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Body fluid identification and donor association of mock case samples: Results of two EDNAP collaborative exercises

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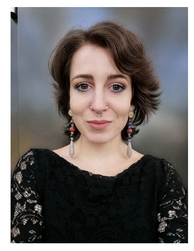

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1. Introduction

- mRNA profiling has emerged as a promising technique for body fluid identification (BFID)
- Coding region SNPs within body fluid specific transcripts facilitate the association between a body fluid and its donor
- Two RNA BFID-cSNP-assays have been developed[1]:
  - the BSS-cSNP-assay for BFID of all forensically relevant body fluids and skin, incl. cSNPs for blood, saliva and semen
  - the 6F-cSNP-assay for BFID incl. cSNPs for blood, saliva, semen, vaginal secretion, menstrual blood, and skin
- Each BFID-cSNP RNA assay comes with a concomitant DNA-cSNP assay for donor genotyping

2. Principle of the Method

Assigning a body fluid to a donor is a three-step-process:

- STR analysis to obtain the number of contributors
- mRNA profiling to determine the body fluid(s) present
- Comparison of the DNA and RNA cSNP genotypes

3. Aims

In two consecutive collaborative exercises organized by the Zurich Institute of Forensic Medicine (ZIFM), both BFID-cSNP assays were evaluated with respect to the following aspects:

- The robustness and reproducibility across different laboratories using two sets of stains provided by the ZIFM:
  - 6 participating laboratories in exercise 3
  - 12 participating laboratories in exercise 4
- The performance on stains prepared by participants (own stains)
- The effect of different sequencing platforms

4. EDNAP mRNA MPS Exercises

The EDNAP exercises 3 and 4 entailed the analysis of:

- 16 provided stains (single source and/or mixed, Fig. 1 + 2)
- 8 own stains (single source and/or mixed) incl. the respective DNA reference profiles (results not shown)

- Participating laboratories were given detailed instructions for stain analysis
- All the data from the participating laboratories was collected and analysed at the ZIFM.

5. Laboratory Workflow

RNA/DNA Co-Extraction → RT → cDNA STR-Analysis → Library Preparation → Sequencing: S5/MiSeq → Data Analysis

References

1. Hanson, Erin, et al. "Targeted S5 RNA sequencing assay for the identification and direct association of common body fluids with DNA donors in mixtures." *International journal of legal medicine* 137.1 (2023): 13-32.

6. Results

- Sequencing results were compared among participants (Fig. 3)
- Stain compositions were predicted by considering the proportion of body-fluid-specific markers:
  - 13/16 correctly predicted stains in exercise 3
  - 11/16 correctly predicted stains in exercise 4
- Sequencing results were compared across platforms in exercise 4 (Fig. 4)
- The cSNP genotypes were compared to the DNA reference profiles to associate body fluids with their donors (Fig. 5)

Stain N°	BF/T	Amount	Stain Provided
1	SE	10 µl	piece of fabric (boxer shorts)
2	BL-MB	1/2 Swab + 25 µl	1/2 swab
3	SE	50 µl	artificial cotton
4	SA-SE	50 µl + 25 µl	part of a T-shirt
5	BL	50 µl	1 swab
6	SK	1 swab	1 swab
7	BL-BL	25 µl + 25 µl	part of a T-shirt
8	SA	Licked plastic spoon	spoon
9	SA-SA	25 µl + 25 µl	1 swab
10	BL-SA	25 µl +25 µl	1 swab
11	SA	50 µl	part of a T-shirt
12	VAG	1/2 swab	1/2 swab
13	BL	Nose bleed on tissue	part of a tissue
14	SA-SE	25 µl + 25 µl	piece of fabric (boxer shorts)
15	MB	1/2 swab	1/2 swab
16	SE-VAG	½ Swab+ 25 µl SE	1/2 swab

Fig. 1: Composition of the provided stains in exercise 3. The colour code indicates high input stains (dark blue), low input stains (light blue), and mixtures (orange).

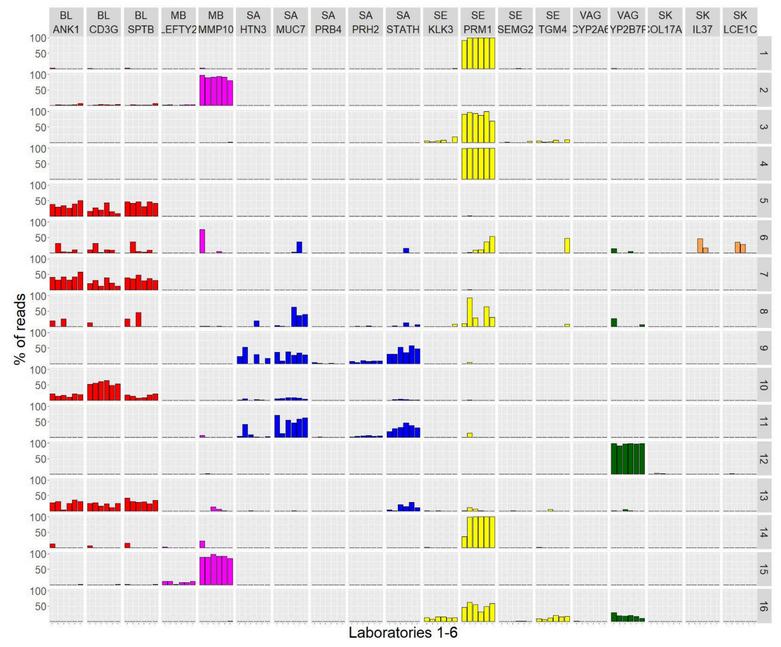

Fig. 3: Sequencing results of all laboratories participating in exercise 3. Markers are depicted as proportions of the total number of reads, and colored in the body-fluid-specific colors (red for BL, pink for MB, blue for SA, yellow for SE, green for VAG, and orange for SK).

Stain N°	BF/T	Amount	Stain Provided
1	SK	1 swab	1 swab
2	MB-BL	1 swab + 25 µl	1/4 swab
3	VAG-SA	1 swab + 25 µl	1/4 swab
4	MB-SA	1 swab + 25 µl	1/4 swab
5	BL-SE	25 µl + 25 µl	part of T-Shirt
6	SE-SE	25 µl + 25 µl	1 swab
7	MB-SA	1 swab + 50 µl	1/4 swab
8	SK-SA	1 swab + 25 µl	1 swab
9	VAG	cotton part of a slip	a piece of it
10	MB	menstrual pad	a part of it
11	SE	50 µl	part of a glove (latex)
12	BL	20 µl	part of a T-Shirt
13	SA-SE	50 µl + 10 µl	artificial cotton
14	VAG-BL	1 swab + 25 µl	1/4 swab
15	SA	50ul	piece of stockings (nylon)
16	VAG-SE	1 swab + 25 µl	1/4 swab

Fig. 2: Composition of the provided stains in exercise 4. The colour code indicates high input stains (dark blue), low input stains (light blue), and mixtures (orange).

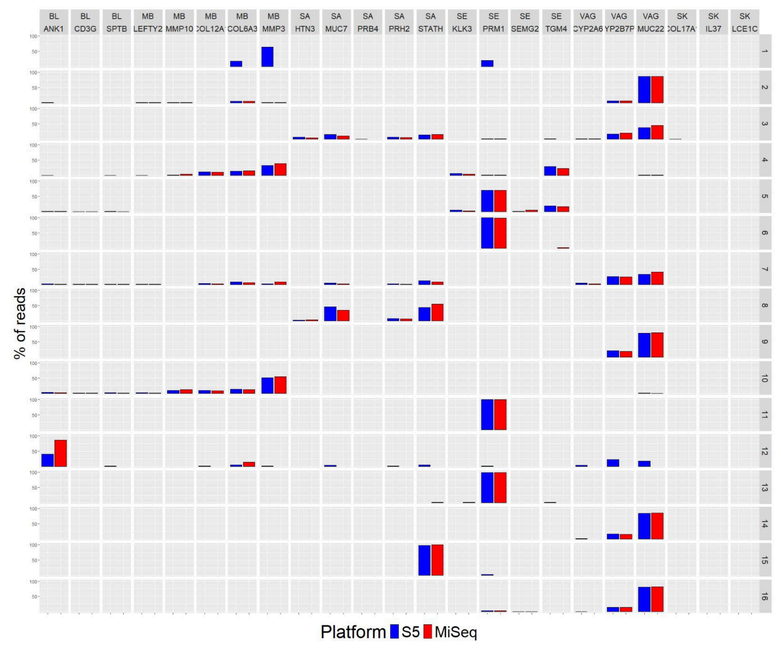

Fig. 4: Sequencing results of one laboratory participating in exercise 4 after stain analysis starting from the same cDNA. Markers are depicted as proportions of the total number of reads.

Ex4_Stain 3	HTN3.0	HTN3.1	HTN3.2	MUC7	PRB4	PRH2	HTN3.4	CYP2A6	MUC22.0	MUC22.1	MUC22.2	MUC22.3	MUC22.4	MUC22.5	MUC22.6	MUC22.7	MUC22.8	MUC22.9
Donor genotype 1	TC	T/T	C/C	C/T	G/G	C/C	C/C	C/C	GT	G/G	T/T	AA/GA	A/G	A/A	CT/TA/TC	C/T	T/A	T/T
Donor genotype 2	CT/CC	C/C	T/C	C/C	G/C	C/C	C/C	C/C	TC/GT	T/G	C/T	AA/GG	A/G	A/G	CA/CC	C/C	A/C	T/C
Lab7 MiSeq - Genotype	TC/TC	T/T	C/C	C/T	G/G	C/C	C/C		TC/GT	T/G	C/T	CAA/CGG	A/G	A/G	ACA	C/C	A/A	C/T
Lab7 MiSeq - Read Counts	14464	14464\14464	14464\14464	21108\17201	2418\2418	8824\8824	14464\14464		8858\8081	8858\8081	8858\8081	27716\18812	27716\18812	27716\18812	30115	30115\30115	30115\30115	8858\8081
Lab8 S5 - Genotype	TC/TC	T/T	C/C	C/T	G/G	C/C	C/C		TC/GT	T/G	C/T	CGG/CAA	G/A	G/A	ACA	C/C	A/A	C/T
Lab8 S5 - Read Counts	33244	33244\33244	33244\33244	47296\36046	3064\3064	33033\33033	33244\33244		11954\10099	11954\10099	11954\10099	57295\51908	57295\51908	57295\51908	70883	70883\70883	70883\70883	11954\10099
Lab9 S5 - Genotype	TC/TC	T/T	C/C	C/T	G/G	C/C	C/C		TC/GT	T/G	C/T	CAA/CGG	A/G	A/G	ACA	C/C	A/A	C/T
Lab9 S5 - Read Counts	2536	2536\2536	2536\2536	68781\41249	298\298	60076\60076	2536\2536		6614\3587	6614\3587	6614\3587	30194\23648	30194\23648	30194\23648	36149	36149\36149	36149\36149	6614\3587

Fig. 5: Example for donor association of a provided stain (stain 3, exercise 4). DNA cSNP genotypes in the markers of interest are compared to the RNA cSNP genotypes (results of three exemplary laboratories, no results reported for CYP2A6). The respective donor genotype for the body fluid of interest is marked in light blue.

7. Conclusion

- Very promising results, i.e. the majority of the stains in both exercises were typed correctly
- Results were quite consistent across different laboratories
- The 6F-BFID-cSNP assay performed well on both sequencing platforms
- Laboratories with limited RNA experience also reported good results
- For body fluid identification difficulties arise because of various misleading reads:
  - If a stain has low reads in general, reads in target markers are low as well → harder to interpret
- Performance of the association of a body fluid and a donor is dependent on how many markers are detected per body fluid:
  - Not every marker includes a cSNP with the power to exclude a donor