



Advances in Forensic Tissue Identification

Directly Associating Body Fluids to Individual DNA Donors in Mixed Body Fluid Stains by MPS Analysis of Coding Region SNPs





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Green Mountain DNA
Conference 2024



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Overview

1. Can there be added value to a DNA profile?
2. Cellular phenotyping by RNA profiling
3. Assigning body fluids to DNA donors by cSNP typing
4. Conclusions

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1. Can there be added value to a DNA profile?



Forensic science helps establish facts that may be in question

Activity and presence produce traces that are fundamental vectors of information

DNA profile = Who? but not What/How etc.

Value of the DNA profile depends upon context

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1. Can there be added value to a DNA profile?

why  **?**




Irrelevance? **activity = what or how?**

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1. Can there be added value to a DNA profile?

social intercourse versus sexual intercourse





For example, does the victim's DNA profile on a suspect originate

- from cellular deposits on her hands (skin) or from vaginal cellular secretions?
- from her peripheral blood or from menstrual blood?

Thus, the identity of the CELLULAR PHENOTYPE responsible for a crime scene DNA profile could inform about possible activities that are likely, or not likely, to have occurred during the deposition of the crime sample.

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1. Can there be added value to a DNA profile?



Hierarchy of Propositions

Level	Question/Issue	Results	Example of Pairs of Propositions
Activity	Did the POI perform the activity?	<ul style="list-style-type: none"> Presence/absence of DNA at different locations Quantity/quality of the DNA (DNA profiling comparison) Presumptive tests Multiple traces from the same activity 	<ul style="list-style-type: none"> Mr. A and Ms. B had penile-vaginal intercourse. Mr. A and Ms. B only partook in social activities as described in the case information.
Source	Is the POI the source of the biological material?		<ul style="list-style-type: none"> Mr. Smith was the driver, and Mr. Jones was the passenger at the relevant time. Mr. Jones was the driver, and Mr. Smith was the passenger at the relevant time.
Sub-Source	Is the POI the source of the DNA?	<ul style="list-style-type: none"> DNA profiling comparison 	<ul style="list-style-type: none"> Mr. A is the source of the blood. An unknown individual is the source of the blood.
Sub-Sub-Source	Is the POI the source of part of the mixture?		<ul style="list-style-type: none"> Mr. A is the source of the DNA. An unknown individual is the source of the DNA. Mr. A is the major contributor of the DNA mixture.

To move up the hierarchy towards activity shouldn't you need BFID? ("Source" or even "Sub-Activity")

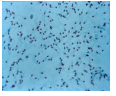


Source: 2024 Forensic DNA Interpretation and Human Factors: Improving the Practice Through a Systems Approach, NIST Interagency/External Report (NISTIR)

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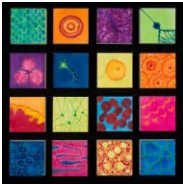
2. Cellular phenotyping by RNA profiling



for **saliva vaginal secretions skin menstrual blood internal organs** need **"second generation" more-specific molecular markers**

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2. Cellular phenotyping by RNA profiling



- Transcriptome
 - Terminally differentiated cells have a pattern of gene expression that is unique to each cell type
 - Presence and relative abundance of specific mRNA or miRNAs
- Epigenome/Methylome
- Proteome
- Microbiome

DNA → transcription → RNA → translation → protein

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2. Cellular phenotyping by RNA profiling

Basic Human Transcriptomics

- Primary transcription is the major driver of cellular specificity
 - the transcriptome is an indicator of cellular phenotype
- > 85% of the genome is transcribed (not in a single tissue)
 - mostly ncRNA, only ~2-3% mRNA
- Approx. 20,000 human genes
 - Vary more across tissues (47% total variance) than individuals (4% total variance)
 - Only ~1% (200) genes exclusively expressed in single tissue
 - 12% of genes enriched in a single tissue (> 5X compared to other tissues)
 - 10% of transcripts in a tissue come from expression-elevated genes (DE in several tissues compared to most)
 - 50% of genes possess tissue dependent isoforms
 - Almost half of all genes (44%) expressed in all tissues

Sources: Human Protein Atlas/Genotype Tissue Expression (GTEx) project/ENCODE

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2. Cellular phenotyping by RNA profiling

Why is transcriptomics useful in forensic genetics?

- RNA of sufficient quality/quantity for analysis can be recovered from crime samples
- DNA and RNA can be co-extracted
- RNA converted to DNA (cDNA): same downstream chemistry and analytical platform
- Unlike gDNA (2 copies per autosomal locus/cell) multiple judiciously selected loci are copied 100s to 1000s into RNA species (in certain body fluids/tissues)
- Digital gene expression (direct counting of transcripts) facilitates quantitative analysis
- Genomic information is directly encoded and faithfully reproduced in RNA transcripts
 - MPS technology enables genomic encoded RNA sequences to be accessed
 - Coding region SNPs

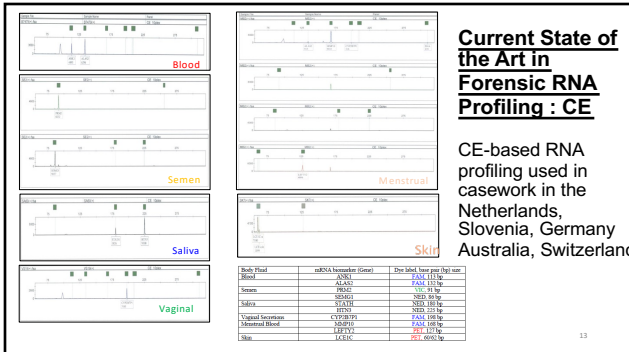
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Reported Body Fluid Specific mRNA Biomarkers

Blood	Semen	Saliva	Vaginal Secretions	Menstrual Blood	Skin	Housekeeping Genes
ALAS2*	PRM1	HTN3*	MUC4	MMP7	LCE1C*	B2M*
SPTB	PRM2*	STATH*	HBD1	MMP10*	LCE1D	UBC
HMBS (PBGD)	MSP	PRB4	ESR1	MMP11	LCE2D	UCE
CD3G	TGM4*	SPRR3	CYP2B7P1*	CK19	CCL27*	GAPDH
HBB	PSA (KLK3)	SPRR1A	MIOZ1	PR	IL1F7	G6PDH
CASP2	SEMG1	KRT4	FUT6	LEFTY2*	LOR	TEF
AMICA1	SEMG2	KRT6A	DKK4	MSX1	CDSN	
C10R1		KRT13	SFTA2	SFRP4	KRT9	
ALOX5AP		MUC7	IL19*		CST6	
AQP9			<i>Lcris</i>		DSC1	
CSR1			<i>Lgus</i>			
NCF52			<i>Ljen</i>			
MINDA						
ABHGAP26						
GYPB						
ANK1*						
HBA						

*bacteria core loci?
 *Ballantyne lab - most frequently used markers no commercial kits

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Contents lists available at ScienceDirect
Forensic Science International: Genetics
 journal homepage: www.elsevier.com/locate/fsig

2. Cellular phenotyping by RNA profiling

RNA/DNA co-analysis from human menstrual blood and vaginal secretion stains: Results of a fourth and fifth collaborative EDNAP exercise

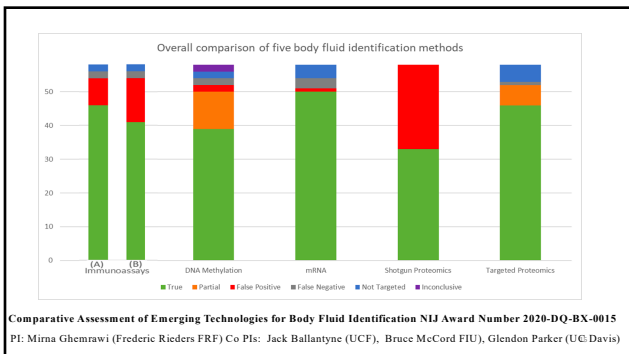
Collaborative studies

- EDNAP
- FoRNAP

Research paper
 mRNA profiling of mock casework samples: Results of a FoRNAP collaborative exercise

Authors: Andrea Patriza Salzman, Malte Bamberg, Cornelius Courts, Guro Derum, Anica Goch, Thomas Hladky, Gavriel Hadzi, Maximilian Heis, Peter M. Schneider, Tjits Sijts, Margreet van den Berge, Peter Wiegand, Cordula Haas

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MPS: BFID/Organ Tissue Assays

CE versus MPS

- Limited multiplex capability (some multiplexes in use require three separate amps)
- Quantitative analysis more challenging (rfu versus counting of transcripts)
- No access to complete sequencing information
- However, CE more sensitive than NGS

Targeted RNA-Seq BFID

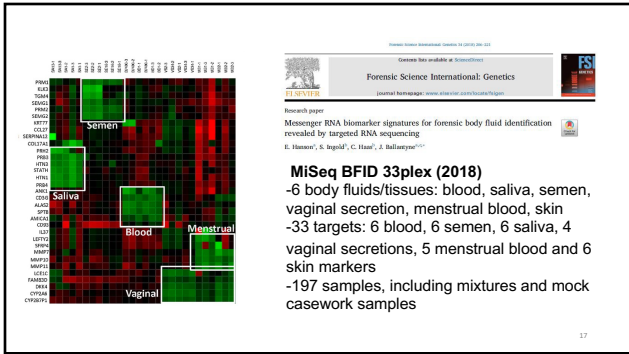
Body fluid

- "Off the shelf" Illumina MiSeq BFID 33plex (6 body fluids) (Hanson et al 2018)
- **Bespoke Life Technologies Ion S5/Chef 36 plex (6 body fluids) (Hanson et al 2023)**

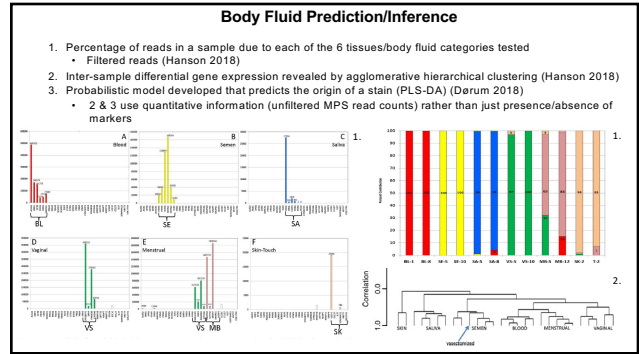
Organ tissue

- ORGAN 46plex Illumina MiSeq (10 organ tissues) (Hanson et al 2017)

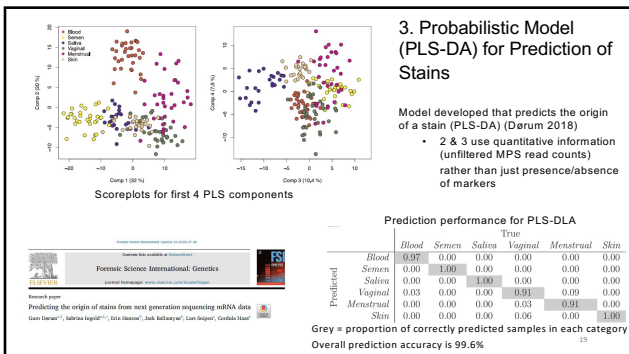
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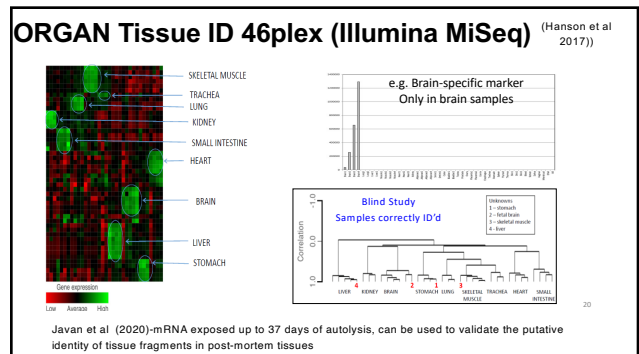
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3. Assigning body fluids to DNA donors by cSNP typing



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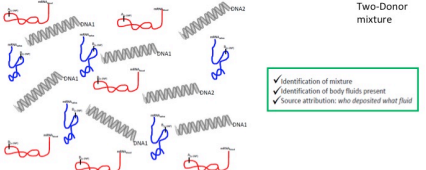
The Problem: how can cell types and DNA donors be associated?

- Need to associate body fluid with a donor DNA profile (needed to evaluate source versus sub-source level propositions)
- "If there is uncertainty about the association of the body fluid with the DNA profile, then the strength of the evidence is always reduced" (Gill, 2014)
- "Association fallacy" (Gill, 2014)
- "discourage" associating cell types and donors from peak heights when performing RNA and DNA profiling (Sijen et al 2013)

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Two-Donor mixture



Identification of mixture
 Identification of body fluids present
 Source attribution: who deposited what fluid

Case:	DNA	RNA-SNP _{blood}	RNA-SNP _{saliva}	
Observed	13,15,17,18	1200 (A allele count-650) (B allele count-550)	800 (A allele count-10 ~ background noise) (B allele count-790)	
Interpretation	13,15 17,18 17,17 15,18 13,18 17,17	Blood present Donor Genotype: AB	Saliva present Donor Genotype: BB	
Reference:	DNA	DNA cSNP blood	DNA cSNP saliva	Body fluid source
Donor 1	13,15	AB	AA	Blood
Donor 2	17,18	BB	BB	Saliva

Association of DNA profiles in mixtures with specific body fluids using cSNPs in bf-specific mRNA transcripts

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Genes	BFID-cSNP-BSS		BFID-cSNP-6F	
	BFID	cSNP	BFID	cSNP
BD	ANK1	4	4	2
	CD3G	1	1	1
	SPTB	4	4	4
SE	PRM1	1	1	1
	SEMG2	1	1	1
	KLK3	2	2	2
	TGM4	4	4	4
SA	HTN3	3	3	3
	PRB4	1	1	1
	PRH2	1	1	1
	MUC7	1	1	1
VS	STATH	1	1	1
	CYP2A6	1	1	1
	MUC22	1	1	1
	CYP2B7P1	1	1	1
MB	MMP10	1	1	1
	MMP3	1	1	1
	COL5A3	1	1	1
	COL12A1	1	1	1
	LEFTY2	1	1	1
SK	LCE1C	1	1	1
	COL17A1	1	1	1
	IL37	1	1	1
Total	19 genes (BFID)	23 cSNPs (11 genes)	23 genes (BFID)	46 cSNPs (20 genes)
	cSNP microarrays: 3 hi-local, 1 tri-local		cSNP microarrays: 8 hi-local, 3 tri-local	

Dual Function Targeted S5 RNA Sequencing Assay for the Identification and Direct Association of Common Body Fluids with DNA Donors in Mixtures

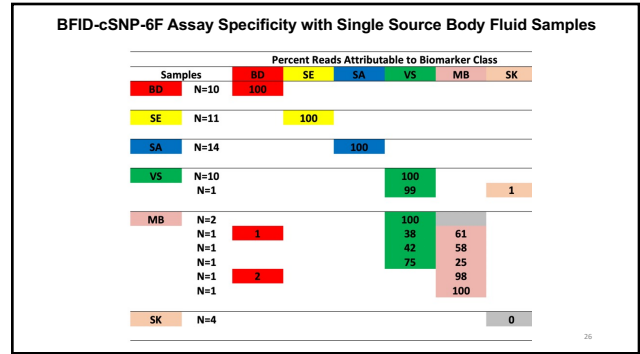
Associated gDNA-cSNP assays for reference samples..cSNP genotypes obtained for all mRNA targets

Targeted S5 RNA sequencing assay for the identification and direct association of common body fluids with DNA donors in mixtures

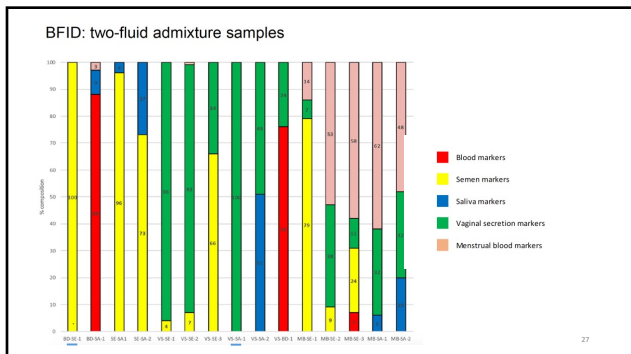
Original Article: <https://doi.org/10.1093/bioinformatics/btq208>

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A "RECIPROCAL" mixture pair comprises the following two mixtures (semen/saliva): DNA donor A / body fluid 1 - DNA donor B / body fluid 2
DNA donor A / body fluid 2 - DNA donor B / body fluid 1

BFID-cSNP-BSS

D1 ref	Semen (D1) - Saliva (D2)				D1 ref	Saliva (D1) - Semen (D2)				D1 ref		
	Observed	cSNP	Genotype	Coverage		Observed	cSNP	Genotype	Coverage			
CG	ANK1_r0504734	CC	CC	0	CG	ANK1_r0504734	CC	0	CG	ANK1_r0504734	CC	0
CC	ANK1_r07818734	CC	CC	0	CC	ANK1_r07818734	CC	0	CC	ANK1_r07818734	CC	0
TT	CD3G_r0753069	TT	TT	0	TT	CD3G_r0753069	TT	0	TT	CD3G_r0753069	TT	0
AG	SPTB_r0259386	AG	AG	0	AG	SPTB_r0259386	AG	0	AG	SPTB_r0259386	AG	0
TT	SPTB_r0741487	TT	TT	0	TT	SPTB_r0741487	TT	0	TT	SPTB_r0741487	TT	0
GG	SPTB_r0741488	GG	GG	0	GG	SPTB_r0741488	GG	0	GG	SPTB_r0741488	GG	0
TT	SPTB_r0259386	TT	TT	0	TT	SPTB_r0259386	TT	0	TT	SPTB_r0259386	TT	0
AA	PRM1_r0733068	AA	AA	34,703	AA	PRM1_r0733068	CC	59,684	CC	PRM1_r0733068	CC	59,684
AC	SEMG2_r0231906	AC	AC	9073	AC	SEMG2_r0231906	CC	4959	AC	SEMG2_r0231906	CC	4959
TT	KLK3_r011573	TT	TT	23,473	TT	KLK3_r011573	TT	6742	TT	KLK3_r011573	TT	6742
AA	KLK3_r0115736	AA	AA	23,551	AA	KLK3_r0115736	AA	6760	AA	KLK3_r0115736	AA	6760
CT	TGM4_r0199540	CT	CT	40,669	CT	TGM4_r0199540	CT	15,074	CT	TGM4_r0199540	CT	15,074
AG	TGM4_r0199541	AG	AG	24,789	AG	TGM4_r0199541	AG	14,736	AG	TGM4_r0199541	AG	14,736
CT	TGM4_r0749195	CT	CT	21,647	CT	TGM4_r0749195	CT	4874	CT	TGM4_r0749195	CT	4874
AG	TGM4_r0749195	AG	AG	21,095	AG	TGM4_r0749195	AG	4907	AG	TGM4_r0749195	AG	4907
CC	HTN3_r0184997	CC	CC	59,961	CC	HTN3_r0184997	CC	26,202	CC	HTN3_r0184997	CC	26,202
TT	HTN3_r0136115	TT	TT	57,600	TT	HTN3_r0136115	TT	25,642	TT	HTN3_r0136115	TT	25,642
CC	HTN3_r0760764	CC	CC	60,241	CC	HTN3_r0760764	CC	20,668	CC	HTN3_r0760764	CC	20,668
CC	PRB4_r01052308	CC	CC	14,613	CC	PRB4_r01052308	CC	9709	CC	PRB4_r01052308	CC	9709
CT	PRB4_r0772391	CT	CT	22,477	CT	PRB4_r0772391	CT	3258	CT	PRB4_r0772391	CT	3258
CC	MUC7_r0236948	CC	CC	10,022	CC	MUC7_r0236948	CC	2486	CC	MUC7_r0236948	CC	2486

-All three binary mixture possibilities of the commonly encountered BL, SE, SA (BL/SA, BL/SE, SE/SA) were tested for 'DNA association' using cSNPs

-cSNP analysis correctly associated one of the body fluids with one donor and the other body fluid with the other donor, while at the same time excluding the alternative possibility

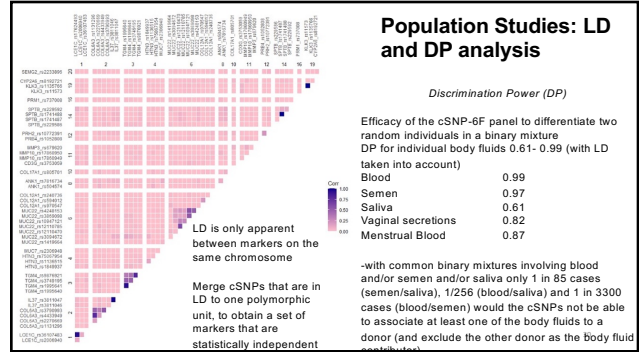
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cSNP: donor association for 2-person mixtures

BFID-cSNP-6F

Mixture	Gene	cSNP	Donor 1	Obs	Donor 2
VSS-BD-1 Donor 1 - FS Donor 2 - BD	ANKK1	rs594574	CG	CC	CG
		rs7816734	GG	AG	GG
	CD3G	rs3753659	TT	TT	TT
	SPTB	rs1741488, rs1741487	CATG	TCGTG	TCGTG
		rs229592	AA	AG	AA
MUC22		rs386998, rs4248153	GG/GA	GG/GA	GG/AA
		rs4419664, rs3094672	CACA	CACA	CA/CC
		rs12110470, rs12110785	TC/CC	TC/TC	GT/GC
		rs10947121	CC	CC	TT
		rs157573, rs1135766	TACG	TACG	TACG
VSS-SE-3 Donor 1 - FS Donor 2 - SE	KLK13	rs57008	CG	TT	TT
	PRM1	rs2233896	CC	CC	CC
	SEM62	rs1995640	CT	CT	CT
	TGM4	rs1995641	AG	AG	AG
	MUC22	rs386998, rs4248153	AA/GG	AA/GG	AA/AA
VSS-SA-2 Donor 1 - FS Donor 2 - SA	H1NS	rs1869927, rs1136515	CT/CT	CT/CT	CT/CT
	MUC7	rs75067954	CC	CC	CC
	PRB4	rs1052808	CG	GG	GG
	PRH2	rs1077239	CT	CT	CT
	MUC22	rs386998, rs4248153	AA/AA	AA/AA	AG/GG
	rs4419664, rs3094672	CT/TA	CT/TA	CA/TA	
	rs12110470, rs12110785	CT/CT	GT/CT	GT/CT	
	rs10947121	TT	TT	CT	

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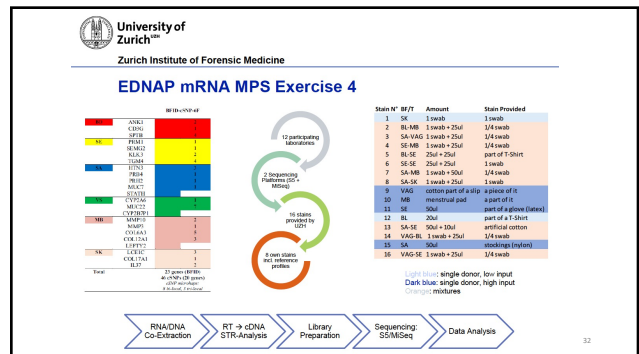
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Bespoke (TF) Software Solution for cSNP and BFID calling


Targets	Percentage	Coverage
Blood	99.26	100.00
Menstrual	0.72	79.2
Saliva	0	0
Semen	0.02	29.1
skin	0	0
Vaginal	0	0
Total Coverage		100.776

SN Target	total/all	target/gene	mh counts	target	gene	mh.gt	mh counts	r_s_65	position	chr	coverage
Blood_01_ANKK1_1650574_3369	15.24	15.16	15111	Blood	ANKK1	TG/TC	8409/7103	-0.96174	3369	CG	7925/8409
Blood_02_ANKK1_17810734_3981	17.60	17.78	17962	Blood	ANKK1	GC	17962	0.7823734	3981	CG	17962/17962
Blood_03_CD3G_1873959_470	11.69	11.17	11395	Blood	CD3G	CT	11395	0.9730939	470	CT	11395/11395
Blood_04_SPTB_21741488_1741487_5339_3545	15.51	15.61	15186	Blood	SPTB	ACA/ATG	7646/6100	0.7414488	5339	CT	7646/6100
Blood_05_SPTB_21741488_1741487_5339_3545	15.51	15.61	15186	Blood	SPTB	ACA/ATG	7646/6100	0.7414488	5339	CT	7646/6100
Blood_06_SPTB_21741488_1741487_5339_3545	15.51	15.61	15186	Blood	SPTB	ACA/ATG	7646/6100	0.7414488	5339	CT	7646/6100
Blood_07_SPTB_21741488_1741487_5339_3545	15.51	15.61	15186	Blood	SPTB	ACA/ATG	7646/6100	0.7414488	5339	CT	7646/6100
Blood_08_SPTB_21741488_1741487_5339_3545	15.51	15.61	15186	Blood	SPTB	ACA/ATG	7646/6100	0.7414488	5339	CT	7646/6100
Blood_09_SPTB_21741488_1741487_5339_3545	15.51	15.61	15186	Blood	SPTB	ACA/ATG	7646/6100	0.7414488	5339	CT	7646/6100
Blood_10_SPTB_21741488_1741487_5339_3545	15.51	15.61	15186	Blood	SPTB	ACA/ATG	7646/6100	0.7414488	5339	CT	7646/6100
Menstrual_01_LEP172	0.03	4.1	30	Menstrual	LEP172	CC	30	0	117802950	1255	0.03
Menstrual_02_MMP9	0	0	0	Menstrual	MMP9		0	0	117802950	1255	0
Menstrual_03_COL12A1	0	0	0	Menstrual	COL12A1		0	0	117802950	1255	0
Menstrual_04_COL12A1	0	0	0	Menstrual	COL12A1		0	0	117802950	1255	0
Menstrual_05_COL12A1	0	0	0	Menstrual	COL12A1		0	0	117802950	1255	0

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University of Zurich
 Zurich Institute of Forensic Medicine



Summary

- Overall promising results
- Some participants did not (fully) follow the recommendations
- Laboratories with limited RNA experience also achieved good results
- Results were quite consistent across different laboratories
- The cSNP panels performed well on both sequencing platforms
- Comparison with Cologne cSNP panel (31 body fluid markers, 80 cSNPs)

> Manuscript draft will be circulated among participants soon
 > Poster presentation at 30. ISFG congress

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Sum Up and Final Thoughts

- **The cell is a bag of RNA (Stephen Quake 2021)**
- **RNA profiling can identify with a high degree of specificity the cellular phenotypes of the biological material yielding crime scene DNA profiles**
- **Genomic information is directly encoded and faithfully reproduced in mRNA transcripts. MPS technology enables this genomic encoded RNA sequences to be accessed.**
- **Direct association of common body fluids with DNA donors in mixtures can be accomplished via cSNP sequencing analysis**
 - cSNPs represent an additional class of SNPs useful in forensic genetics (in addition to Identity, Ancestry, Lineage Marker and PhenotypeSNPs)
 - cSNPs are an addition to the DNA mixture deconvolution toolbox
 - movement towards increasing #RNA-SNPs towards human identification?
- **Access to the RNA and DNA cSNP assay primer pools and analysis software should be made available in the near future via ThermoFisher Community Panels**

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Cordula Haas
 Guro Dørum (right),
 Sabrina Ingold (left)
 Nadescha Hänggi (insert)
 (Zurich)

Erin Hanson
UCF

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ThermoFisher
 Robert Lagacé


 Chantal Roth

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Relevant Grants and Awards

Messenger RNA Profiling: A Prototype Method to Supplant Conventional Methods for Semen Identification
FBI JFB103287/A3C0328700 (UCF 11-64-6021) (2003-2005)

Who and How: Comprehensive RNA-based Bodyfluid Assay to Provide Context to a Recovered DNA Profile
Department of the Army W911NF-16-2-0018 (2012-2014)

How it got there: associating individual DNA profiles with specific body fluids in mixtures using targeted digital gene expression and RNA-SNP identification National Institute of Justice 2014-DN-BX-K019 (2015 -2017)

Human Organ Tissue Identification by Targeted RNA Deep Sequencing to Aid in the Investigation of Shooting and Other Traumatic Bodily Injury Incidents National Institute of Justice 2016-DN-BX-0165 (2017-2019)

Comparative Assessment of Emerging Technologies for Body Fluid Identification
National Institute of Justice 2020-DQ-BX-0015 (2021-2023)

Transfer, Persistence and DNA Source Attribution of Trace Biological Material in Digital Penetration Assault Cases National Institute of Justice 15PNIJ-21-GG-04147-RESS (2022-2024)

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Thank you for your attention!



Any Questions?

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