems, Bologna, Italien



+ nucleoli / DAPI

DAPI stained nucleolus



Fluorescent marked intracellular / surface antibodies

Bright field channel:
cell size, symmetry, shape

Forensic Sci Int Genet. 2017 Jul;29:225-241. doi: 10.10

Isolation and genetic analysis o ... cal mixtures: The precision of a digital approach.

Fontana F[1], Rapone C[2], Bregola G[3], Aversa R[3], de ... tto R[3], Medoro G[3], Giorgini G[3], Manaresi N[3], Berti A[2].

Author information

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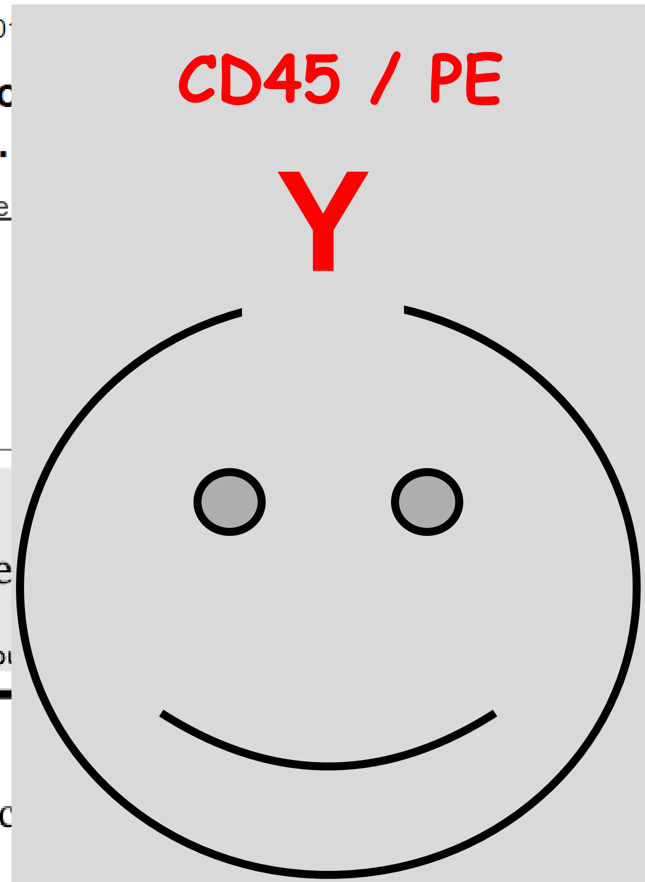

ELSEVIER
Fore ... ics
jou
FSI GENETICS

Original research paper

Enhanced DNA mixture dec ... les using the DEPArray™ system

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11.12.2017 | Case reports | Ausgabe 2/2018

Application of DEPArray^{TM} technology for the isolation of white blood cells from cell mixtures in chimerism analysis

Zeitschrift: Rechtsmedizin > Ausgabe 2/2018

Autoren: PD Dr. K. Anslinger, B. Bayer, D. von Máriássy

Abstract

After allogeneic bone marrow or blood stem cell transplantation, STR profiling is often used for monitoring chimerisms. For the evaluation reference samples taken from the patient before the transplantation and/or taken from the donor are needed. In the case described here, no reference samples were available. Reporting the chimerism based on posttransplantation samples did not yield conclusive results; therefore, white blood cells and epithelial cells obtained from a buccal swab taken from the patient were separated using DEPArray^{TM} technology and STR profiling was carried out resulting in distinct reference profiles.

Int J Legal Med. 2018 Aug 18. doi: 10.1007/s00414-018-1912-7. [Epub ahead of print]

Whose blood is it? Application of DEPArray™ technology for the identification of individual/s who contributed blood to a mixed stain.

Anslinger K[1], Bayer B[2].

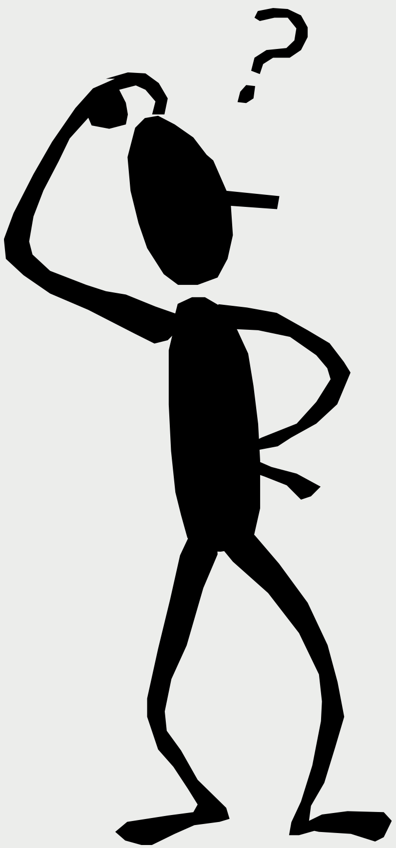
Author information

Abstract

The interpretation and statistical evaluation of mixed DNA profiles often presents a particular challenge in forensic DNA investigations. Only in specific combinations can single cellular components of a mixture be assigned to one contributor. In this study, the DEPArray™ technology, which enables image-assisted immunofluorescent-sorting of rare single cells using dielectrophoretic (DEP) forces, was applied together with different preliminary tests to identify the individual/s who contributed blood to a given mixture. The technique was successfully applied in two routine casework samples. In order to ascertain how old a stain can be and still be processed successfully, white blood cells from two 10- and one 27-year-old stains were investigated. Depending on the stain's age, the associated DNA degradation level and the number of target cells successfully isolated, the final profile reflects a compromise between the gain of information due to isolation of pure cells of a specific cell type from a single contributor and the loss of discriminatory power due to incomplete profiles caused by DNA degradation.

KEYWORDS: Cell separation; Cold case; DEPArray™ technology; Mixed DNA profiles; White blood cells

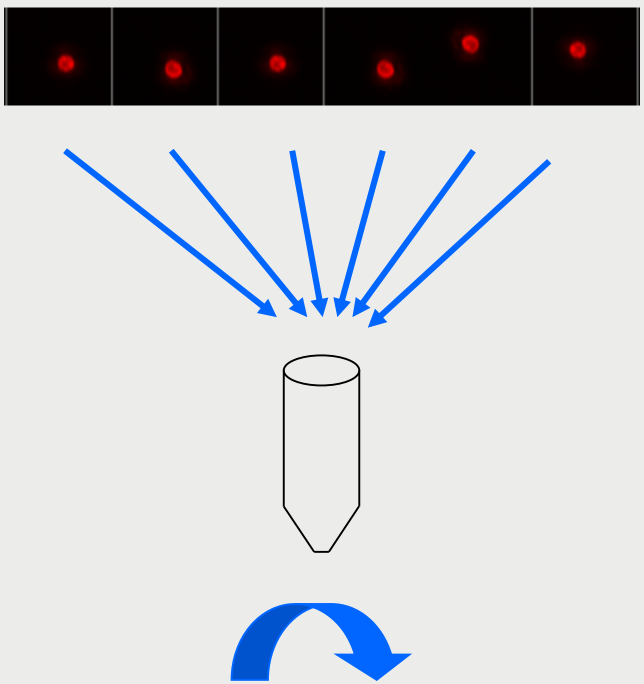
PMID: 30121738 DOI: 10.1007/s00414-018-1912-7



But what if a mixture contains blood from more than one person ?

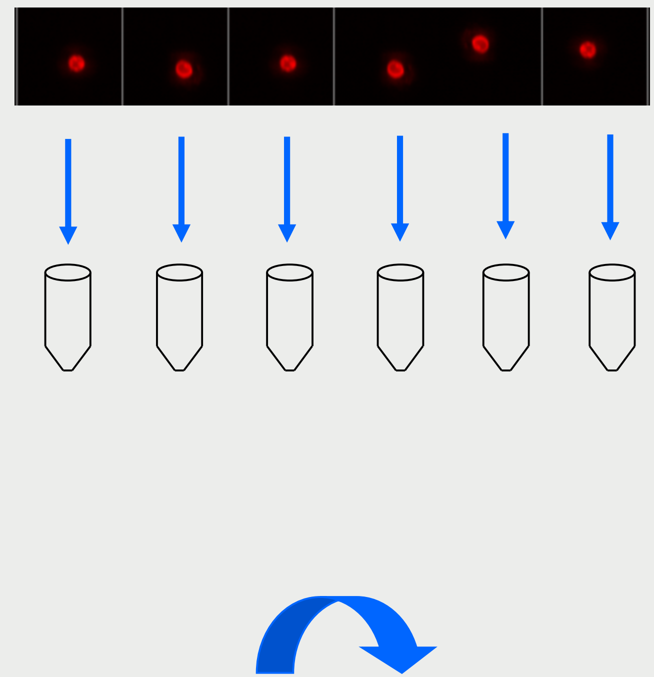
Can we use the DEPArray™ Technology for the deconvolution of blood-/blood mixtures ?

So far: separation of cell pools



STR-Profiling of cell pool ≥ 5 cells

New: separation of single cells



Single Cell STR-Profiling

Single Cell STR-Profiling

- not very common in forensic DNA investigations
- more commonly used:
  collection of individual skin flakes or "bio-particles"
  (➡ more than one single cell)

➡ very small amount of DNA

- special extraction and amplification methods are needed
- appearance of profiles are influenced
- special interpretation guidelines must be used

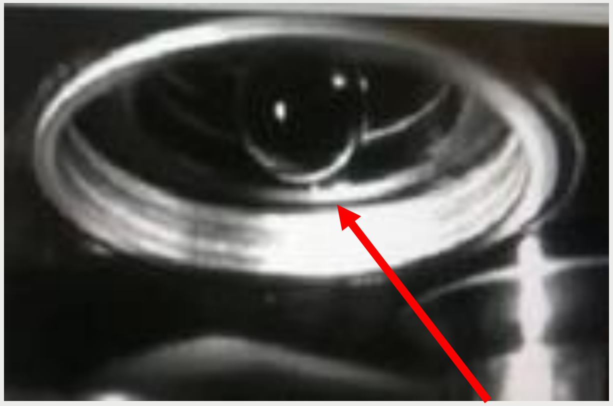
Methods:

DNA extraction:

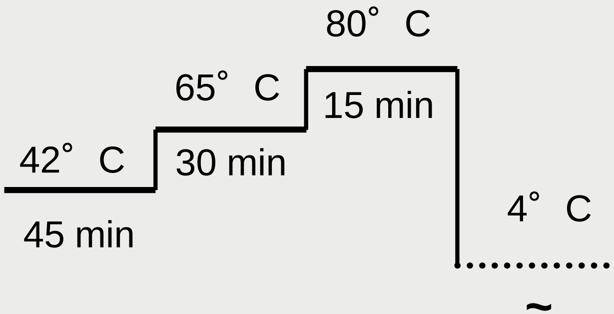
DEPArray™ Lyse Prep Kit (Menarini, Silicon Biosystems)

→ volume reduction to 1µl

→ „Single-tube" lysis kit
+ 2µl LyseMix (enzyme/DTT)



Incubation:



PCR: PP ESXfast and Y23, Promega
32 cycle programs ("In-house" validations)

Study Design

Validation
Investigation of two fresh (unmixed) blood samples

→ quality and number of obtained profiles?
→ appearance of profiles?
→ interpretation guidelines?

Blood-/blood mixtures of 2 or 3 contributors

→ mock sample (fresh blood samples from 3 contributors)
→ case work sample: stain on a knife (2 contributors stain 2)
→ GENAP 55 (2 contributors)

Validation

- 5µl blood from two different donors (A and B) were added to the staining procedure
- collection of 59 single WBCs
- further processing immediately after staining
  ➞ 43 WBCs (autosomal and Y-chrom. STRs)
- further processing after one week storage by 7° C
  ➞ 16 WBCs (autosomal STRs)

Results Validation I:
WBCs processed immediately after staining

Results: 27 WBCs profiled with autosomal STRs

- 26 partial and 1 full profile
- worst profile still showed >50% of the expected alleles
- average profile completeness: 82% (donor A) and 86% (donor B)

16 WBCs profiled with Y-chrom. STRs

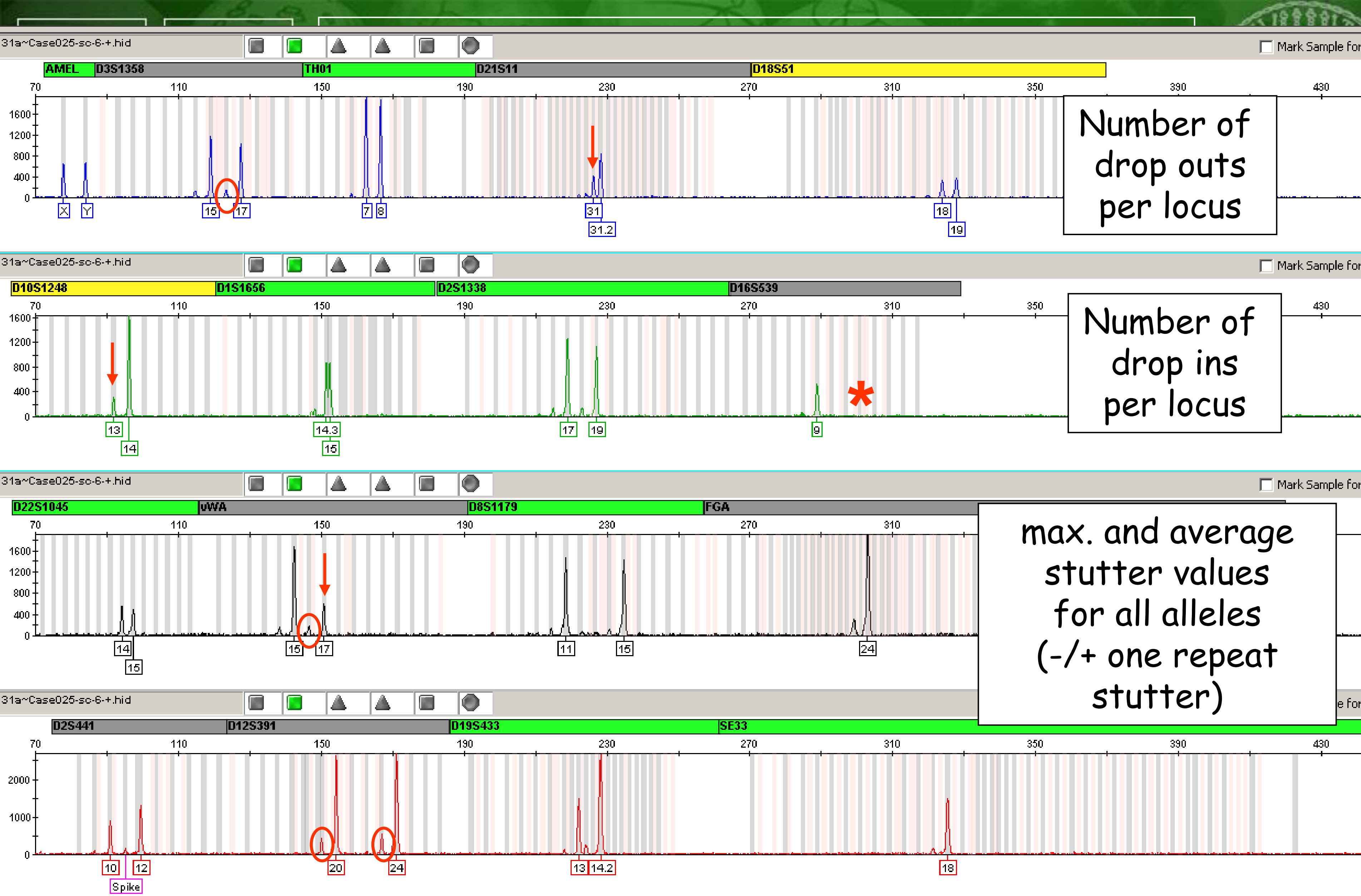
- 16 partial profiles
- average profile completeness: 86%

Results Validation II:
WBCs stored by 7° C for one week

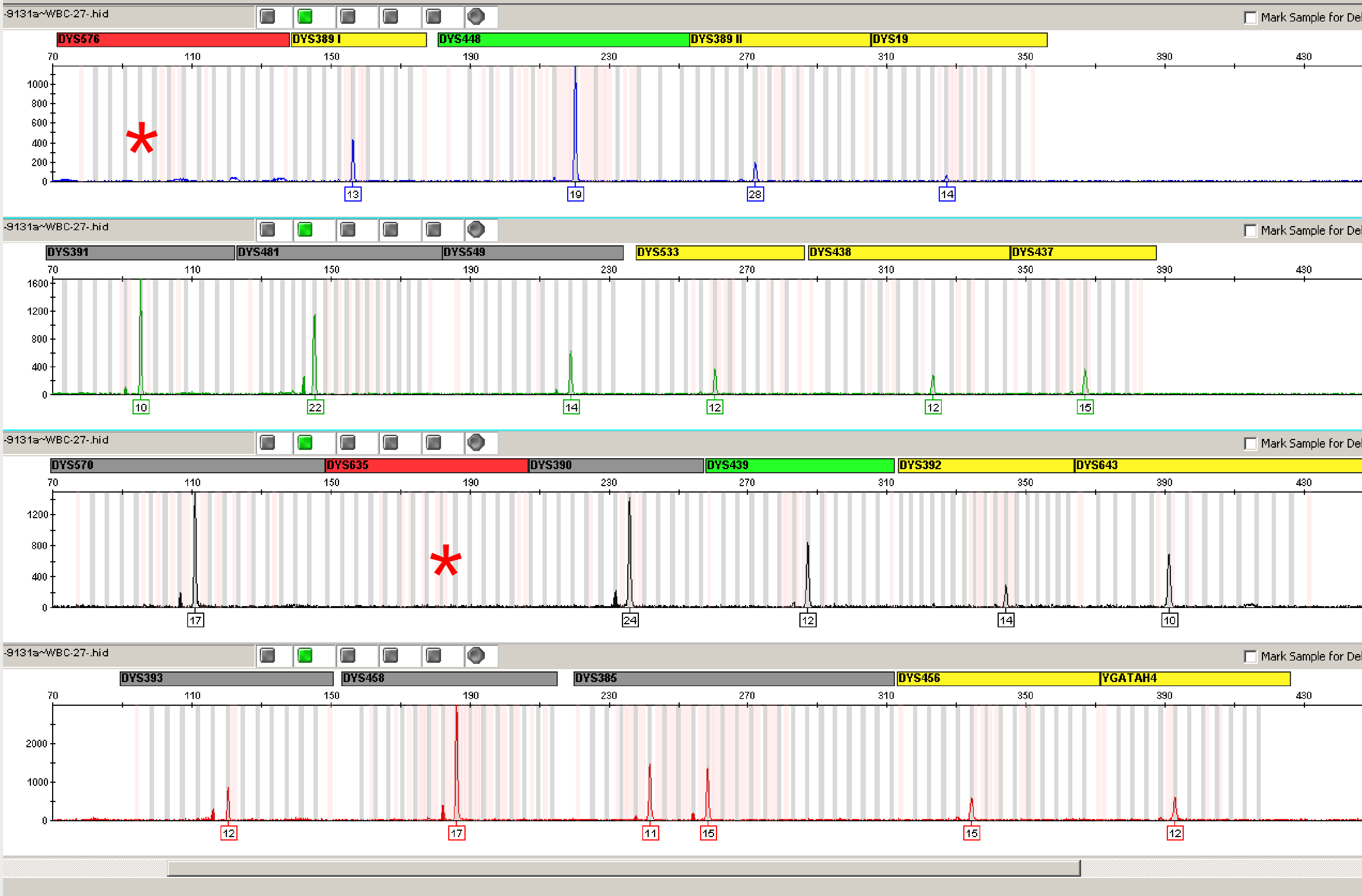
Results: 16 WBCs profiled with autosomal STRs

- 16 partial profiles
- average profile completeness: 83% (donor B)

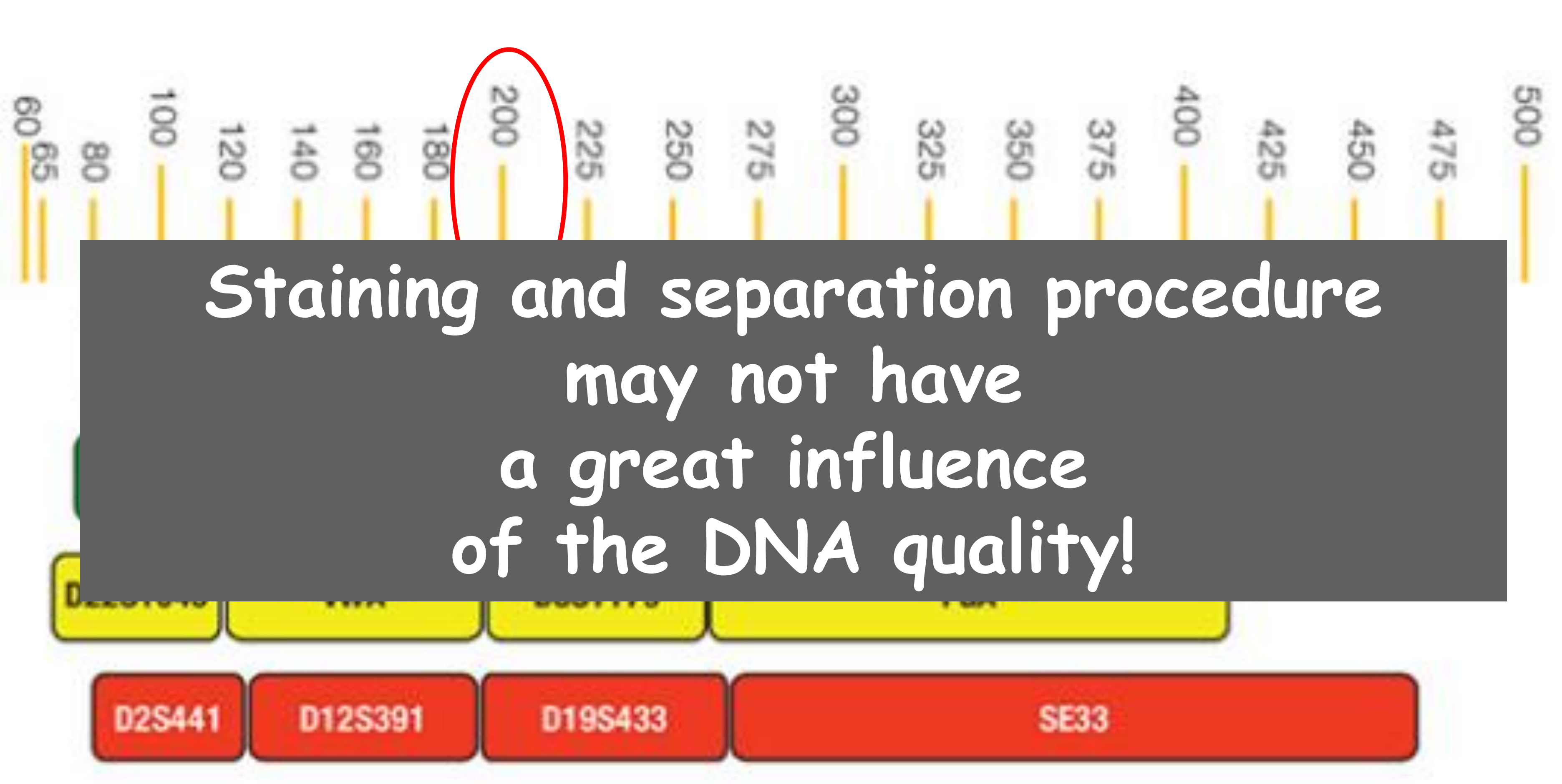
To sum up: Approach leads to meaningful results
Storage does not have a significant influence


31a~Case025-sc-6-+.hid
Mark Sample for D
AMEL
D3S1358
TH01
D21S11
D18S51
X
Y
15
17
7
8
31
31.2
18
19
Number of drop outs per locus
D10S1248
D1S1656
D2S1338
D16S539
13
14
14.3
15
17
19
9
Number of drop ins per locus
D22S1045
vWA
D8S1179
FGA
14
15
15
17
11
15
24
max. and average stutter values for all alleles (-/+ one repeat stutter)
D2S441
D12S391
D19S433
SE33
10
12
Spike
20
24
13
14.2
18

Number of drop outs per locus



Partial profiles donor B: 61 drop outs



Staining and separation procedure may not have a great influence of the DNA quality!

Minus one repeat stutter

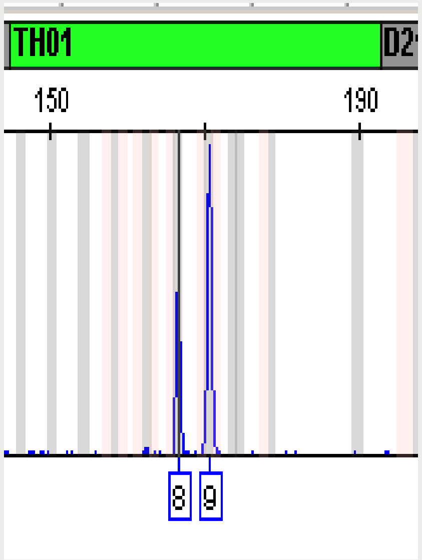
- For routine case work, marker specific stutter values are used
- Not useful for single cell STR-profiling

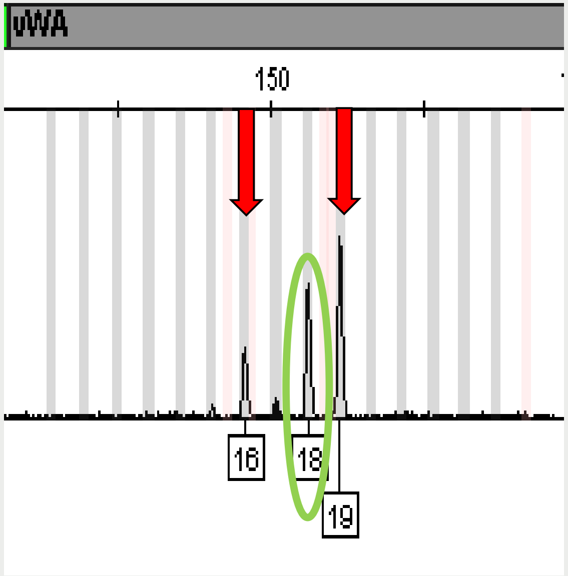
→ depending on the marker >60% n-1 stutter would be labeled

↕

- Maximum and average n-1 stutter values were determined
- New (intra-laboratory) thresholds for single cell profiles were worked out, so that the *majority* of peaks could be recognized as stutter
- However, extreme values are not covered …

Drop in or increased n-1 stutter?





Dealing with artefact

Decreased PCR-cycle number → decreased artifacts

but also to

→ increased number of allelic drop out

- LT protocol:
  several PCRs were used to create a consensus profiles

- Single cell STR profiling:
  combining the results of several single cell profiles to a consensus profile

11 partial profiles donor A

DNA-Systeme	D3S1358	VWA	FIBRA	THO1	SE33	D8S1179	D21S11	D18S51	Amel	D16S539	D2S1338	D19S433	D22S1045	D1S1656	D10S1248	D2S441	D12S391	Number of detected alleles
reference Pool 5 WBCs	15 / 17	15 / 17	24	7 / 8	18	11 / 15	31 / 31.2	18 / 19	X / Y	9 / 12	17 / 19	13 / 14.2	14 / 15	14.3 / 15	13 / 14	10 / 12	20 / 24	32
SC-1 WBC	15 / 17	15	24	7	18	11 / 15	31 / 31.2	18 / 19	X / Y	9 / 12	17	13 / 14.2	14 / 15	15	13 / 14	10 / 12	20 / 24	28
SC-2 WBC	15 / 17	15 / 17	24	8	18	11 / 15	31 / 31.2	18 / 19	X	9	17 / 19	13	15	14.3 / 15	14	10 / 12	20 / 24	26
SC-3 WBC	15		24	7 / 8	18	15	31 / 31.2	18 / 19	Y			13		14.3 / 15	13 / 14		20 / 24	18
SC-4 WBC	17	15 / 17	24	7 / 8	18	11 / 15	31 / 31.2	18 / 19	Y	12	19		14 / 15	14.3 / 15	13 / 14	12	20 / 24	25
SC-5 WBC	15 / 17	15 / 17	24	7 / 8	18	11	31 / 31.2	18 / 19	Y	9	19	13 / 14.2	14 / 15	15	13 / 14	10 / 12	20 / 24	27
SC-6 WBC	15 / 17	15 / 17	24	7 / 8	18	11 / 15	31 / 31.2	18 / 19	X / Y	9	17 / 19	13 / 14.2	14 / 15	14.3 / 15	13 / 14	10 / 12	20 / 24	31
SC-7 WBC	15 / 17	15 / 17	24	7 / 8	18	11 / 15	31	18 / 19	X / Y	12		13 / 14.2	14	14.3 / 15	13 / 14	12	20 / 24	26
SC-8 WBC	15 / 17	14 / 15 / 17		7 / 8					X / Y		17 / 19	13 / 14.2	14 / 15	15	13 / 14	10 / 12	20 / 24	21
SC-9 WBC	15 / 17	15 / 17	24	8	18	11 / 15	31 / 31.2	18 / 19	X / Y	9 / 12	17 / 19	13 / 14.2	14 / 15	14.3 / 15	13 / 14	10 / 12	20 / 24	31
SC-10 WBC	15 / 17	15 / 17	24	7 / 8	18	11 / 15	31 / 31.2	18	X / Y	9 / 12	17 / 19	13 / 14.2	14 / 15	14.3 / 15	13 / 14	10	20 / 24	30
SC-11 WBC	15 / 17	15	24	7	18	11	31	18 / 19	X	12	17 / 19	13 / 14.2	14 / 15	14.3 / 15	13 / 14	10 / 12	20	25

- average profile completeness: 82%

- each single allele was at least detectable 6 times
- one allele in minus-one stutter position was classified as drop in (79%)
  → unique event
- Combining the results:
  → a full and clear profile could be deduced

Blood stain on the blade of a knife

Complete profiles could be derived for both persons !

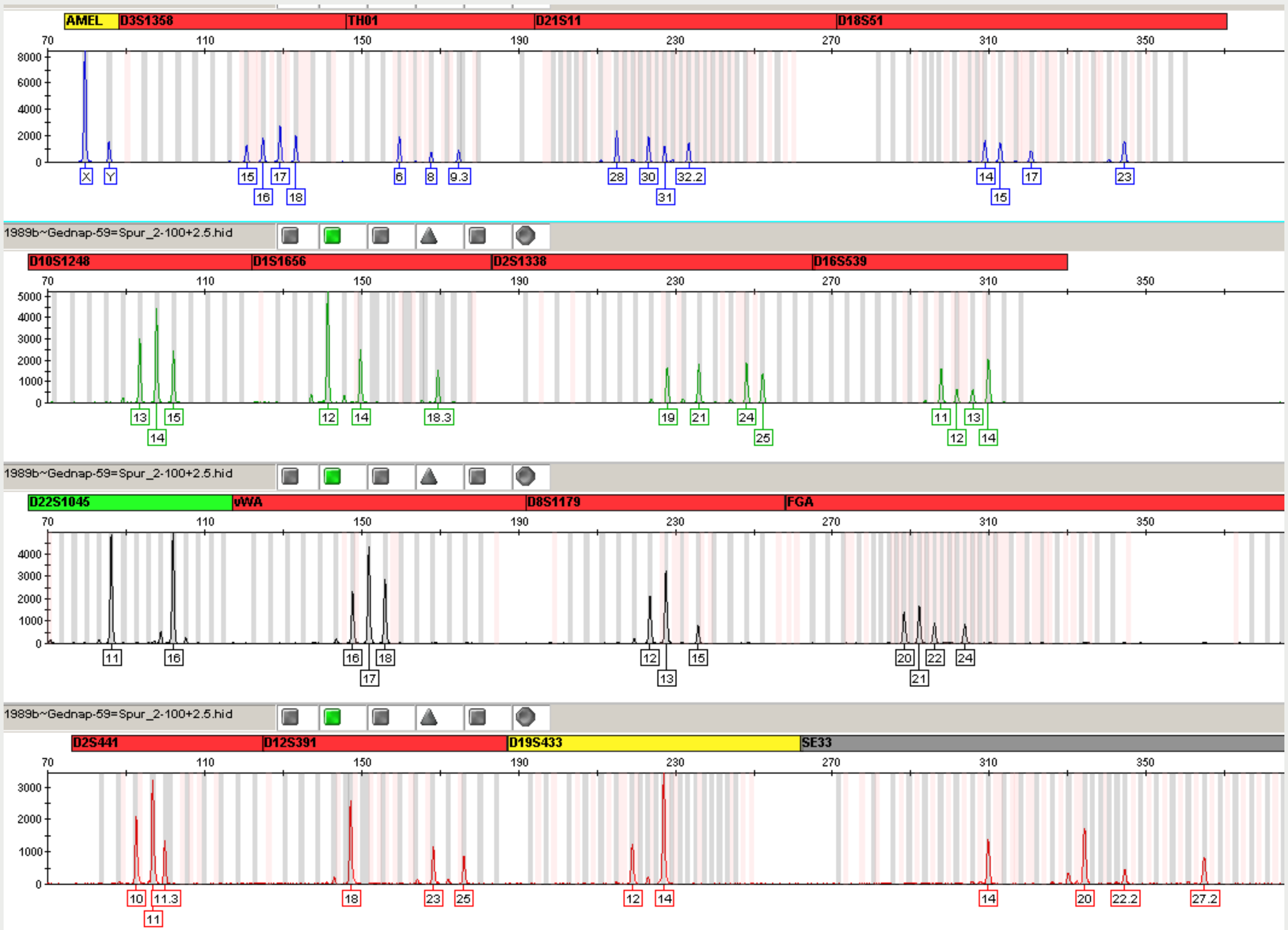
- age... ks
- routine S... ...ling

→ mixture 2 ... ...utors
(victim and sus...

Results of single cell profiling:
- recovering 17 WBCs
- 4 cells no detectable alleles
- 13 partial profiles:
  6x victim (11-27 of the expected 29 alleles)
  7x suspect (14-28 of the expected 30 alleles)
  every single allele detected at least 3 times
  + 3 drop in alleles (each occured only once)

GEDNAP 55 – stain 2

blood on cellulose



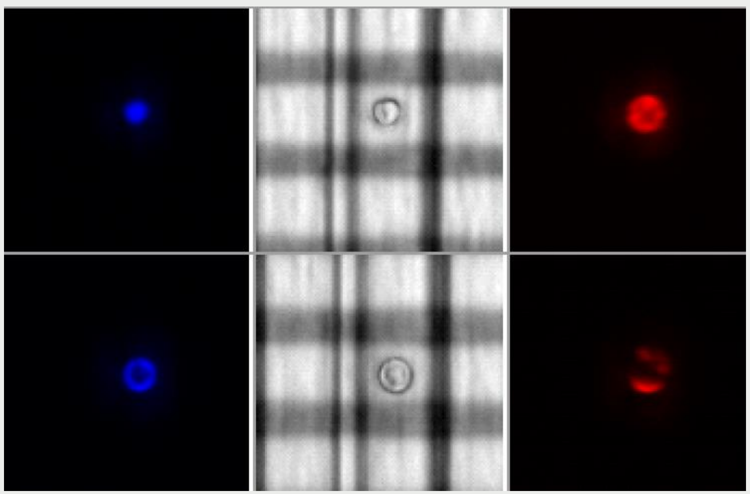
Deduced partial male profile was confirmed by the organizer of the GEDNAP proficiency test!

Deduced partial male profile was confirmed by the organizer of the GEDNAP proficiency test!

Looking for an explanation …

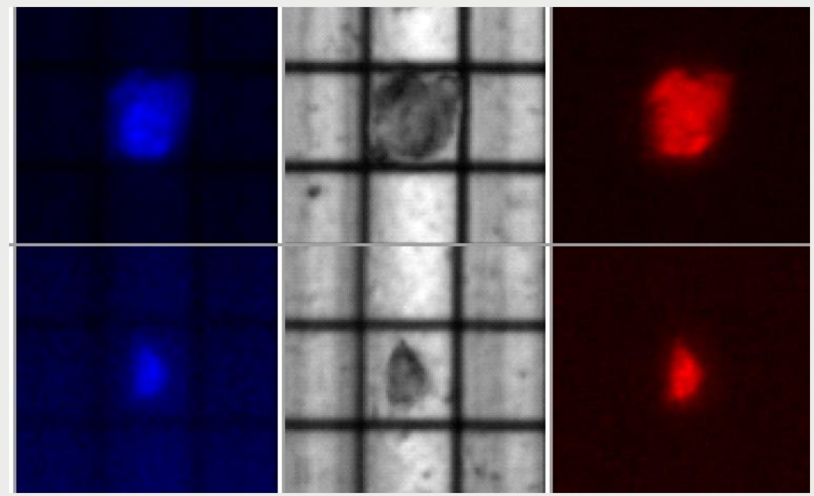
WBCs fresh blood sample

DAPI Bright field PE



WBCs sample GEDNAP sample

DAPI Bright field PE



→ treated with surfactants → damage of cell membrane?

Mixture – fresh blood of 3 contributors

DNA-Systeme	D3S1358	VWA	FIBRA	THO1	SE33	D8S1179	D21S11	D18S51	Amel	D16S539	D2S1338	D19S433	D22S1045	D1S1656	D10S1248	D2S441	D12S391	Number of detected true alleles
Reference A	***15 / 17***	***16 / 17***	***21 / 25***	***7 / 9***	***19***	***10 / 15***	***30 / 30.2***	***12 / 18***	***X / Y***	***12***	***17 / 23***	***13 / 16.2***	***14 / 16***	***11 / 17.3***	***14 / 16***	***11***	***22 / 23***	***31***
SC-1 WBC	15 / 17	16 / 17	21 / 25	7 / 9	19	10 / 15	30 / 30.2	12 / 18	X	12	23	13 / 16.2	14	17.3	14	11	22	25
SC-2 WBC	15 / 17	16 / 17	21 / 25	7 / 9	19	10 / 15	30 / 30.2	12 / 18	X / Y	12	17	13 / 16.2	16	11 / 17.3	14 / 16	11	22 / 23	29
SC-4 WBC	15	16 / 17	21 / 25	6 / 7	19	10 / 15	30 / 30.2	12	X / Y	12	17	15 / 16.2		11 / 14		11	22 / 23	21
SC-5 WBC	15 / 17	16 / 17	21 / 25	7 / 9	19	10 / 15	29 / 30 / 30.2	12 / 18	Y	12	23	13	14 / 16	11 / 17.3	14 / 16		/ 23	28
SC-14 WBC	15 / 17	16	21 / 25	7 / 9	19	10		12 / 18 / 19	X / Y	12	17 / 23	13 / 16.2	13 / 14	11 / 17.3	14 / 16	3/6	/ 23	26
SC-15 WBC		16	21 / 25	9	19	10	30 / 30.2	12 / 18	X	12	23	13 / 16.2	14 / 16	11	14 / 16	11	22 / 23	23
Deduced profile A	***15 / 17***	***16 / 17***	***21 / 25***	***7 / 9***	***19***	***10 / 15***	***30 / 30.2***	***12 / 18***	***X / Y***	***12***	***17 / 23***	***13 / 16.2***	***14 / 16***	***11 / 17.3***	***14 / 16***	***11***	***22 / 23***	***31***
Reference B	***15 / 18***	***17 / 18***	***20 / 24***	***6 / 9.3***	***20 / 27.2***	***13***	***29 / 31.2***	***12 / 14***	***X / Y***	***9 / 11***	***20 / 25***	***14.2 / 15***	***11 / 14***	***11 / 14***	***13 / 15***	***14 / 15***	***19 / 21***	***33***
SC-9 WBC	15 / 18		20 / 24	9.3	20 / 27.2	13	29	14	Y	9 / 11	20 / 25		14	11 / 14	13 / 15	14 / 15	19 / 21	24
SC-10 WBC	15 / 18	17	20 / 24	6 / 9.3	20 / 27.2	13	29	12 / 14	X / Y	9 / 11	20	15	11 / 14	11	13	2/3	/ 21	26
SC-11 WBC	15 / 18	17 / 18	20 / 24	6	20 / 27.2	13 / 15	29 / 31.2	11 / 12 / 14	X / Y	9 / 11	25	14.2	11 / 14	11 / 14	13 / 15		/ 21	29
Deduced profile B	***15 / 18***	***17 / ?***	***20 / 24***	***6 / 9.3***	***20 / 27.2***	***13***	***29***	***12 / 14***	***X / Y***	***9 / 11***	***20 / 25***		***11 / 14***	***11 / 14***	***13 / 15***	***14 / 15***	***19 / 21***	***29***
Reference C	***16 / 18***	***18***	***22 / 24***	***6***	***15 / 26.2***	***14***	***30***	***13 / 14***	***X***	***12***	***17 / 21***	***14***	***16***	***11***	***14***	***11***	***17 / 19.3***	***23***
SC-3 WBC	16 / 18	18	22 / 24	6	15 / 26.2	14	30	13 / 14	X	12	17	14	16	11	14	11	17 / 19.3	22
SC-6 WBC	16 / 18	18	21 / 24	6	15 / 26.2		30	13 / 14	X	12	17	14	16	11	14	11	17 / 19.3	20
SC-7 WBC	16 / 18	18	22	6	15 / 26.2	14	30	13	X	12	17 / 20 / 21	14	16	11	14		/ 19.3	21
SC-8 WBC	18	18	22 / 24	6	26.2	14	30	14	X	12	17 / 21	14	16	11	14	5/8	/ 19.3	20
SC-12 WBC	16 / 18	18	22 / 24	6	15 / 18 / 26.2	14	30	13 / 14	X	12	17 / 21		16	11	14		/ 19.3	22
SC-13 WBC	16 / 18	18	22 / 24	6	15 / 26.2	14	30	13 / 14	X	12	17 / 21	14	16	11	14	11	16 / 17	22
SC-16 WBC	16 / 18	18	24		15	14	30	13 / 14	X	12	17	14	16	11	14	11	19.3	18
SC-17 WBC	16	17 / 18	24	6	15 / 26.2	14	30	13 / 14	X	12	21	14	16	11	14	11	17 / 19.3	20
Deduced profile C	***16 / 18***	***18***	***22 / 24***	***6***	***15 / 26.2***	***14***	***30***	***13 / 14***	***X***	***12***	***17 / 21***	***14***	***16***	***11***	***14***	***11***	***17 / 19.3***	***23***

Original article

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Conclusions

→ **STR profiling of single cells isolated by DEPArray™ technology …**

- can be used successfully for the deconvolution of mixtures composed of cells of different as well as the same cell type
- it is well suited for the investigation of balanced mixtures
- depends very much on the quality of cells (cell membrane)

→ **Partial profiles, attributed to the same person, should always be combined to consensus sequences**

- based on the occurrence of artifacts, single profiles should be handled with greatest care
- how many single cells / partial profiles are needed for deducing a full and clear profile depends on the DNA quality (profile completeness)
- standards should be defined

Birgit Bayer

Menarini, Silicon Biosystems

… and you, for your attention !