AGENDA FOR THE EDNAP MEETING

LISBON 27 SEPTEMBER 2022

Expected duration: 09.00 - 17.00

Coffee: 10.30-11.00 – Lunch: 12.30-13.30 – Coffee: 15.30-16.00

Host:
Chairman: Niels Morling

Welcome	Carlos Farinha
To Commemorate the Dead	Niels Morling
Update on activities	
Methylated DNA and age exercise	Denise Synderc. Court
Exercise no. Three on mRNA typing with MPS	Cordula Haas
Proposition for exercise no. 4 on cSNPs for vaginal secretion, menstrual blood, and skin	Cordula Haas
mtDNA quantification exercise	Arnoud Kal
The series of exercises relating to DNA transfer	Baas Kokshoorn
Collaborative exercise on detection of mtDNA heteroplasmy by MPS	Walther Parson
Updates from other groups	
The VISAGE project	Walther Parson
ISFG	Walther Parson
EMPOP	Walther Parson
ENFSI	Sander Kneppers
Presentations	
MPSproto: Analysis of mixtures using a novel open-source probabilistic genotyping model	Peter Gill
Bayesian network for combined analysis of mRNA vaginal mucosa and STR markers	Peter Gill
An improved method for estimating the amount of DNA	Peter Gill
Future activities	
Please see above about mRNA exercise no. 4	
Next EDNAP meeting	Niels Morling
The date and place of the next EDNAP meeting is to be decided	Niels Morling
Any other business	

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EUROPEAN DNA PROFILING GROUP (EDNAP) MEETING

Lisbon, Portugal

27 September 2022

Host: Sandra Cristina Costa
Chairman: Niels Morling

A list of participants is attached.

Welcome
Carlos Farinha welcomed members to Lisbon.

To Commemorate the Dead Niels Morling
Niels Morling uttered words of remembrance of Peter Schneider (31 May 1955 – 9 September 2022), who passed away after a long illness. Peter Schneider was one of the founding members of EDNAP.

Update on exercises

Second exercise on methylated DNA and age Denise Syndercombe Court
Denise Syndercombe Court informed members that a manuscript will be circulated as soon as possible.

Exercise no. 3 on mRNA typing with MPS Cordula Haas
Cordula Haas gave a brief overview of the results (presentation attached). Jack Ballantyne, Cordula Haas, and their groups have collaborated with Thermo Fischer Scientific on an extended cSNP assay, BFID-cSNP-6F, with 23 body fluid markers and 46 cSNPs (a manuscript is submitted), which will be tested in exercise no. 4 (cf. below). When the results of exercises 3 and 4 on mRNA typing with MPS are analysed, it will be discussed if there is enough data for publication.

mtDNA quantification exercise Arnoud Kal
Arnoud Kal gave a summary of the results. The colleagues in NFI will discuss if they find the results should be published (presentation attached).

The series of exercises relating to DNA transfer Baas Kokshoorn
Bas Kokshoorn summarised the framework of the series of collaborative exercises that will be organised by Bas Kokshoorn, The Netherlands, Bianca Szkuta, and Roland van Oorschot, Australia. Members who have expressed interest in participation will be approached again (presentation attached).

Collaborative exercise on detection of mtDNA heteroplasmy by MPS Walther Parson
Walther Parson provided an update on the heteroplasmy exercise. All results and raw data were sent to Innsbruck, where the team is currently analyzing the data. An update will be provided at the next EDNAP meeting.

Updates from other groupsP

The VISAGE project Walther Parson
Walther Parson gave an update on work on Forensic DNA Phenotyping within the EU-funded projects VISAGE and INFER (presentation attached).

EMPOP Walther Parson
Walther Parson gave an update on mtDNA and EMPOP (presentation attached).

ISFG Walther Parson
Walther Parson gave an update on the activities of the ISFG (presentation attached).

ENFSI Sander Kneppers
Sander Kneppers reported from the ENFSI DNA Working Group (presentation attached).

Presentations
MPSproto: Peter Gill
Peter Gill presented a new open-source probabilistic genotyping tool for the analysis of mixtures and non-mixtures (presentation attached).

mRNA & STRs Peter Gill
Peter Gill presented a bayesian network tool for the combined analysis of mRNA vaginal mucosa and STR markers (presentation attached).

DNA quantification with an improved method Peter Gill
Peter Gill presented a new DNA quantification method based on the RFUs of electropherograms (presentation attached).

Future activities

New collaborative exercise on mRNA and cSNP typing using TFS S5 Cordula Haas
Cordula Haas presented an updated proposal for a collaborative exercise on identifying donors of body fluids using mRNA and cSNPs with the IonTorrent S5 assay. The exercise will most likely begin in late 2022. EDNAP members, who are interested in participation, should contact Cordula Hass as soon as possible.

Next meetings Niels Morling
The date and place of the next EDNAP meeting have not yet been decided.

Any other business Niels Morling
There was no other business.

Closing of the meeting Niels Morling
The meeting closed with sincere thanks to Sandra Cristina Costa and all colleagues who organised the meeting.

The minutes and attachments are found at the EDNAP website:
http://www.isfg.org/EDNAP/Meetings, including:

- Agenda
- List of participants
- Group photo
- Minutes
- Presentations
  - Niels Morling: To commemorate Peter Schneider
  - Cordula Haas: Update on collaborative exercises on mRNA NGS
  - Arnoud Kal: Update on the mtDNA quantification exercise
  - Walther Parson: The VISAGE project
  - Walther Parson: EMPOP report
  - Walther Parson: ISFG report
  - Bas Kokshoorn: Series of exercises relating to DNA transfer
  - Sander Kneppers: Report from the ENFSI DNA Working Group
  - Peter Gill: MPSproto
  - Peter Gill: mRNA vaginal mucosa and STR markers
  - Peter Gill: DNA quantification.

Peter Matthias Schneider

31 May 1955 – 9 September 2022

EDUCATION, POSITIONS, AND PRIZE

1983: MSc biology - University of Bonn

1984-1986: Research fellow - Harvard Medical School

1987: PhD - University of Mainz

1996: Dr.rer.nat. - University of Mainz

1996: Assistant professor – University of Mainz

2004: Full professor and head of the Division of Forensic Molecular Genetics, Institute of Legal Medicine, University of Cologne

2006: The prize of the German Konrad Händel Foundation for his outstanding scientific achievements and his merits in the field of the administration of justice

BOARD MEMBERSHIPS AND HONORARY APPOINTMENTS

1989:	Founding member of the European DNA Profiling (EDNAP) Group
Since 2000:	Executive board member of the International Society for Forensic Genetics (ISFG)
Since 2000:	Member of the German Stain Commission, a joint commission of Institutes of Legal Medicine and Forensic Science, and chairman of the commission since 2010
2004–2007:	President of the ISFG
Since 2007:	Associate editor of Forensic Science International: Genetics[20]
2008–2011:	Vice president of the ISFG
2009–2018:	Member of the German Commission on Genetic Testing at the Robert Koch Institute
Since 2014:	Secretary of the ISFG
Since 2020:	Member of the Committee on Investigative Genetic Genealogy of the Scientific Working Group on DNA Analysis Methods (SWGDAM)

LARGER COLLABORATIVE PROJECTS

2002-2005:
High Throughput Analysis of Single Nucleotide Polymorphisms for the Identification of Persons – SNPforID

2012–2016:
European Forensic Genetics Network of Excellence - EUROFORGEN-NoE.

2017 -2022:
Work package leader in the VISible Attributes Through GEnomics -- VISAGE Consortium (Horizon 2020 funded EU project)

Peter Matthias Schneider

31 May 1955 – 9 September 2022

University of Zurich
Zurich Institute of Forensic Medicine

EDNAP mRNA MPS collaborative exercise 3 - IonTorrent S5 (BFID-cSNP-BSS*)

*BSS stands for
blood, semen, saliva

Cordula Haas, Nadescha Hänggi, Rob Lagace, Erin Hanson, Jack Ballantyne

EDNAP Meeting, 27. September 2022, Lisbon

EUROFORGEN Network of Excellence
EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

EDNAP mRNA MPS Exercise 3

- **BFID-cSNP-BSS RNA assay**
  - identification of blood, saliva, semen, vaginal secretion, menstrual blood, skin
  - including cSNPs to associate specific mRNA transcripts to an individual (blood, saliva, semen)
- **BFID-cSNP-BSS DNA assay** for reference persons

  (→ cSNP genotypes)
- Protocols and primer pools were provided by UZH

Gene	cSNP
ANK1	Blood_01_ANK1
	Blood_02_ANK1
CD3G	Blood_03_CD3G
SPTB	Blood_05_SPTB
	Blood_04.0_SPTB
	Blood_04.1_SPTB
	Blood_06_SPTB
PRM1	Semen_02_PRM1
TGM4	Semen_04_TGM4
	Semen_05_TGM4
	Semen_06.0_TGM4
	Semen_06.1_TGM4
SEMG2	Semen_03_SEMG2
KLK3	Semen_01.0_KLK3
	Semen_01.1_KLK3
HTN3	Saliva_01.0_HTN3
	Saliva_01.1_HTN3
	Saliva_01.2_HTN3
PRB4	Saliva_03_PRB4
PRH2	Saliva_04_PRH2
MUC7	Saliva_02_MUC7
STATH	Saliva_05_STATH
CYP2B7P	CYP2B7P1
CYP2A6	CYP2A6
MMP10	MMP10
LEFTY2	LEFTY2
LCE1C	LCE1C
COL17A1	COL17A1
IL37	IL37

Targets in primer pool BSS

EDNAP mRNA MPS Exercise 3

- 16 stains provided by UZH
- 8 own single source and/or mixed body fluid stains

  up to 8 own reference DNA samples (for assignment with donor)
- RNA extraction (manual or kit), DNase treatment, RNA quant, RT, manual or automated library prep, sequencing
- DNA extraction of reference samples, DNA quant, manual or automated library prep, sequencing
- Participating Laboratories:
  - Institute of Forensic Medicine, University Medical Center Cologne, University of Cologne, Germany
  - National Center for Forensic Science, University of Central Florida (UCF), USA
  - Institute of Forensic Sciences, DNA department, Bavarian State Criminal Police Office, Germany
  - Departement of Forensic Sciences, Oslo University Hospital, Norway
  - Institute of Legal Medicine, Innsbruck Medical University, Austria
  - Institute of Forensic Medicine, University of Zurich, Switzerland
  - LKA Wiesbaden did not hand in results

Composition of Stains n° 1-16

Nr	BF	Details stain
1	SE	10 µl Boxer
2	BL-MB	1/2 Swab + 25 µl
3	SE	50 µl Zellette
4	SA-SE	T-shirt (50 µl + 25 µl)
5	BL	50 µl swab
6	SK	swab
7	BL-BL	25 µl + 25 µl on T-shirt
8	SA	Licked plastic spoon
9	SA-SA	25 µl + 25 µl on Swab
10	BL-SA	25 µl +25 µl
11	SA	50 µl T-shirt
12	VAG	1/2 swab
13	BL	Nose bleed on tissue
14	SA-SE	Boxer (25 µl + 25 µl)
15	MB	1/2 swab
16	SE-VAG	½ Swab (25 µl SE)

Light blue: single donor, low input
Dark blue: single donor, high input
Orange: mixtures

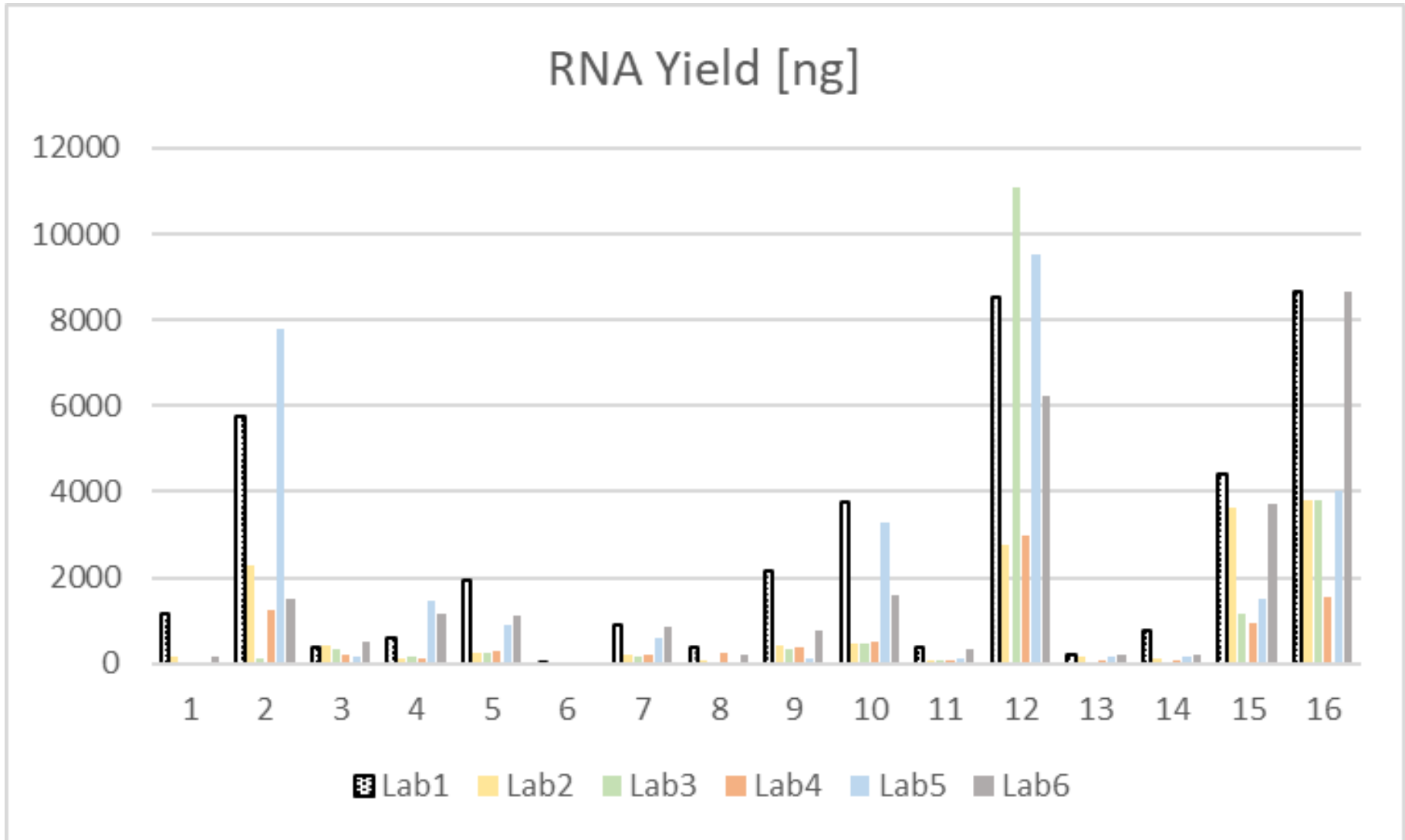
Methods & Quantification Results

Laboratory Methods

- DNA extraction of reference samples: any Kit
- DNA quantification: e.g. Quantifiler® Trio DNA Quantification Kit

- RNA or DNA/RNA co extraction of stains
- DNAse treatment: TURBO DNA-free Kit
- RNA quantification (recommended)
- Reverse Transcription (RNA): SuperScript™ IV VILO™ Master Mix

- **Manual** library preparation (RNA and DNA): Ion AmpliSeq™ library Kit 2.0 or Precision ID Library Kit
- **Automated** library preparation on IonChef (RNA and DNA): Precision ID DL8 kit or Ion AmpliSeq™ Kit for Chef DL8
- Ion Chef template preparation and Ion S5 sequencing
  - Ion S5™ Precision ID Chef & Sequencing Kit or Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef
  - 2x 510 or 1x 520 chips


RNA Yield [ng]
12000
10000
8000
6000
4000
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0
1
2
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10
11
12
13
14
15
16
Lab1
Lab2
Lab3
Lab4
Lab5
Lab6

Data Analysis Methods

- Ion Torrent's TMAP alignment program > aligned BAM/BAI Files

- multiple sequence alignment algorithm:
  - all SNPs positions of the targeted microhaplotype need to be present
  - removes contaminating genomic DNA (alignment gap parameters)
  - the sequences are phased and the microhaplotype genotypes identified
  → sequence coverage and cSNP genotypes

- Body fluid identification:
  - Threshold (0.5% of total reads) to identify sporradic reads
  (put back to zero in mh counts corrected)

- Assignment of body fluids with donors:
  - Comparison of cSNP genotypes based on RNA-Seq with DNA references (DNA genotypes)

Results of Body Fluid Identification for stains n° 1-16

BFID - Stains n° 1-4

Actual Body Fluids: SE MB-BL SE SA-SE

mh counts corrected	Lab1_1	Lab2_1	Lab3_1	Lab4_1	Lab5_1	Lab6_1	Lab1_2	Lab2_2	Lab3_2	Lab4_2	Lab5_2	Lab6_2	Lab1_3	Lab1_3.2	Lab2_3	Lab3_3	Lab4_3	Lab5_3	Lab6_3	Lab1_4	Lab1_4.2	Lab2_4	Lab3_4	Lab4_4	Lab5_4	Lab6_4
Blood_01_ANK1	75	0	0	0	0	0	458	7802	5344	3596	2111	40731	0	0	0	0	0	0	0	80	0	0	0	0	0	0
Blood_02_ANK1	72	0	0	0	0	0	0	5925	0	7317	1947	42511	0	0	0	0	0	0	0	90	0	0	0	0	0	0
Blood_03_CD3G	72	0	0	0	0	0	0	6936	10204	12845	3220	45910	0	0	0	0	0	0	0	75	0	0	0	0	0	0
Blood_04_SPTB	59	0	0	0	0	0	0	5073	0	0	0	21990	0	0	0	0	0	0	0	68	0	0	0	0	0	0
Blood_05_SPTB	90	0	0	0	0	0	0	7613	5687	6102	3764	41938	0	0	0	0	0	0	0	132	73	0	0	0	0	0
Blood_06_SPTB	0	0	0	0	0	0	0	2956	0	0	0	9651	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_01_LEFTY2	0	0	0	0	0	0	815	11077	2170	5215	3841	28924	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_02_MMP10	121	0	0	0	0	0	69912	429769	252563	517502	167715	1000255	0	0	0	0	0	0	574	0	0	0	0	0	0	0
Saliva_01_HTN3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saliva_02_MUC7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saliva_03_PRB4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saliva_04_PRH2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saliva_05_STATH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_01_KLK3	0	0	0	0	0	5594	0	0	0	0	0	0	1360	2874	2816	23870	14661	0	19619	0	0	0	0	0	0	0
Semen_02_PRM1	5212	467415	542649	175175	252016	626626	0	0	0	0	0	0	11900	64382	165359	554802	192577	8426	75454	9392	11312	4548	204133	175825	308273	619222
Semen_03_SEMG2	0	0	0	909	0	0	0	0	0	0	0	0	0	598	1441	0	0	0	4137	127	84	0	0	0	0	0
Semen_04_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	368	1504	0	13089	11730	0	3906	0	0	0	0	0	0	0
Semen_05_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	346	1699	920	0	2611	0	5716	81	0	0	0	0	0	0
Semen_06_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_01_COL17A1	0	0	0	0	0	0	0	0	0	0	0	9101	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_02_IL37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_03_LCE1C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal_01_CYP2A6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal_02_CYP2B7P1	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Number of Reads	5768	467415	542649	176084	252016	632220	71185	477151	275968	552577	182598	1241011	13974	71057	170536	591761	221579	8426	109406	10045	11469	4548	204133	175825	308273	619222

Predicted Body Fluids: SE MB/ MB-BL SE SE, SA missing

BFID - Stains n° 5-8

Actual Body Fluids: BL SK BL SA

mh counts corrected	Lab1_5	Lab2_5	Lab3_5	Lab4_5	Lab5_5	Lab6_5	Lab1_6	Lab2_6	Lab3_6	Lab4_6	Lab5_6	Lab6_6	Lab1_7	Lab2_7	Lab3_7	Lab4_7	Lab5_7	Lab6_7	Lab1_8	Lab1_8.2	Lab2_8	Lab3_8	Lab4_8	Lab5_8	Lab6_8
Blood_01_ANK1	18944	30362	264808	42106	43353	104847	0	397	37	14	0	0	15264	193668	276953	42371	26905	103086	38	0	0	6	0	0	0
Blood_02_ANK1	21879	21256	91691	67002	67053	4625	0	303	11	34	11	0	13953	146075	45023	99602	48995	4428	58	5	0	0	0	0	0
Blood_03_CD3G	17574	47864	193875	183817	40114	19213	5	721	13	188	9	0	14585	327537	91427	176501	40668	23212	64	6	0	0	83	0	0
Blood_04_SPTB	11832	20721	80767	29810	26828	6145	0	217	9	26	0	0	4846	96702	38788	26781	14053	4154	47	0	0	0	0	0	0
Blood_05_SPTB	29806	39722	399559	97440	93931	83555	0	470	49	29	10	0	20405	223585	331782	99302	48910	53474	59	6	0	11	0	0	0
Blood_06_SPTB	8734	13989	0	3949	7584	0	0	125	0	0	0	0	2441	56378	0	8165	4331	0	24	0	0	0	0	0	0
Menstrual_01_LEFTY2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0
Menstrual_02_MMP10	0	0	0	0	0	0	38	0	0	104	0	0	0	0	0	0	0	0	6	144	53	0	195	0	0
Saliva_01_HTN3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0	2941	0	0
Saliva_02_MUC7	0	0	0	0	0	0	0	0	0	49	38	0	0	0	0	0	0	0	20	0	97	0	10136	16	4188
Saliva_03_PRB4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	113	0	0
Saliva_04_PRH2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69	0	537	0	65
Saliva_05_STATH	0	0	0	0	0	0	0	0	0	269	0	0	0	0	0	0	0	0	0	0	116	0	1964	0	679
Semen_01_KLK3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	807
Semen_02_PRM1	0	2556	0	0	0	0	0	37	96	190	38	17	0	10931	0	0	0	0	50	0	4708	7	0	29	3168
Semen_03_SEMG2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_04_TGM4	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	781
Semen_05_TGM4	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	5	0	0	0	0	0	85
Semen_06_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_01_COL17A1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_02_IL37	0	0	0	0	0	0	0	0	495	294	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_03_LCE1C	0	0	0	0	0	0	0	0	373	497	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal_01_CYP2A6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal_02_CYP2B7P1	0	0	0	0	0	0	7	0	0	105	0	0	0	0	0	0	0	0	133	6	0	0	0	0	616
Total Number of Reads	108769	176470	1030700	424124	278863	218385	50	2270	1083	1799	106	32	71494	1054876	783973	452722	183862	188354	509	175	5080	24	15969	45	10389

Predicted Body Fluids: BL Difficult! skin, blood? BL SA

BFID - Stains n° 9-12

Predicted Body Fluids: SA BL-SA SA VAG

mh counts corrected	Lab1_9	Lab2_9	Lab3_9	Lab4_9	Lab5_9	Lab6_9	Lab1_10	Lab2_10	Lab3_10	Lab4_10	Lab5_10	Lab6_10	Lab1_11	Lab1_11.2	Lab2_11	Lab3_11	Lab4_11	Lab5_11	Lab5_11	Lab1_12	Lab2_12	Lab3_12	Lab4_12	Lab5_12	Lab6_12
Blood_01_ANK1	0	0	0	0	0	0	693	1736	39201	8181	2856	35281	0	0	0	0	0	0	0	0	41	0	0	0	0
Blood_02_ANK1	0	0	0	0	0	0	900	1213	5465	14366	3671	19766	0	6	0	0	0	0	0	0	0	0	0	0	0
Blood_03_CD3G	0	0	0	0	0	0	3902	12066	161555	143576	14621	161872	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood_04_SPTB	0	0	0	0	0	0	345	984	3412	6043	843	27872	0	8	0	0	0	0	0	0	0	0	0	0	0	0
Blood_05_SPTB	0	0	0	0	0	0	710	1313	16679	12496	3747	35442	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Blood_06_SPTB	0	0	0	0	0	0	316	744	0	1854	827	1688	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_01_LEFTY2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_02_MMP10	0	0	0	0	0	0	0	0	0	0	0	0	216	47	0	0	0	0	0	0	78	0	1529	0	0
Saliva_01_HTN3	6059	8955	0	30897	0	51496	128	1070	0	5184	546	0	149	0	2664	48881	2469	0	6399	0	0	0	0	0	0
Saliva_02_MUC7	9488	1436	191932	28940	6075	86195	445	1383	23694	21296	2207	13424	2784	194	786	340408	151776	31142	208966	0	0	0	0	0	0
Saliva_03_PRB4	838	129	0	1635	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	0	0	0
Saliva_04_PRH2	1888	551	51450	7729	1486	26548	0	0	1420	1834	0	1747	62	0	218	29400	18780	1579	15255	0	0	0	0	0	0
Saliva_05_STATH	7884	5147	267186	37830	10375	148819	70	539	11967	7799	540	3611	715	134	1761	201324	148252	19790	101528	0	0	0	0	0	0
Semen_01_KLK3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_02_PRM1	0	675	0	0	0	0	0	412	0	0	0	0	0	0	841	0	0	0	0	0	51	0	0	0	0	0
Semen_03_SEMG2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_04_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_05_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_06_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_01_COL17A1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	177	221	9344	0	1349	2533
Skin_02_IL37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_03_LCE1C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	149	0	0	0	0	0
Vaginal_01_CYP2A6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2305	2843
Vaginal_02_CYP2B7P1	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	26779	5973	606456	246124	238697	364797
Total Number of Reads	26157	16893	510568	107031	17936	313058	7509	21460	263393	222629	29858	300703	3926	404	6307	620013	321277	52511	332148	26956	6513	615800	247653	242351	370173

Predicted Body Fluids: SA BL-SA SA VAG

BFID - Stains n° 13-16

Predicted Body Fluids: BL SA-SE MB SE-VAG

mh counts corrected	Lab1_13	Lab2_13	Lab3_13	Lab4_13	Lab5_13	Lab6_13	Lab1_14	Lab1_14.2	Lab2_14	Lab3_14	Lab4_14	Lab5_14	Lab6_14	Lab1_15	Lab2_15	Lab3_15	Lab4_15	Lab5_15	Lab6_15	Lab1_16	Lab2_16	Lab3_16	Lab4_16	Lab5_16	Lab6_16
Blood_01_ANK1	1052	5250	49	8015	9023	7258	10	48	0	0	0	0	0	0	0	0	0	0	13114	0	0	0	0	0	0
Blood_02_ANK1	40	3820	13	189	0	8240	18	83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood_03_CD3G	963	7938	298	7879	2853	12671	10	70	0	0	0	0	0	0	0	0	0	0	8064	0	0	0	0	0	0	0
Blood_04_SPTB	195	3009	16	170	144	3175	9	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood_05_SPTB	1479	5589	540	9485	5811	14542	20	91	0	0	0	0	0	707	0	0	0	0	8771	0	0	0	0	0	0	0
Blood_06_SPTB	0	698	0	0	0	0	8	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_01_LEFTY2	0	0	0	0	0	0	0	35	0	0	0	0	0	13854	110508	10121	34218	44602	103317	0	0	0	0	0	0	0
Menstrual_02_MMP10	0	0	273	1810	199	0	8	226	729	0	0	0	0	114133	920132	886796	399233	578083	776501	0	0	0	0	0	5917
Saliva_01_HTN3	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saliva_02_MUC7	0	0	0	442	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saliva_03_PRB4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saliva_04_PRH2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saliva_05_STATH	142	363	414	4863	7122	5432	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_01_KLK3	22	0	17	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	9040	6569	58447	35600	43038	50195	0
Semen_02_PRM1	49	3326	111	254	0	0	16	368	109640	483995	176138	126620	228487	0	0	0	0	0	0	32687	52123	215279	75917	181028	223154	0
Semen_03_SEMG2	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	633	0	0	4853	6531	0	0
Semen_04_TGM4	0	0	76	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	2924	2268	37706	16668	19788	55682	0
Semen_05_TGM4	0	0	21	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	3027	2576	10050	20414	26766	8396	0
Semen_06_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	385	1037	0	11450	9180	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	385	1037	0	11450	9180	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	385	1037	0	11450	9180	0	0
Skin_01_COL17A1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_02_IL37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_03_LCE1C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	706	0	0	0	0	0
Vaginal_01_CYP2A6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1046	658	0	2732	3595	0	0
Vaginal_02_CYP2B7P1	55	0	92	218	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	20522	15756	68131	47318	60324	41762	0
Total Number of Reads	3997	29993	1948	33325	25152	51318	110	1019	110369	483995	176138	126620	228487	128694	1030640	896917	433451	622685	909767	71034	83767	389613	237852	368610	385106	0

Predicted Body Fluids: BL SE, SA missing MB SE-VAG

Assignment of Body Fluids with Donors - stains n° 1-16

Single Donor Stains

Stain_1	Semen_02_PRM1	Semen_04_TGM4	Semen_05_TGM4	Semen_06.0_TGM4	Semen_06.1_TGM4	Semen_03_SEMG2	Semen_01.0_KLK3	Semen_01.1_KLK3
SE	PRM1	TGM4	TGM4	TGM4	TGM4	SEMG2	KLK3	KLK3
Donor genotype	TT	CT	AG	CC	GG	AC	CT	AG
Lab1_1	T=5212	T=15	A=6	-	-	-	C=13	G=13
Lab2_1	T=467415	C=52	G=66 A=15	-	-	C=94 A=50	C=234 T=206	G=234 A=206
Lab3_1	T=542649	C=445 T=300	G=787 A=129	C=18	G=18	C=533 A=276	T=1571 C=1026	A=1571 G=1026
Lab4_1	T=175175	C=76 T=46	G=94 A=83	C=13	G=13	C=590 A=319	T=346 C=275	A=346 G=275
Lab5_1	T=252016	-	-	-	-	-	-	-
Lab6_1	T=626626	C=595 T=352	G=1012 A=601	C=42	G=42	C=1359 A=490	T=2910 C=2684	A=2910 G=2864

Stain 1 (low input):
- high number of reads in some markers
- RNA cSNP genotype reflects donor genotype

Stain_3	Semen_02_PRM1	Semen_04_TGM4	Semen_05_TGM4	Semen_06.0_TGM4	Semen_06.1_TGM4	Semen_03_SEMG2	Semen_01.0_KLK3	Semen_01.1_KLK3
SE	PRM1	TGM4	TGM4	TGM4	TGM4	SEMG2	KLK3	KLK3
Donor genotype	TT	TT	AA	TT	AA	AC	CT	AG
Lab1_3	T=11900	T=368	A=346	-	-	C=18 A=8	T=694 C=666	A=694 G=666
Lab2_3	T=165359	T=584	A=920	T=259	A=259	C=848 A=593	T=1460 C=1356	A=1460 G=1356
Lab3_3	T=554802	T=13089	A=1730	-	-	C=164 A=66	C=13352 T=10518	G=13352 A=19518
Lab4_3	T=175175	C=76 T=46	G=94 A=83	C=13	G=13	C=590 A=319	T=346 C=275	A=346 G=275
Lab5_3	T=8426	T=9	-	-	-	-	-	-
Lab6_3	T=75454	T=3906	A=5716	T=242	A=242	C=2472 A=1665	C=10949 T=8670	G=10949 A=8670

Stain 3 (high input):
- high number of reads in some markers
- RNA cSNP genotype reflects donor genotype
  (discrepancies due to low number of reads)

Single Donor Stains

Stain_5	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_05_SPTB	Blood_04.0_SPTB	Blood_04.1_SPTB	Blood_06_SPTB
BL	ANK1	ANK1	CD3G	SPTB	SPTB	SPTB	SPTB
Donor genotype	CG	GG	TT	CC	AA	CC	AA
Lab1_5	C=9528 G=9416	G=21879	T=17574	C=29806	A=11832	C=11832	A=8734
Lab2_5	G=15935 C=14427	G=21256	T=47864	C=39722	A=20721	C=20721	A=13989
Lab3_5	C=135285 G=129523	G=91691	T193875	C399559	A=80767	C=399559	A=2730
Lab4_5	C=21667 G=20439	G=67002	T=183817	C=97440	A=29810	C=29810	A=3949
Lab5_5	C=22824 G=20529	G=67053	T=40114	C=93931	A=26828	C=26828	A=7584
Lab6_5	C=53051 G=51796	G=4625	T=19213	C=83555	A=6145	C=6145	A=363

Stain_13	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_05_SPTB	Blood_04.0_SPTB	Blood_04.1_SPTB	Blood_06_SPTB
BL	ANK1	ANK1	CD3G	SPTB	SPTB	SPTB	SPTB
Donor genotype	CG	GG	TT	CT	AA	CC	AG
Lab1_13	G=649 C=403	G=40	T=963	T=954 C=525	A=195	C=195	A=13
Lab2_13	G=2874 C=2376	G=3820	T=7938	C=2860 T=2729	A=3009	C=3009	A=698
Lab3_13	G=28 C=21	G=13	T=298	C=540	A=16	C=16	-
Lab4_13	G=5117 C=2898	G=189	T=7879	T=5589 C=3896	A=158 G=12	C=158 T=12	A=9
Lab5_13	G=4522 C=4501	G=91	T=2853	C=2967 T=2844	A=144	C=144	-
Lab6_13	C=3819 G=3439	G=8240	T=12671	C=8086 T=6456	A=3175	C=3175	A=159

Stain 5 (high input):
- high number of reads in all markers
- RNA cSNP genotype reflects donor genotype

Stain 13 (low input):
- relatively high number of reads execpt in SPTB
- RNA cSNP genotype reflects donor genotype

Single Donor Stains

Stain_8	Saliva_01.0_HTN3	Saliva_01.1_HTN3	Saliva_01.2_HTN3	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_02_MUC7	Saliva_05_STATH
SA	HTN3	HTN3	HTN3	PRB4	PRH2	MUC7	STATH
Donor genotype	TT	CC	CC	CG	CT	CT	
Lab1_8	-	-	-	-	-	C=13 T=7	-
Lab1_8.2	-	-	-	-	-	-	-
Lab2_8	T=37	-	-	-	C=69	C=97	-
Lab3_8	-	-	-	-	-	-	-
Lab4_8	T=2941	C=2941	C=2941	C=118	T=338 C=199	C=6691 T=3445	-
Lab5_8	-	-	-	-	-	C=16	-
Lab6_8	-	-	-	-	-	-	-

Stain 8 (low input):
- high number of reads in Lab 4
- RNA cSNP genotype mostly reflects donor genotype

Stain_11	Saliva_01.0_HTN3	Saliva_01.1_HTN3	Saliva_01.2_HTN3	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_02_MUC7	Saliva_05_STATH
SA	HTN3	HTN3	HTN3	PRB4	PRH2	MUC7	STATH
Donor genotype	CC	TT	CC	GG	CC	CT	
Lab1_11	C=149	T=149	C=149	G=14	C=62	T=1504 C=1280	-
Lab1_11.2	-	-	-	-	-	T=194	-
Lab2_11	C=2664	-	-	G=37	C=218	C=394 T=392	-
Lab3_11	C=48881	T=48881	C=48881	G=1745	C=29400	C=187784 T=152624	-
Lab4_11	C=2469	T=2469	C=2469	G=16	C=18780	C=86479 T=65297	-
Lab5_11	C=8	T=8	C=8	-	C=1579	C=16947 T=14195	-
Lab6_11	C=6399	T=6399	C=6933	G=206	C=15255	C=116980 T=91986	-

Stain 11 (high input):
- decent number of reads
- RNA cSNP genotype reflects donor genotype

Single Donor Stains

Stain_6	Skin_01_COL17A1	Skin_02_IL37	Skin_03_LCE1C
SK	COL17A1	IL37	LCE1C
Lab1_6	-	-	-
Lab2_6	-	-	-
Lab3_6	-	-	-
Lab4_6	-	-	-
Lab5_6	-	-	-
Lab6_6	-	-	-

Stain 6:
- no SKIN cSNPs in panel

Stain_12	CYP2A6	CYP2B7P1
VAG	CYP2A6	CYP2B7P1
Lab1_12	-	-
Lab2_12	-	-
Lab3_12	-	-
Lab4_12	-	-
Lab5_12	-	-
Lab6_12	-	-

Stain 12:
- no VAG cSNPs in panel

Stain_15	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_05_SPTB	Blood_04.0_SPTB	Blood_04.1_SPTB	Blood_06_SPTB	MMP10	MMP10	LEFTY2
MB	ANK1	ANK1	CD3G	SPTB	SPTB	SPTB	SPTB	MMP10	MMP10	LEFTY2
Donor genotype	CG	GG	TT	CC	AG	CT	AA			
Lab1_15	-	G=20	-	C=36	-	-	-	-	-	-
Lab2_15	C=361 G=170	G=334	T=1635	C=702	G=135 A=106	C=135 T=106	A=252	-	-	-
Lab3_15	C=2294 G=2120	G=49	T=3880	C=3566	G=33 A=25	T=33 C=25	-	-	-	-
Lab4_15	G=245 C=235	G=1211	T=1999	C=1132	G=318 A=268	T=318 C=268	A=299	-	-	-
Lab5_15	C=820 G=809	G=2202	T=2400	C=3078	A=515 G=505	C=510 T=505	A=586	-	-	-
Lab6_15	C=6906 G=6208	G=459	T=8064	C=8771	A=458 G=452	C=458 T=452	A=45	-	-	-

Stain 15:
- high number of reads for some markers
- no MB cSNPs in panel

Mixed Stains

A mixed stain can contain…

…two different body fluids from the same donor

…two different body fluids from two different donors

…the same type of body fluid from two different donors

Stain_9	Saliva_01.0_HTN3	Saliva_01.1_HTN3	Saliva_01.2_HTN3	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_02_MUC7	Saliva_05_STATH
SA-SA	HTN3	HTN3	HTN3	PRB4	PRH2	MUC7	STATH
donor 1	CT	CT	CC	GG	CC	CC	
donor 2	CC	TT	CC	CG	CT	CT	
Lab1_9	C=4235 T=1824	C=4235 T=1824	C=4235	G=747 C=91	C=1663 T=225	C=8761 T=727	-
Lab2_9	C=5896 T=3059	C=5896 T=3059	C=5896	G=129	C=506 T=45	C=1267 T=169	-
Lab3_9	C=204 T=49	C=204 T=49	C=204	-	C=45185 T=6265	-	-
Lab4_9	C=21889 T=9008	C=21889 T=9008	C=21889	G=1549 C=86	C=6913 T=816	C=26044 T=2896	-
Lab5_9	-	-	-	-	C=1380 T=106	C=5630 T=445	-
Lab6_9	C=35674 T=15822	C=35674 T=15822	C=35674	G=651 C=37	C=24714 T=1834	C=78255 T=7940	-

Stain 9:
- high number of reads in some markers
- RNA cSNP genotype reflects sum of donor genotypes

Mixed Stains

Stain_2	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_05_SPTB	Blood_04.0_SPTB	Blood_04.1_SPTB	Blood_06_SPTB	MMP10	MMP10	LEFTY2	CYP2A6	CYP2B7P1
MB-BL	ANK1	ANK1	CD3G	SPTB	SPTB	SPTB	SPTB	MMP10	MMP10	LEFTY2	CYP2A6	CYP2B7P1
donor 1	CG	GG	TT	CC	AG	CT	AA					
donor 2	CC	GG	TT	CT	AA	CC	AG					
Lab1_2	C= 295 G= 163	G=333	T=355	C=317 T=42	A=46 G=23	C=46 T=23	A=22 G=7	-	-	-	-	-
Lab2_2	C=6953 G=849	G=5925	T=6936	C=5227 T=2386	A=4673 G=400	C=4673 T=400	A=2054 G=902	-	-	-	-	-
Lab3_2	C=4210 G=1134	G=36	T=10204	C=4167 T=1520	A=47 G=22	C=47 T=22		-	-	-	-	-
Lab4_2	C=2764 G=832	G=7317	T=12845	C=4845 T=1257	A=1287 G=571	C=1287 T=571	A=1090 T=1257	-	-	-	-	-
Lab5_2	C=1644 G=467	G=1947	T=3220	C=2571 T=1193	A=772 G=130	C=772 T=130	A=398 G=144	-	-	-	-	-
Lab6_2	C=32179 G=8552	G=42511	T=45910	C=31060	A=16540 G=5450	C=16540 T=5450	A=6957 G=2694	-	-	-	-	-

Stain 2:
- high number of reads
- no MB, VAG cSNPs in panel
- BL RNA cSNP genotype reflects sum of donor genotypes

Stain_7	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_05_SPTB	Blood_04.0_SPTB	Blood_04.1_SPTB	Blood_06_SPTB
BL-BL	ANK1	ANK1	CD3G	SPTB	SPTB	SPTB	SPTB
donor 1	CG	GG	TT	CC	AG	CT	AA
donor 2	CC	GG	TT	CT	AA	CC	AG
Lab1_7	C= 11817 G= 3447	G=13953	T=14585	C=16031 T=4374	A=3723 G=1123	C=3723 T=1123	A=1793 G=648
Lab2_7	C=153908 G=39760	G=146075	T=327537	C=171354 T=52231	A=70271 G=26431	C=70271 T=26431	A=41841 G=14537
Lab3_7	C=221347 G=55606	G=45023	T=91427	C=254521 T=77261	A=28778 G=10010	C=28778 T=10010	A=689 G=206
Lab4_7	C=33980 G=8391	G=99602	T=176501	C=76120 T=23182	A=19712 G=7069	C=19712 T=7069	A=6410 G=1755
Lab5_7	C=21068 G=5837	G=48995	T=40668	C=37435 T=11475	A=10564 G=3489	C=10564 T=3489	A=3381 G=950
Lab6_7	C=81521 G=21565	G=4428	T=23212	C=41863 T=11611	A=3031 G=1123	C=3031 T=1123	A=280 G=87

Stain 7:
- high number of reads
- RNA cSNP genotype reflects sum of donor genotypes

Stain_10	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_05_SPTB	Blood_04.0_SPTB	Blood_04.1_SPTB	Blood_06_SPTB	Saliva_01.0_HTN3	Saliva_01.1_HTN3	Saliva_01.2_HTN3	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_02_MUC7	Saliva_05_STATH
BL-SA	ANK1	ANK1	CD3G	SPTB	SPTB	SPTB	SPTB	HTN3	HTN3	HTN3	PRB4	PRH2	MUC7	STATH
donor 1	CG	GG	TT	CC	AG	CT	AG	CC	CT	CC	GG	CC	CC	
donor 2	CG	GG	TT	CC	AA	CC	AA	TT	CC	CC	CG	CT	CT	
Lab1_10	C=436 G=257	G=900	T=3902	C=710	A=181 G=164	C=181 T=164	A=165 G=151	T=128	C=128	-	G=11	C=62	C=366 T=79	-
Lab2_10	G=884 C=852	G=1213	T=12066	C=1313	A=562 G=442	T=562 C=442	A=417 G=327	T=1070	C=1070	C=1070	-	T=52 C=33	C=897 486	-
Lab3_10	C=22549 G=16652	G=5465	T=161555	C=16679	A=1923 G=1489	C=1923 T=1489	G=156 A=128	T=562 C=16	C=562 T=16	C=562	-	C=868 T=552	C=16995 T=6699	-
Lab4_10	C=4509 G=3672	G=14366	T=143576	C=12496	A=3066 G=2977	T=3066 C=2977	G=1054 A=800	T=5184	C=5184	C=5184	C=63	C=1002 T=832	C=14876 T=6420	-
Lab5_10	C=1582 G=1274	G=3671	T=14621	C=3747	A=471 G=372	C=471 T=372	G=500 A=327	T=546	-	-	C=44 G=6	-	C=1440 T=767	-
Lab6_10	C=19820 G=15461	G=19766	T=161872	C=35442	A=14385 G=13487	C=14385 T=13487	G=1003 A=685	T=303	C=303	C=303	-	T=1000 C=747	C=9277 T=4147	-

Stain 10:
- decent number of reads
- RNA cSNP genotype reflects sum of donor genotypes

Mixed Stains

Stain_4	Semen_02_PRM1	Semen_04_TGM4	Semen_05_TGM4	Semen_06.0_TGM4	Semen_06.1_TGM4	Semen_03_SEMG2	Semen_01.0_KLK3	Semen_01.1_KLK3	Saliva_01.0_HTN3	Saliva_01.1_HTN3	Saliva_01.2_HTN3	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_02_MUC7	Saliv+B41:P41a_05_STATH
SA-SE	PRM1	TGM4	TGM4	TGM4	TGM4	SEMG2	KLK3	KLK3	HTN3	HTN3	HTN3	PRB4	PRH2	MUC7	STATH
donor 1	TT	TT	AA	CT	AG	CC	TT	AA	CT	CT	CC	GG	CC	CC	
Lab1_4	T=9392		A=81	-	-	C=127	T=5	G=7	-	-	-	-	-		-
Lab1_4.2	T=11312	T=10	A=8			C=84	C=7	A=5						C=9	
Lab2_4	T=4548	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lab3_4	T=204133	T=6	A=53	-	-	C=32	T=34	A=34	-	-	-	-	-	-	-
Lab4_4	T=175825	-	A=293	-	-	C=259	T=66	A=66	C=18 T=15	T=18 C=15	C=18	-	-	-	-
Lab5_4	T=308273	-	-	-	-	-	T=60	A=60	-	-	-	-	-	-	-
Lab6_4	-	-	-	-	-	-	C=10949 T=8670	G=10949 A=8670	T=27 C=14	C=27 T=14	C=27	G=26	C=92	C=208 T=64	-

Stain 4:
- overall low number of reads
- RNA cSNP genotype poorly reflects DNA genotypes

Stain_14	Semen_02_PRM1	Semen_04_TGM4	Semen_05_TGM4	Semen_06.0_TGM4	Semen_06.1_TGM4	Semen_03_SEMG2	Semen_01.0_KLK3	Semen_01.1_KLK3	Saliva_01.0_HTN3	Saliva_01.1_HTN3	Saliva_01.2_HTN3	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_02_MUC7
SA-SE	PRM1	TGM4	TGM4	TGM4	TGM4	SEMG2	KLK3	KLK3	HTN3	HTN3	HTN3	PRB4	PRH2	MUC7
donor 1	TT	CT	AG	CT	AG	CC	TT	AA	CC	CT	CC	CG	CT	CC
donor 2	TT	CC	GG	CT	AG	CC	TT	AA	CT	CT	CC	GG	CC	CC
Lab1_14	T=16	-	-	-	-	-	-	-	-	-	-	-	-	-
Lab1_14.2	T=368	T=15	A=6	-	-	-	-	-	-	-	-	-	-	-
Lab2_14	T=109640	C=15	-	-	-	-	-	-	-	-	-	-	-	-
Lab3_14	T=483995	C=1160 T=58	G=575	-	-	-	T=520 C=433	A=520 G=433	-	-	-	-	-	C=281
Lab4_14	T=176138	C=308 T=31	G=22 A=6	-	-	-	T=72 C=16	A=72 G=16	-	-	-	-	-	C=72
Lab5_14	T=126620	T=61	-	-	-	-	-	-	-	-	-	-	-	-
Lab6_14	T=228487	C=68	-	-	-	-	-	-	-	-	-	-	-	-

Stain 14:
- high number of reads only in one marker
- RNA cSNP genotypes hardly reflects donor genotypes

Stain_16	Semen_02_PRM1	Semen_04_TGM4	Semen_05_TGM4	Semen_06.0_TGM4	Semen_06.1_TGM4	Semen_03_SEMG2	Semen_01.0_KLK3	Semen_01.1_KLK3	CYP2A6	CYP2B7P1
SE-VAG	PRM1	TGM4	TGM4	TGM4	TGM4	SEMG2	KLK3	KLK3	CYP2A6	CYP2B7P1
donor 1	TT	TT	AA	TT	AA	AC	CT	AG		
donor 2	TT	CC	GG	CC	GG	CC	CC	GG		
Lab1_16	T=32687	T=2924	A=3027	T=385	A=385	A=349 C=284	C=5308 T=3732	G=5308 A=3732	-	-
Lab2_16	T=52123	T=2268	A=2576	T=1037	A=1037	C=197 A=117	C=4237 T=2332	G=4237 A=2332	-	-
Lab3_16	T=215279	T=784	A=1010	-	-	C=19 A=5	C=31282 T=27165	G=31282 A=27165	-	-
Lab4_16	T=75917	T=16668	A=20414	T=11450	A=11450	C=2719 A=2134	C=19113 T=16487	G=19113 A=16487	-	-
Lab5_16	T=181028	T=19788	A=26766	T=9180	A=9180	C=3713 A=2818	C=25524 T=17514	G=25524 A=17514	-	-
Lab6_16	T=223154	T=55682	A=8151 G=245	-	-	C=164 A=82	C=29081 T=21114	G=29081 A=21114	-	-

Stain 16:
- high number of reads in some markers
- no VAG cSNPs in panel
- SE RNA cSNP genotype reflects donor genotype (except Semen_05_TGM4)

Results for the Body Fluid Identification for the Own Stains (8 per laboratory)

BFID RNA Results – Laboratory 3 Stains n° 1-8

- mh counts: raw data, used to calculate the 0.5% threshold for correction
- mh counts corrected: everything below the 0.5% threshold set to 0

	Lab3_1	Lab3_1	Lab3_2	Lab3_2	Lab3_3	Lab3_3	Lab3_4	Lab3_4	Lab3_5	Lab3_5	Lab3_6	Lab3_6	Lab3_7	Lab3_7	Lab3_8	Lab3_8
MH Target	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected
Blood_01_ANK1	0	0	0	0	0	0	0	0	0	0	137	0	0	0	0	0
Blood_02_ANK1	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0
Blood_03_CD3G	0	0	0	0	0	0	0	0	6	6	0	0	0	0	0	0
Blood_04_SPTB	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0
Blood_05_SPTB	0	0	0	0	10	0	0	0	0	0	17	0	0	0	0	0
Blood_06_SPTB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_01_LEFTY2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_02_MMP10	0	0	0	0	16	0	0	0	0	0	5	0	16	0	0	0
Saliva_01_HTN3	0	0	0	0	0	0	3146	3146	0	0	33	0	98233	98233	0	0
Saliva_02_MUC7	786	786	0	0	24	0	15409	15409	0	0	0	0	24357	24357	0	0
Saliva_03_PRB4	0	0	0	0	0	0	7	0	0	0	0	0	629	0	0	0
Saliva_04_PRH2	343	343	0	0	9	0	4366	4366	0	0	8	0	41730	41730	0	0
Saliva_05_STATH	4586	4586	0	0	42	0	20031	20031	5	5	123	0	428320	428320	9	9
Semen_01_KLK3	6	0	0	0	69459	69459	352	352	9	9	120869	120869	15	0	0	0
Semen_02_PRM1	16	0	41	41	279262	279262	89	0	27	27	218220	218220	130	0	0	0
Semen_03_SEMG2	0	0	0	0	69705	69705	216	0	0	0	7611	7611	7	0	0	0
Semen_04_TGM4	15	0	9	9	293681	293681	427	427	44	44	352453	352453	199	0	0	0
Semen_05_TGM4	0	0	0	0	116148	116148	295	295	6	6	181412	181412	169	0	0	0
Semen_06_TGM4	0	0	0	0	528	0	0	0	0	0	174	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	528	0	0	0	0	0	174	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	528	0	0	0	0	0	174	0	0	0	0	0
Skin_01_COL17A1	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0
Skin_02_IL37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_03_LCE1C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal_01_CYP2A6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal_02_CYP2B7P1	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0
Total number of reads	5752	5715	50	50	829971	828255	44338	44026	97	97	881431	880565	593805	592640	9	9
Threshold	28.76		0.25		4149.855		221.69		0.485		4407.155		2969.025		0.045	

Predicted Body Fluids: SA ? SE SA-SE ? SE SA ?

BFID RNA Results – Laboratory 5 Stains n° 1-8

- mh counts: raw data, used to calculate the 0.5% threshold for correction
- mh counts corrected: everything below the 0.5% threshold set to 0

MH Target	Lab5_1	Lab5_1	Lab5_2	Lab5_2	Lab5_3	Lab5_3	Lab5_4	Lab5_4	Lab5_5	Lab5_5	Lab5_6	Lab5_6	Lab5_7	Lab6_7	Lab5_8	Lab5_8
	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected	mh counts	mh counts corrected
Blood_01_ANK1	4025	4025	6765	6765	0	0	0	0	13	13	54	0	6219	6219	24	24
Blood_02_ANK1	80	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood_03_CD3G	2565	2565	1396	1396	0	0	274	0	0	0	0	0	236	236	0	0
Blood_04_SPTB	48	48	15	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood_05_SPTB	1244	1244	7296	7296	0	0	0	0	0	0	0	0	901	901	0	0
Blood_06_SPTB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_01_LEFTY2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menstrual_02_MMP10	0	0	0	0	622	622	2673	2673	0	0	635	0	7	0	0	0
Saliva_01_HTN3	0	0	0	0	195	0	34392	34392	0	0	5800	5800	0	0	0	0
Saliva_02_MUC7	0	0	0	0	39377	39377	54757	54757	9	9	63837	63837	765	765	7	7
Saliva_03_PRB4	0	0	0	0	0	0	292	0	0	0	52	0	0	0	0	0
Saliva_04_PRH2	0	0	0	0	193	0	7123	7123	0	0	6729	6729	144	144	0	0
Saliva_05_STATH	0	0	0	0	26600	26600	126248	126248	12	12	96388	96388	1174	1174	0	0
Semen_01_KLK3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_02_PRM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_03_SEMG2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_04_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_05_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen_06_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semen-gDNA_01_TGM4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_01_COL17A1	0	0	0	0	220	0	1984	1984	0	0	8	0	11	0	0	0
Skin_02_IL37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin_03_LCE1C	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
Vaginal_01_CYP2A6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal_02_CYP2B7P1	13	0	111	111	0	0	0	0	0	0	0	0	0	0	0	0
Total number of reads	7975	7962	15583	15568	67207	66599	227743	227177	34	34	173515	172754	9457	9439	31	31
Threshold	39.875		77.915		336.035	332.995	1138.715		0.17		867.575		47.285		0.155	

Predicted Body Fluids: BL BL SA SA-MB ? SA SA-BL ?

Assignment of Body Fluids with a Donor: Own Stains (8 per laboratory)

Assignment of Body Fluid with Donor – Own Stains Laboratory 3 (Stains 1-3)

- Supposed body fluid according to BFI are framed
- Matching RNA + DNA genotype in green, discrepancies in lilac
- Supposed donor in light blue

- Co-extracted DNA of stains was analyzed instead of DNA of reference persons
- Single stains: incomplete DNA reference profiles
- Mixed stains: mixed DNA profile, assignment with donor not possible
- DNA 1 belongs to RNA from stain 1 and so forth

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2		Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab3_1_RNA	0	0	0	0	0	0	0	C/C (786)	0	C/T (216/127)	0	C/C (6)	T/G (9/7)	0	C/C (9)	0	0	0
Lab3_1_DNA	C/C	G/G	T/T	C/C	A/A	C/C	C/C	C/C	C/G	C/T	C/C	C/T	G/T	A/C	C/T	A/G	C/T	A/G
Lab3_2_DNA	0	0	0	C/C	A/A	C/C	0	0	C/C	0	0	0	G/T		C/T	0	0	A/A
Lab3_3_DNA	0	0	T/T	C/C	A/A	C/C	C/C	0	C/G	C/T	C/C	0	G/T	A/C	C/T	G/G	C/T	A/G
Lab3_4_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/G	C/T	C/C	0	G/T	0	C/T	A/A	0	A/G
Lab3_5_DNA	0	G/G	T/T	C/C	A/A	C/T	C/C	0	C/G	T/T	C/C	C/C	G/T	0	C/T	A/G	T/T	A/G
Lab3_6_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/C	C/T	C/C	0	G/G	A/C	C/T	0	T/T	A/G
Lab3_7_DNA	0	0	0	C/C	A/A	C/T	C/C	0	0	C/C	C/C	0	G/G	A/A	C/T	0	T/T	A/A
Lab3_8_DNA	0	0	0	C/C	A/A	C/T	0	0	G/G	C/C	0	C/C	G/G	0	C/T	0	T/T	A/A

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2		Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab3_2_RNA	0	0	0	0	0	0	0	0	0	0	0	0	T/T (41)	0	C/C (9)	0	0	0
Lab3_1_DNA	C/C	G/G	T/T	C/C	A/A	C/C	C/C	C/C	C/G	C/T	C/C	C/T	G/T	A/C	C/T	A/G	C/T	A/G
Lab3_2_DNA	0	0	0	C/C	A/A	C/C	0	0	C/C	0	0	0	G/T		C/T	0	0	A/A
Lab3_3_DNA	0	0	T/T	C/C	A/A	C/C	C/C	0	C/G	C/T	C/C	0	G/T	A/C	C/T	G/G	C/T	A/G
Lab3_4_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/G	C/T	C/C	0	G/T	0	C/T	A/A	0	A/G
Lab3_5_DNA	0	G/G	T/T	C/C	A/A	C/T	C/C	0	C/G	T/T	C/C	C/C	G/T	0	C/T	A/G	T/T	A/G
Lab3_6_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/C	C/T	C/C	0	G/G	A/C	C/T	0	T/T	A/G
Lab3_7_DNA	0	0	0	C/C	A/A	C/T	C/C	0	0	C/C	C/C	0	G/G	A/A	C/T	0	T/T	A/A
Lab3_8_DNA	0	0	0	C/C	A/A	C/T	0	0	G/G	C/C	0	C/C	G/G	0	C/T	0	T/T	A/A

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2		Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab3_3_RNA	0	0	0	C/C (15)	0	C/C (10)	0	C/T (19/5)	0	C/C (9)	0	C/T (35330/34129)	G/T (144901/134361)	C/A (46485/23220)	C/T (277091/16590)	G/A (109436/6712)	C/T (497/31)	G/A (497/31)
Lab3_1_DNA	C/C	G/G	T/T	C/C	A/A	C/C	C/C	C/C	C/G	C/T	C/C	C/T	G/T	A/C	C/T	A/G	C/T	A/G
Lab3_2_DNA	0	0	0	C/C	A/A	C/C	0	0	C/C	0	0	0	G/T		C/T	0	0	A/A
Lab3_3_DNA	0	0	T/T	C/C	A/A	C/C	C/C	0	C/G	C/T	C/C	0	G/T	A/C	C/T	G/G (10)	C/T	A/G
Lab3_4_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/G	C/T	C/C	0	G/T	0	C/T	A/A	0	A/G
Lab3_5_DNA	0	G/G	T/T	C/C	A/A	C/T	C/C	0	C/G	T/T	C/C	C/C	G/T	0	C/T	A/G	T/T	A/G
Lab3_6_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/C	C/T	C/C	0	G/G	A/C	C/T	0	T/T	A/G
Lab3_7_DNA	0	0	0	C/C	A/A	C/T	C/C	0	0	C/C	C/C	0	G/G	A/A	C/T	0	T/T	A/A
Lab3_8_DNA	0	0	0	C/C	A/A	C/T	0	0	G/G	C/C	0	C/C	G/G	0	C/T	0	T/T	A/A

Assignment of Body Fluid with Donor – Own Stains Laboratory 3 (Stains 4-8)

- Supposed body fluid according to BFI are framed
- Matching RNA + DNA genotype in green, discrepancies in lilac
- Supposed donor in light blue

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2		Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab3_4_RNA	0	0	0	0	0	0	C/C (3146)	C/C (15409)	G/G (7)	/T (3163/120	0	C/C (352)	G/T (68/21)	C/C (216)	C/C (427)	G/G (295)	0	0
Lab3_1_DNA	C/C	G/G	T/T	C/C	A/A	C/C	C/C	C/C	C/G	C/T	C/C	C/T	G/T	A/C	C/T	A/G	C/T	A/G
Lab3_2_DNA	0	0	0	C/C	A/A	C/C	0	0	C/C	0	0	0	G/T	0	C/T	0	0	A/A
Lab3_3_DNA	0	0	T/T	C/C	A/A	C/C	C/C	0	C/G	C/T	C/C	0	G/T	A/C	C/T	G/G	C/T	A/G
Lab3_4_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/G (21/10)	C/T	C/C	0	G/T	0	C/T (45/17)	G/G	0	A/G
Lab3_5_DNA	0	G/G	T/T	C/C	A/A	C/T	C/C	0	C/G	T/T	C/C	C/C	G/T	0	C/T	A/G	T/T	A/G
Lab3_6_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/C	C/T	C/C	0	G/G	A/C	C/T	0	T/T	A/G
Lab3_7_DNA	0	0	0	C/C	A/A	C/T	C/C	0	0	C/C	C/C	0	G/G	A/A	C/T	0	T/T	A/A
Lab3_8_DNA	0	0	0	C/C	A/A	C/T	0	0	G/G	C/C	0	C/C	G/G	0	C/T	0	T/T	A/A

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2		Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab3_5_RNA	0	0	T/T (6)	0	0	0	0	0	0	0	0	C/C (9)	T/G (15/12)	0	C/T (38/6)	G/G (6)	0	0
Lab3_1_DNA	C/C	G/G	T/T	C/C	A/A	C/C	C/C	C/C	C/G	C/T	C/C	C/T	G/T	A/C	C/T	A/G	C/T	A/G
Lab3_2_DNA	0	0	0	C/C	A/A	C/C	0	0	C/C	0	0	0	G/T		C/T	0	0	A/A
Lab3_3_DNA	0	0	T/T	C/C	A/A	C/C	C/C	0	C/G	C/T	C/C	0	G/T	A/C	C/T	G/G	C/T	A/G
Lab3_4_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/G	C/T	C/C	0	G/T	0	C/T	A/A	0	A/G
Lab3_5_DNA	0	G/G	T/T	C/C	A/A	C/T	C/C	0	C/G	T/T	C/C	C/C	G/T	0	C/T	A/G	T/T	A/G
Lab3_6_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/C	C/T	C/C	0	G/G	A/C	C/T	0	T/T	A/G
Lab3_7_DNA	0	0	0	C/C	A/A	C/T	C/C	0	0	C/C	C/C	0	G/G	A/A	C/T	0	T/T	A/A
Lab3_8_DNA	0	0	0	C/C	A/A	C/T	0	0	G/G	C/C	0	C/C	G/G	0	C/T	0	T/T	A/A

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2		Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab3_6_RNA	G/C (115/22)	G/G (6)	0	0	0	C/C (17)	C/C (19)	0	0	C/C (8)	0	C/C (120869)	G/T (115594/102626)	C/A (5452/2159)	C/T (263640/88813)	G/A (134950/46462)	T/C (125/49)	A/G (125/49)
Lab3_1_DNA	C/C	G/G	T/T	C/C	A/A	C/C	C/C	C/C	C/G	C/T	C/C	C/T (835\367)	G/T	A/C	C/T	A/G	C/T	A/G
Lab3_2_DNA	0	0	0	C/C	A/A	C/C	0	0	C/C	0	0	0	G/T		C/T	0	0	A/A
Lab3_3_DNA	0	0	T/T	C/C	A/A	C/C	C/C	0	C/G	C/T	C/C	0	G/T	A/C	C/T	G/G	C/T	A/G
Lab3_4_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/G	C/T	C/C	0	G/T	0	C/T	A/A	0	A/G
Lab3_5_DNA	0	G/G	T/T	C/C	A/A	C/T	C/C	0	C/G	T/T	C/C	C/C	G/T	0	C/T	A/G	T/T (7)	A/G
Lab3_6_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/C	C/T	C/C	0	G/G	A/C	C/T	0	T/T (10)	A/G
Lab3_7_DNA	0	0	0	C/C	A/A	C/T	C/C	0	0	C/C	C/C	0	G/G	A/A	C/T	0	T/T	A/A
Lab3_8_DNA	0	0	0	C/C	A/A	C/T	0	0	G/G	C/C	0	C/C	G/G	0	C/T	0	T/T	A/A

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2		Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab3_7_RNA	0	0	0	0	0	0	C/C (50200)	C/T (12371/11986)	G/G (629)	C/C (41730)	0	C/T (8/7)	G/T (76/54)	C/C (7)	C/T (135/64)	G/G (169)	0	0
Lab3_1_DNA	C/C	G/G	T/T	C/C	A/A	C/C	C/C	C/C	C/G	C/T	C/C	C/T	G/T	A/C	C/T	A/G	C/T	A/G
Lab3_2_DNA	0	0	0	C/C	A/A	C/C	0	0	C/C	0	0	0	G/T		C/T	0	0	A/A
Lab3_3_DNA	0	0	T/T	C/C	A/A	C/C	C/C	0	C/G	C/T	C/C	0	G/T	A/C	C/T	G/G	C/T	A/G
Lab3_4_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/G	C/T	C/C	0	G/T	0	C/T	A/A	0	A/G
Lab3_5_DNA	0	G/G	T/T	C/C	A/A	C/T	C/C	0	C/G	T/T	C/C	C/C	G/T	0	C/T	A/G	T/T	A/G
Lab3_6_DNA	0	0	T/T	C/C	A/A	C/T	C/C	0	C/C	C/T	C/C	0	G/G	A/C	C/T	0	T/T	A/G
Lab3_7_DNA	0	0	0	C/C	A/A	C/T	C/C	0	0	C/C	C/C	0	G/G	A/A	C/T	0	T/T	A/A
Lab3_8_DNA	0	0	0	C/C	A/A	C/T	0	0	G/G	C/C	0	C/C	G/G	0	C/T	0	T/T	A/A

- Stain 8: no reads except for STATH (no cSNPs)

Assignment of Body Fluid with Donor – Own Stains Laboratory 5 (Stains 1-4)

- Supposed body fluid according to BFI are framed
- Matching RNA + DNA genotype in green, discrepancies in lilac
- Supposed donor in light blue

- Genotypes in DNA reference profiles set to zero, if the coverage was ≤5 (see brackets)

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_05_HTN3	Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab5_1_RNA	G/C (2490/1535)	A/G (40/40)	T/T (2565)	C/C (48)	0	C/T (643/601)	0	0	0	0	0	0	0	0	0	0	0	0
Lab5_1_DNA	C/G (10/8)	A/G	T/T	C/C	A/G	C/T	C/T	0 (C/C, 3)	G/G	C/C	C/C	C/T	G/T	C/C	T/T	A/A	C/T	A/G
Lab5_2_DNA	0 (C/G, 2/3)	G/G	T/T	C/C	A/G	C/T	C/C	0 (C/C, 3)	C/G	C/C	C/T	C/T	G/G	C/C	C/C	G/G	C/C	G/G
Lab5_3_DNA	0 (C/C, 4)	A/G	T/T	C/C	A/A	C/C	C/C	0 (C/C, 4)	G/G	C/C	C/C	C/T	T/T	A/A	C/T	A/G	C/T	A/A
Lab5_4_DNA	0 (C/C,3)	G/G	T/T	C/T	A/G	C/C	C/C	0	C/G	C/T	C/C	T/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_5_DNA	C/G (23/29)	G/G	C/T	C/C	A/A	C/C	T/T	C/C (10)	G/G	C/C	C/C	C/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_6_DNA	0 (C/C,1)	G/G	C/T	T/T	A/A	T/T	C/C	0 (C/C, 1)	G/G	C/C	C/C	T/T	G/T	C/C	C/T	A/G	C/T	A/A
Lab5_7_DNA	C/C (23)	G/G	T/T	C/C	A/A	C/C	C/C	T/T (8)	C/G	C/C	C/C	C/C	T/T	A/C	C/C	G/G	C/T	A/G
Lab5_8_DNA	C/G (11/11)	G/G	T/T	C/C	A/A	C/C	C/C	C/C (9)	C/G	C/T	C/C	T/T	G/T	C/C	C/C	G/G	C/T	A/G

→Donor 1

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_05_HTN3	Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab5_2_RNA	G/C (3651/3114)	0	T/T (1396)	C/C (15)	0	C/T (3941/3355)	0	0	0	0	0	0	0	0	0	0	0	0
Lab5_1_DNA	C/G (10/8)	A/G	T/T	C/C	A/G	C/T	C/T	0 (C/C, 3)	G/G	C/C	C/C	C/T	G/T	C/C	T/T	A/A	C/T	A/G
Lab5_2_DNA	0 (C/G, 2/3)	G/G	T/T	C/C	A/G	C/T	C/C	0 (C/C, 3)	C/G	C/C	C/T	C/T	G/G	C/C	C/C	G/G	C/C	G/G
Lab5_3_DNA	0 (C/C, 4)	A/G	T/T	C/C	A/A	C/C	C/C	0 (C/C, 4)	G/G	C/C	C/C	C/T	T/T	A/A	C/T	A/G	C/T	A/A
Lab5_4_DNA	0 (C/C,3)	G/G	T/T	C/T	A/G	C/C	C/C	0	C/G	C/T	C/C	T/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_5_DNA	C/G (23/29)	G/G	C/T	C/C	A/A	C/C	T/T	C/C (10)	G/G	C/C	C/C	C/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_6_DNA	0 (C/C,1)	G/G	C/T	T/T	A/A	T/T	C/C	0 (C/C, 1)	G/G	C/C	C/C	T/T	G/T	C/C	C/T	A/G	C/T	A/A
Lab5_7_DNA	C/C (23)	G/G	T/T	C/C	A/A	C/C	C/C	T/T (8)	C/G	C/C	C/C	C/C	T/T	A/C	C/C	G/G	C/T	A/G
Lab5_8_DNA	C/G (11/11)	G/G	T/T	C/C	A/A	C/C	C/C	C/C (9)	C/G	C/T	C/C	T/T	G/T	C/C	C/C	G/G	C/T	A/G

→Donor 1 or 2

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_05_HTN3	Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab5_3_RNA	0	0	0	0	0	0	C/T (103/92)	C/C (103)	0	C/C (193)	0	0	0	0	0	0	0	0
Lab5_1_DNA	C/G (10/8)	A/G	T/T	C/C	A/G	C/T	C/T	0 (C/C, 3)	G/G	C/C	C/C	C/T	G/T	C/C	T/T	A/A	C/T	A/G
Lab5_2_DNA	0 (C/G, 2/3)	G/G	T/T	C/C	A/G	C/T	C/C	0 (C/C, 3)	C/G	C/C	C/T	C/T	G/G	C/C	C/C	G/G	C/C	G/G
Lab5_3_DNA	0 (C/C, 4)	A/G	T/T	C/C	A/A	C/C	C/C	0 (C/C, 4)	G/G	C/C	C/C	C/T	T/T	A/A	C/T	A/G	C/T	A/A
Lab5_4_DNA	0 (C/C,3)	G/G	T/T	C/T	A/G	C/C	C/C	0	C/G	C/T	C/C	T/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_5_DNA	C/G (23/29)	G/G	C/T	C/C	A/A	C/C	T/T	C/C (10)	G/G	C/C	C/C	C/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_6_DNA	0 (C/C,1)	G/G	C/T	T/T	A/A	T/T	C/C	0 (C/C, 1)	G/G	C/C	C/C	T/T	G/T	C/C	C/T	A/G	C/T	A/A
Lab5_7_DNA	C/C (23)	G/G	T/T	C/C	A/A	C/C	C/C	T/T (8)	C/G	C/C	C/C	C/C	T/T	A/C	C/C	G/G	C/T	A/G
Lab5_8_DNA	C/G (11/11)	G/G	T/T	C/C	A/A	C/C	C/C	C/C (9)	C/G	C/T	C/C	T/T	G/T	C/C	C/C	G/G	C/T	A/G

→Donor 1

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_05_HTN3	Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab5_4_RNA	0	0	T/T (274)	0	0	0	C/C (18626)	C/C (54757)	C/G (152/140)	C/C (7123)	0	0	0	0	0	0	0	0
Lab5_1_DNA	C/G (10/8)	A/G	T/T	C/C	A/G	C/T	C/T	0 (C/C, 3)	G/G	C/C	C/C	C/T	G/T	C/C	T/T	A/A	C/T	A/G
Lab5_2_DNA	0 (C/G, 2/3)	G/G	T/T	C/C	A/G	C/T	C/C	0 (C/C, 3)	C/G	C/C	C/T	C/T	G/G	C/C	C/C	G/G	C/C	G/G
Lab5_3_DNA	0 (C/C, 4)	A/G	T/T	C/C	A/A	C/C	C/C	0 (C/C, 4)	G/G	C/C	C/C	C/T	T/T	A/A	C/T	A/G	C/T	A/A
Lab5_4_DNA	0 (C/C,3)	G/G	T/T	C/T	A/G	C/C	C/C	0	C/G	C/T	C/C	T/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_5_DNA	C/G (23/29)	G/G	C/T	C/C	A/A	C/C	T/T	C/C (10)	G/G	C/C	C/C	C/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_6_DNA	0 (C/C,1)	G/G	C/T	T/T	A/A	T/T	C/C	0 (C/C, 1)	G/G	C/C	C/C	T/T	G/T	C/C	C/T	A/G	C/T	A/A
Lab5_7_DNA	C/C (23)	G/G	T/T	C/C	A/A	C/C	C/C	T/T (8)	C/G	C/C	C/C	C/C	T/T	A/C	C/C	G/G	C/T	A/G
Lab5_8_DNA	C/G (11/11)	G/G	T/T	C/C	A/A	C/C	C/C	C/C (9)	C/G	C/T	C/C	T/T	G/T	C/C	C/C	G/G	C/T	A/G

→Donor 2

Assignment of Body Fluid with Donor – Own Stains Laboratory 5 (Stains 5-8)

- Supposed body fluid according to BFI are framed
- Matching RNA + DNA genotype in green, discrepancies in lilac
- Supposed donor in light blue

- Genotypes in DNA reference profiles set to zero, if the coverage was ≤5 (see brackets)

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_05_HTN3	Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab5_5_RNA	C/G (7/6)	0	0	0	0	0	0	C/C (9)	0	0	0	0	0	0	0	0	0	0
Lab5_1_DNA	C/G (10/8)	A/G	T/T	C/C	A/G	C/T	C/T	0 (C/C, 3)	G/G	C/C	C/C	C/T	G/T	C/C	T/T	A/A	C/T	A/G
Lab5_2_DNA	0 (C/G, 2/3)	G/G	T/T	C/C	A/G	C/T	C/C	0 (C/C, 3)	C/G	C/C	C/T	C/T	G/G	C/C	C/C	G/G	C/C	G/G
Lab5_3_DNA	0 (C/C, 4)	A/G	T/T	C/C	A/A	C/C	C/C	0 (C/C, 4)	G/G	C/C	C/C	C/T	T/T	A/A	C/T	A/G	C/T	A/A
Lab5_4_DNA	0 (C/C,3)	G/G	T/T	C/T	A/G	C/C	C/C	0	C/G	C/T	C/C	T/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_5_DNA	C/G (23/29)	G/G	C/T	C/C	A/A	C/C	T/T	C/C (10)	G/G	C/C	C/C	C/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_6_DNA	0 (C/C,1)	G/G	C/T	T/T	A/A	T/T	C/C	0 (C/C, 1)	G/G	C/C	C/C	T/T	G/T	C/C	C/T	A/G	C/T	A/A
Lab5_7_DNA	C/C (23)	G/G	T/T	C/C	A/A	C/C	C/C	T/T (8)	C/G	C/C	C/C	C/C	T/T	A/C	C/C	G/G	C/T	A/G
Lab5_8_DNA	C/G (11/11)	G/G	T/T	C/C	A/A	C/C	C/C	C/C (9)	C/G	C/T	C/C	T/T	G/T	C/C	C/C	G/G	C/T	A/G

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_05_HTN3	Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab5_6_RNA	G/G (54)	0	0	0	0	0	C/C (3000)	C/C (63837)	G/C (31/21)	C/C (6729)	0	0	0	0	0	0	0	0
Lab5_1_DNA	C/G (10/8)	A/G	T/T	C/C	A/G	C/T	C/T	0 (C/C, 3)	G/G	C/C	C/C	C/T	G/T	C/C	T/T	A/A	C/T	A/G
Lab5_2_DNA	0 (C/G, 2/3)	G/G	T/T	C/C	A/G	C/T	C/C	0 (C/C, 3)	C/G	C/C	C/T	C/T	G/G	C/C	C/C	G/G	C/C	G/G
Lab5_3_DNA	0 (C/C, 4)	A/G	T/T	C/C	A/A	C/C	C/C	0 (C/C, 4)	G/G	C/C	C/C	C/T	T/T	A/A	C/T	A/G	C/T	A/A
Lab5_4_DNA	0 (C/C,3)	G/G	T/T	C/T	A/G	C/C	C/C	0	C/G	C/T	C/C	T/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_5_DNA	C/G (23/29)	G/G	C/T	C/C	A/A	C/C	T/T	C/C (10)	G/G	C/C	C/C	C/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_6_DNA	0 (C/C,1)	G/G	C/T	T/T	A/A	T/T	C/C	0 (C/C, 1)	G/G	C/C	C/C	T/T	G/T	C/C	C/T	A/G	C/T	A/A
Lab5_7_DNA	C/C (23)	G/G	T/T	C/C	A/A	C/C	C/C	T/T (8)	C/G	C/C	C/C	C/C	T/T	A/C	C/C	G/G	C/T	A/G
Lab5_8_DNA	C/G (11/11)	G/G	T/T	C/C	A/A	C/C	C/C	C/C (9)	C/G	C/T	C/C	T/T	G/T	C/C	C/C	G/G	C/T	A/G

→Donor 2

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_05_HTN3	Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab5_7_RNA	G/C (3589/2630)	0	T/T (236)	0	0	C/T (485/416)	0	C/C (765)	0	C/C (144)	0	0	0	0	0	0	0	0
Lab5_1_DNA	C/G (10/8)	A/G	T/T	C/C	A/G	C/T	C/T	0 (C/C, 3)	G/G	C/C	C/C	C/T	G/T	C/C	T/T	A/A	C/T	A/G
Lab5_2_DNA	0 (C/G, 2/3)	G/G	T/T	C/C	A/G	C/T	C/C	0 (C/C, 3)	C/G	C/C	C/T	C/T	G/G	C/C	C/C	G/G	C/C	G/G
Lab5_3_DNA	0 (C/C, 4)	A/G	T/T	C/C	A/A	C/C	C/C	0 (C/C, 4)	G/G	C/C	C/C	C/T	T/T	A/A	C/T	A/G	C/T	A/A
Lab5_4_DNA	0 (C/C,3)	G/G	T/T	C/T	A/G	C/C	C/C	0	C/G	C/T	C/C	T/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_5_DNA	C/G (23/29)	G/G	C/T	C/C	A/A	C/C	T/T	C/C (10)	G/G	C/C	C/C	C/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_6_DNA	0 (C/C,1)	G/G	C/T	T/T	A/A	T/T	C/C	0 (C/C, 1)	G/G	C/C	C/C	T/T	G/T	C/C	C/T	A/G	C/T	A/A
Lab5_7_DNA	C/C (23)	G/G	T/T	C/C	A/A	C/C	C/C	T/T (8)	C/G	C/C	C/C	C/C	T/T	A/C	C/C	G/G	C/T	A/G
Lab5_8_DNA	C/G (11/11)	G/G	T/T	C/C	A/A	C/C	C/C	C/C (9)	C/G	C/T	C/C	T/T	G/T	C/C	C/C	G/G	C/T	A/G

→Donor 1 for blood
→Donor 5 for saliva

Genotypes References	Blood_01_ANK1	Blood_02_ANK1	Blood_03_CD3G	Blood_04_SPTB	Blood_05_SPTB	Blood_06_SPTB	Saliva_01_HTN3	Saliva_02_MUC7	Saliva_03_PRB4	Saliva_04_PRH2	Saliva_05_HTN3	Semen_01_KLK3	Semen_02_PRM1	Semen_03_SEMG2	Semen_04_TGM4	Semen_05_TGM4	Semen_06_TGM4	Semen_07_TGM4
Lab_8_RNA	G/C (12)	0	0	0	0	0	0	C/C (7)	0	0	0	0	0	0	0	0	0	0
Lab5_1_DNA	C/G (10/8)	A/G	T/T	C/C	A/G	C/T	C/T	0 (C/C, 3)	G/G	C/C	C/C	C/T	G/T	C/C	T/T	A/A	C/T	A/G
Lab5_2_DNA	0 (C/G, 2/3)	G/G	T/T	C/C	A/G	C/T	C/C	0 (C/C, 3)	C/G	C/C	C/T	C/T	G/G	C/C	C/C	G/G	C/C	G/G
Lab5_3_DNA	0 (C/C, 4)	A/G	T/T	C/C	A/A	C/C	C/C	0 (C/C, 4)	G/G	C/C	C/C	C/T	T/T	A/A	C/T	A/G	C/T	A/A
Lab5_4_DNA	0 (C/C,3)	G/G	T/T	C/T	A/G	C/C	C/C	0	C/G	C/T	C/C	T/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_5_DNA	C/G (23/29)	G/G	C/T	C/C	A/A	C/C	T/T	C/C (10)	G/G	C/C	C/C	C/T	T/T	C/C	C/T	A/G	C/T	A/G
Lab5_6_DNA	0 (C/C,1)	G/G	C/T	T/T	A/A	T/T	C/C	0 (C/C, 1)	G/G	C/C	C/C	T/T	G/T	C/C	C/T	A/G	C/T	A/A
Lab5_7_DNA	C/C (23)	G/G	T/T	C/C	A/A	C/C	C/C	T/T (8)	C/G	C/C	C/C	C/C	T/T	A/C	C/C	G/G	C/T	A/G
Lab5_8_DNA	C/G (11/11)	G/G	T/T	C/C	A/A	C/C	C/C	C/C (9)	C/G	C/T	C/C	T/T	G/T	C/C	C/C	G/G	C/T	A/G

Conclusion and Outlook

Conclusions

Stain n° 1-16:

BFID

- 13/16 stains were predicted correctly
  2/4 low input stains correctly predicted
- 3/16 stains could not be predicted
  2/3 one body fluid was missing
  1/3 skin generally difficult
- Difficulties arise because of various misleading reads
- Are there any misleading reads arising systematically (marked in pink)?

cSNPs

- performance dependent on how many markers are detected per body fluid

Own Stains of the Laboratories:

BFID

- Overall we could predict 21/32 stains (65%)

cSNPs

- performance dependent on how many reads per RNA cSNP were detected
  → the more, the more accurate/complete the reflection of DNA genotypes
- Some labs did not analyze reference persons?

Outlook

New Thermofisher cSNP assay - BFID-cSNP-6F (6 fluids/tissues):

- Includes cSNP markers for vaginal secretion, menstrual blood and skin:
  - menstrual blood (3 genes)
  - vaginal secrection (1 gene)
  - skin (3 genes)

→ additional 18 cSNPs for body fluids + 6 cSNPs for tissue (skin) = 23 BFI markers + 46 cSNPs

→ Separate RNA + DNA assays

Manuscript submitted to FSI Genetics

Outlook

Potential EDNAP mRNA MPS exercise 4 testing BFID-cSNP-6F in winter 2022/23?
- 16 dried stains
- 8 own samples and donor samples (reference)
- 2 primer pools (RNA/DNA)
- on IonTorrent S5

Timeline:

September 2022:	Suggestion for collaborative exercise 4
November 2022:	Shipment of samples, primers, protocols
March 2023:	Submission of results
April/May 2023:	Presentation of results at next EDNAP meeting

→ If you are interested to participate in this exercise, please contact cordula.haas@irm.uzh.ch

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Robert Lagace, Chantal Roth

Netherlands Forensic Institute
Ministry of Justice

EDNAP Exercise mtDNA quantification

Kris van der Gaag
Natalie Weiler
Titia Sijen
Arnoud Kal

EDNAP exercise mtDNA quantification

- Home made assay (cheap!)
- Quantification of autosomal, Y and mtDNA
- Long and short mt probes

DNA	Probe	Bp	Dye
Total DNA	Alu Ya5	127 bp	VIC
Y DNA	DYZ5	137 bp	FAM
mtDNA	16533-180	217 bp	JUN
mtDNA	2502-2571	70 bp	ABY

21 Labs

- 16 x Europe
- 1 x Asia
- 4 x USA

NFI provides:

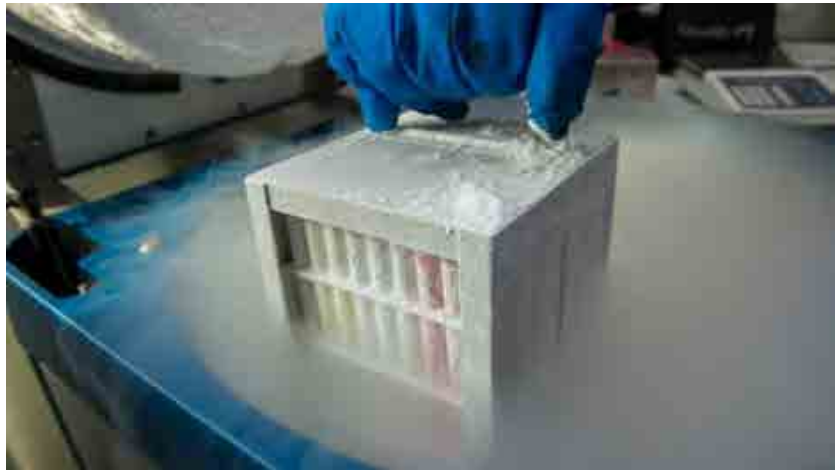
- Primers and probes
- Challenging samples
- Protocols

Labs provide:

- Their own favourite sample
- Their own total/Y/mtDNA quantification method

Challenging Samples

- Control DNA
- Sperm
- Unbalanced mixture male:female
- Fragmented DNA
- Oligo short mt amplicon
- Humic acid inhibited sample



Analysis of the results

- Analysis started but delayed
- Variable results: effect of transit time?
- Unexplaned results – outliers
- Data from 2 labs excluded

- Some examples in the next slides

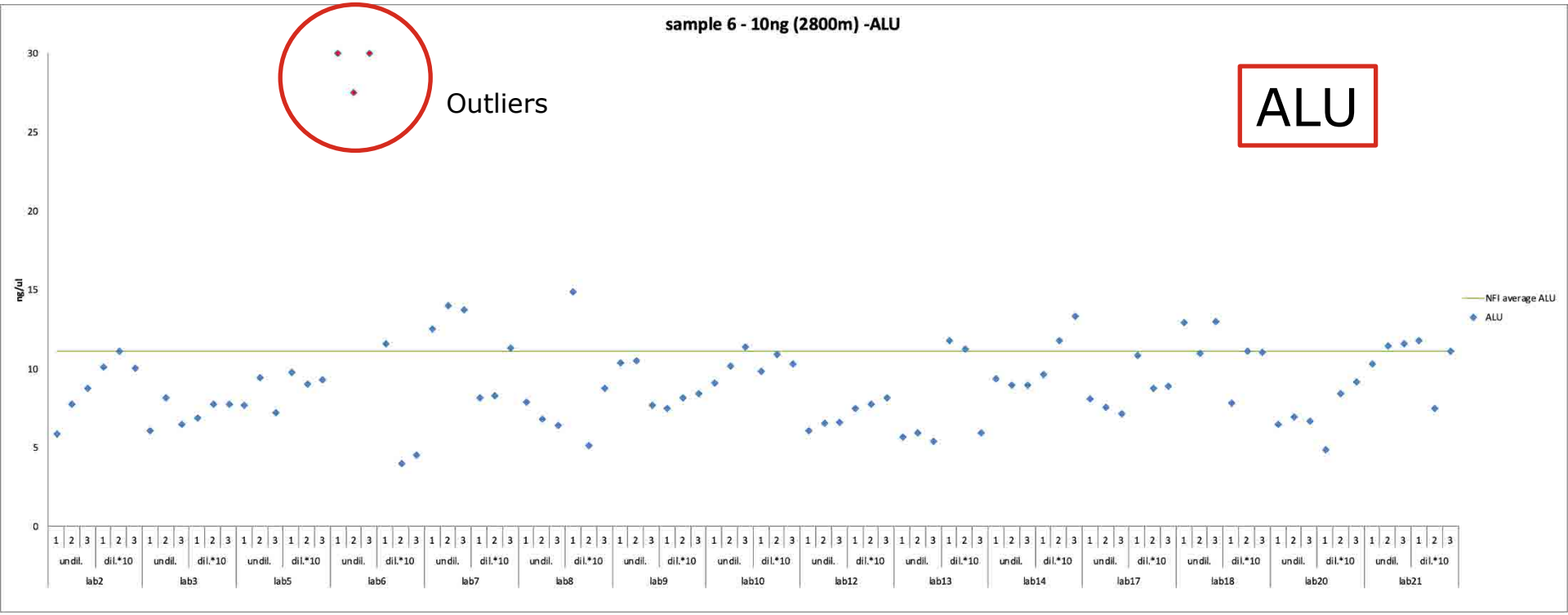
Sample #6

Sample = 10 ng 2800M control DNA (male)

Expected results:

Quant value >0 for ALU, Y, mt short and mt long

Sample #6, 10 ng control DNA 2800M



Similar results for Y, mt long and mt short

Sample #7

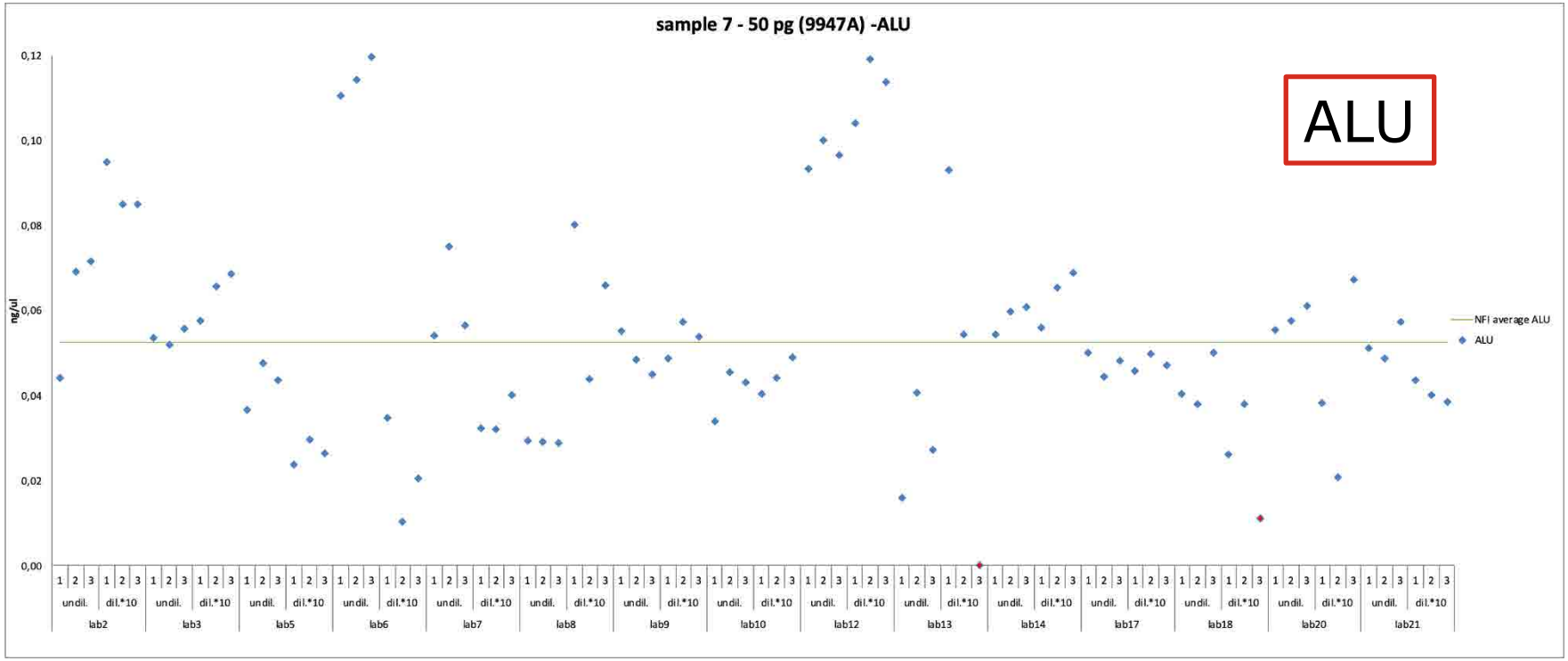
Sample = 50 pg control DNA 9947A (female)

Expected results

Quant value >0 for ALU, mt short and mt long

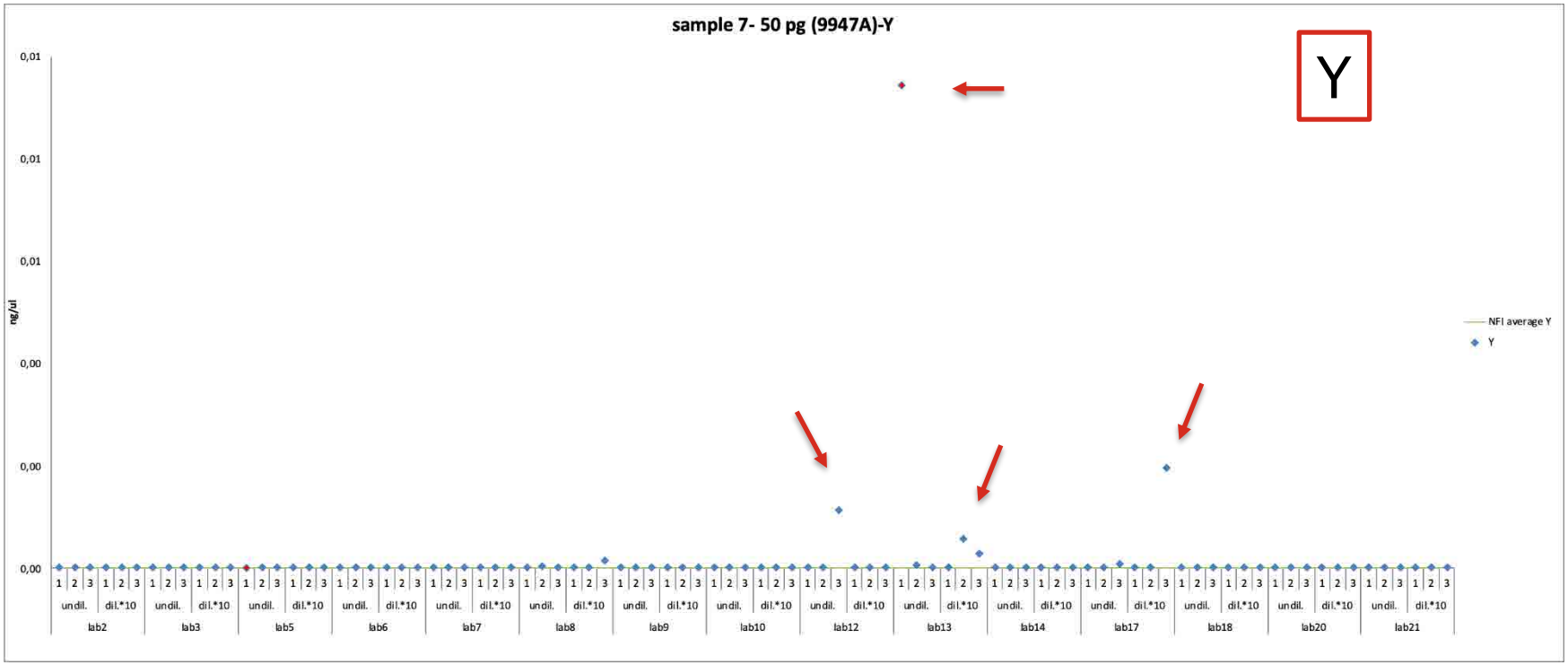
Quant value = 0 for Y

Sample #7 Control DNA 9947A – female



Similar results for mt short and mt long

Sample #7 Control DNA 9947A – female



Unexpected results for Y quant

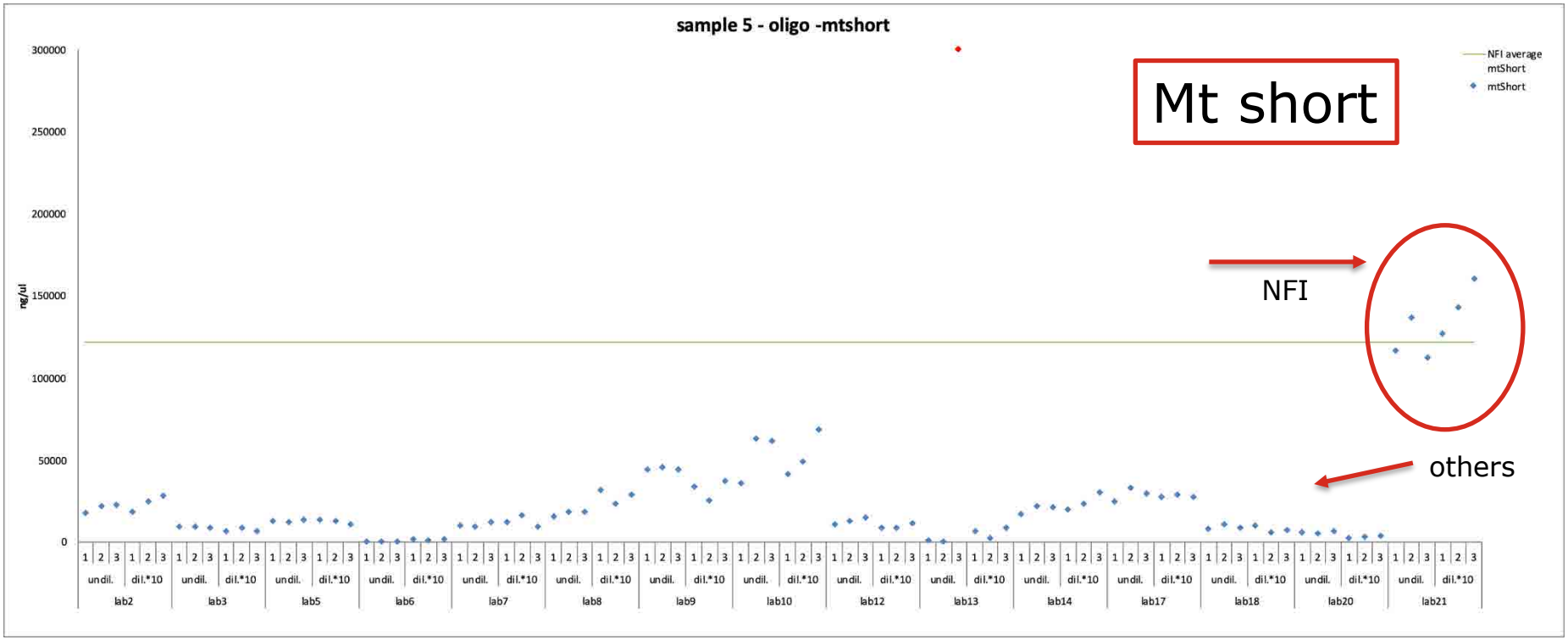
Sample #5

Sample = oligo for the short mtDNA amplicon

Expected results:

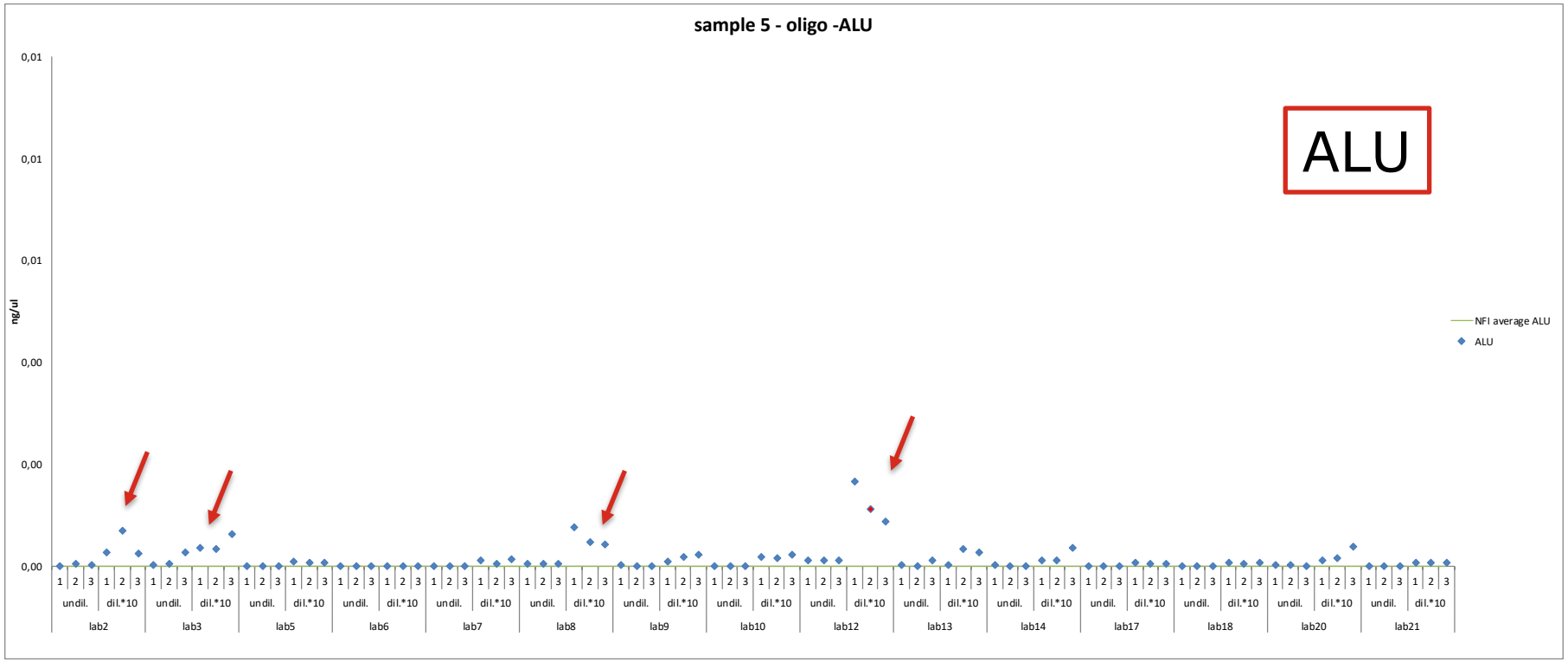
Quant value >0 for mt short
Quant value = 0 for ALU, Y and mt long

Sample #5 oligo for the short mtDNA amplicon



Effect of transit time?

Sample #5 oligo for the short mtDNA amplicon



Unexpected results

Sample #3

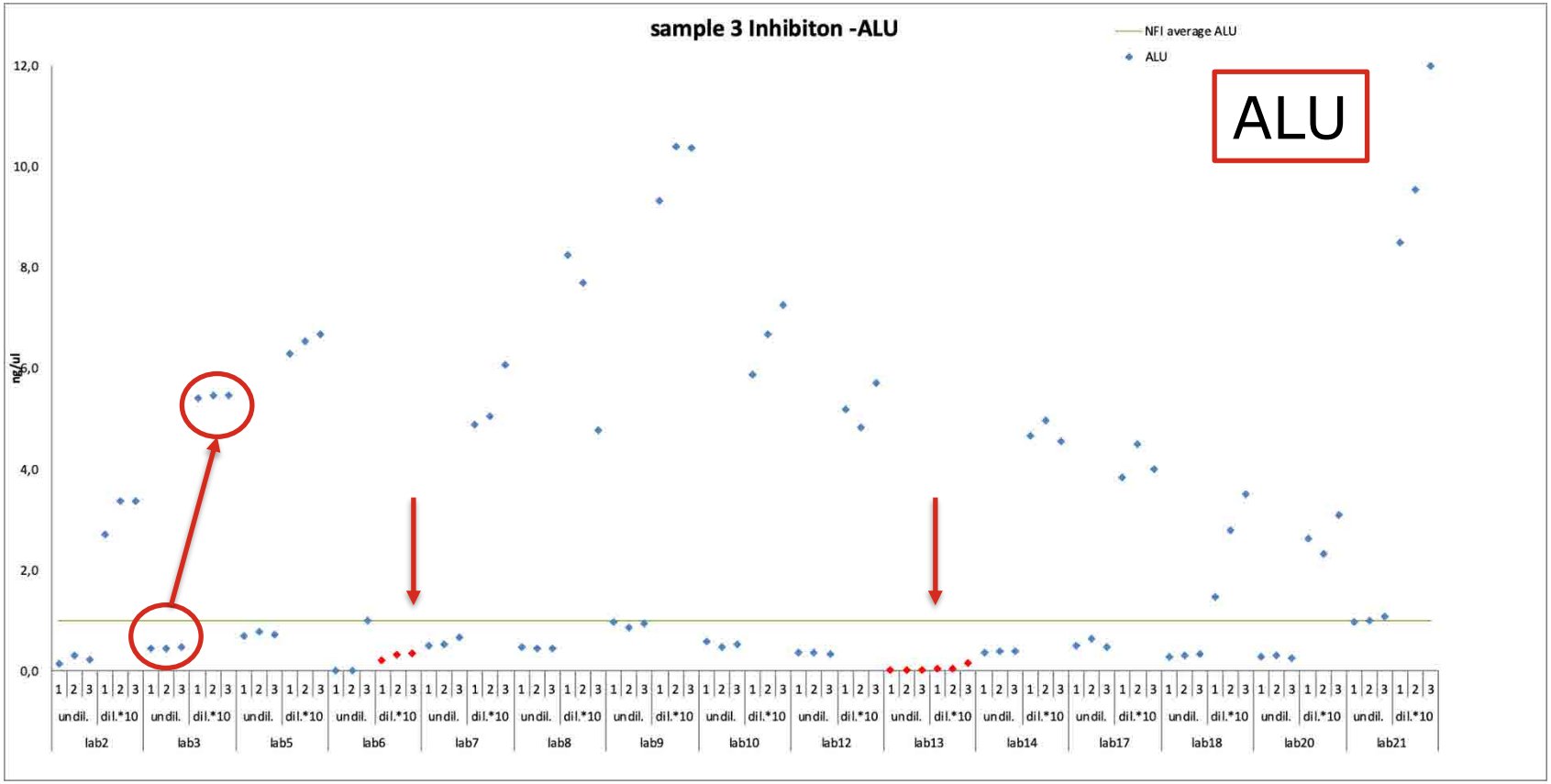
Sample = male DNA + inhibitor humic acid

Expected results:

Quant value >0 for ALU, Y, mt short and mt long

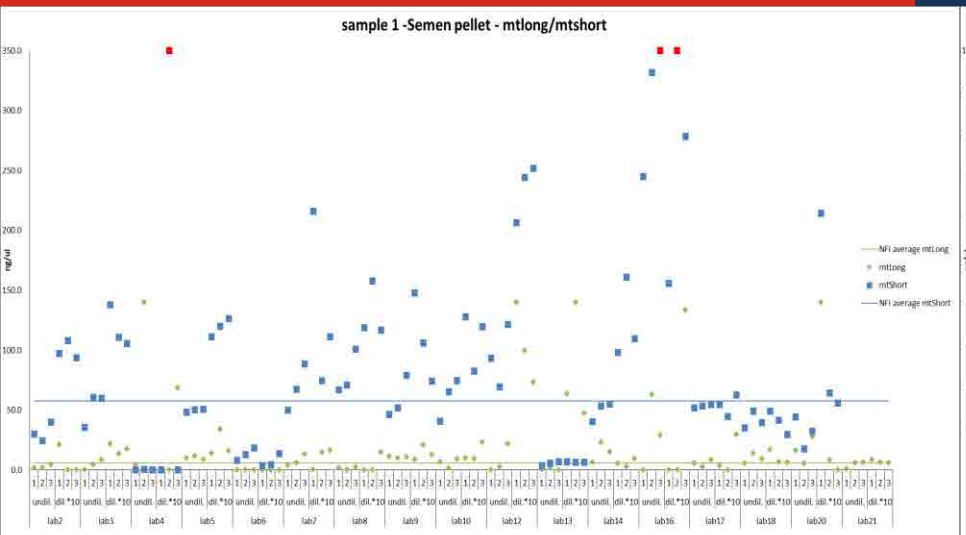
Quant value higher for diluted sample vs undiluted sample

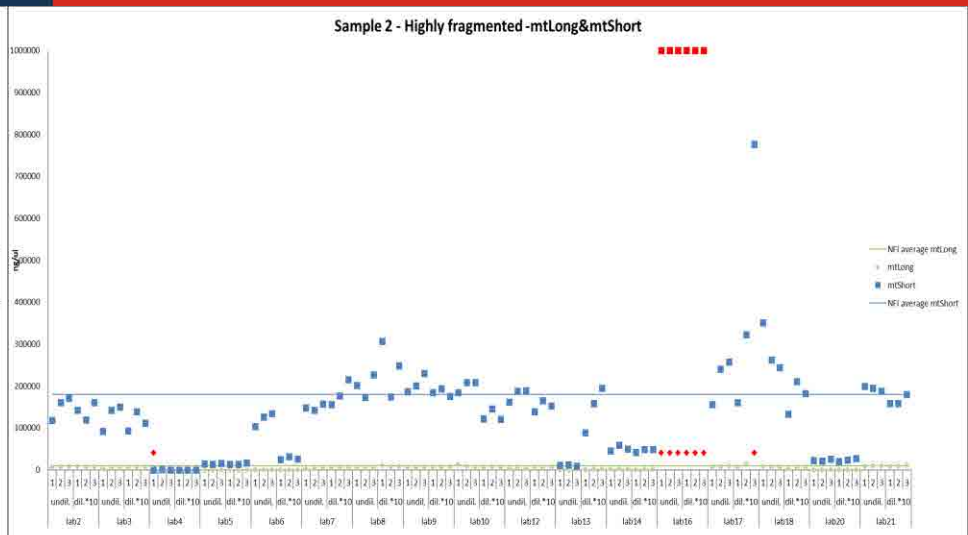
Sample #3 Male DNA + humic acid

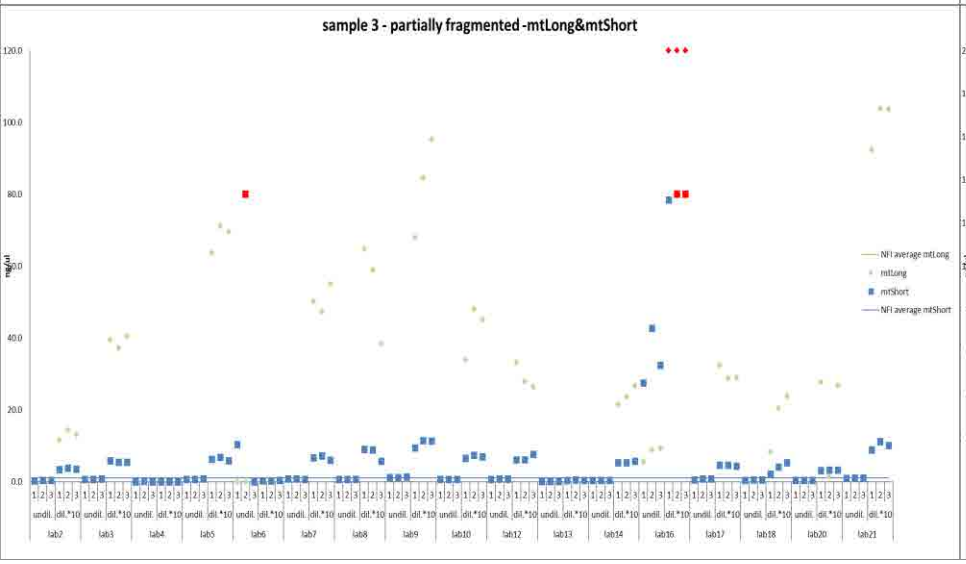


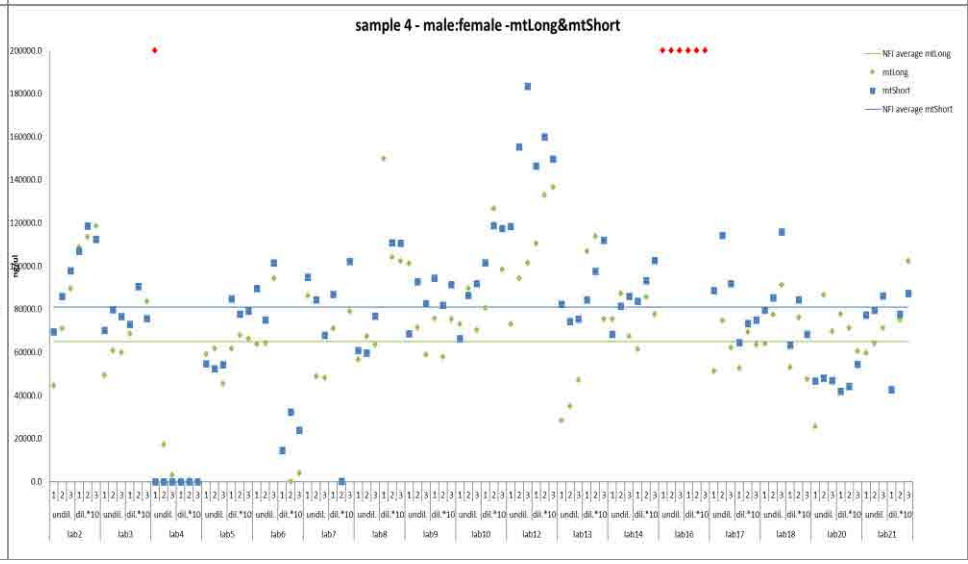
Next steps

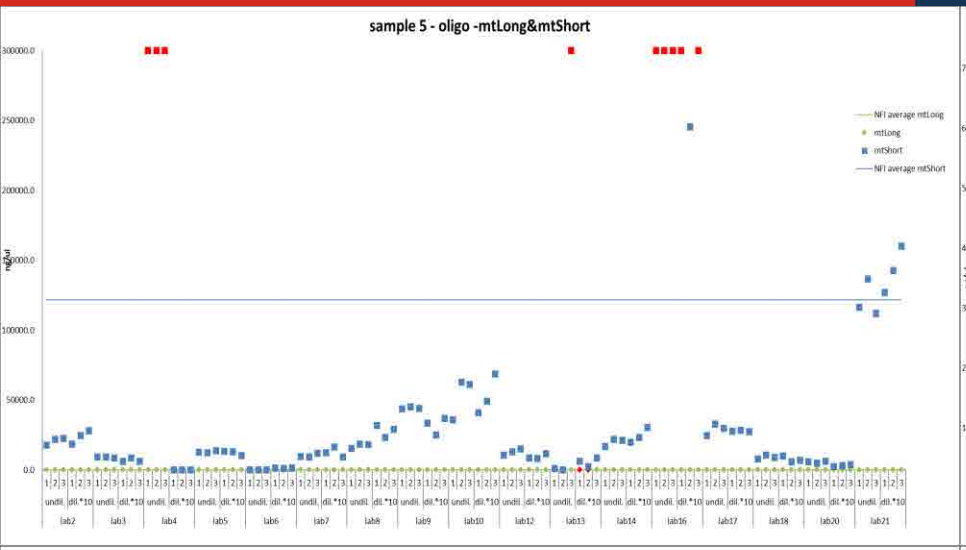
- Further analysis of the data
- Decide if it is worthwhile to publish
- Update at the next EDNAP meeting

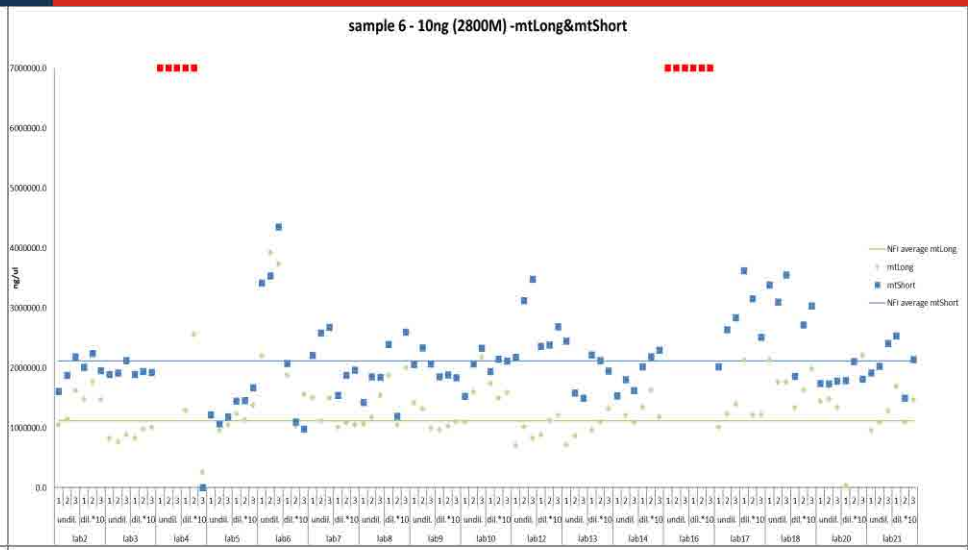

sample 1 -Semen pellet - mtlong/mtshort
ng/ul
NFI average mtLong
mtLong
mtShort
NFI average mtShort

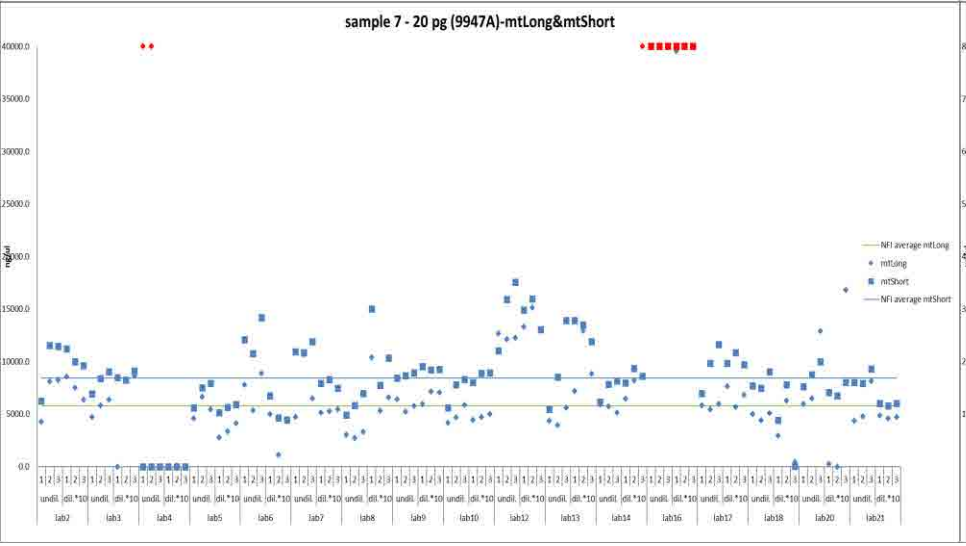

Sample 2 - Highly fragmented -mtLong&mtShort
ng/ul
NFI average mtLong
mtLong
mtShort
NFI average mtShort

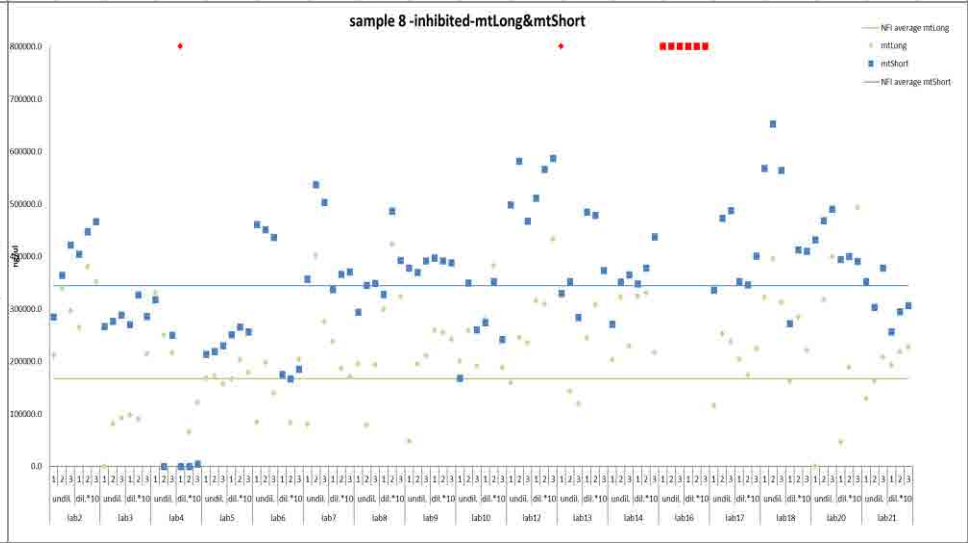

sample 3 - partially fragmented -mtLong&mtShort
ng/ul
NFI average mtLong
mtLong
mtShort
NFI average mtShort


sample 4 - male:female -mtLong&mtShort
ng/ul
NFI average mtLong
mtLong
mtShort
NFI average mtShort


sample 5 - oligo -mtLong&mtShort
NFI average mtLong
mtLong
mtShort
NFI average mtShort
ng/µl


sample 6 - 10ng (2800M) -mtLong&mtShort
NFI average mtLong
mtLong
mtShort
NFI average mtShort
ng/µl


sample 7 - 20 pg (9947A)-mtLong&mtShort
NFI average mtLong
mtLong
mtShort
NFI average mtShort
ng/µl


sample 8 -inhibited-mtLong&mtShort
NFI average mtLong
mtLong
mtShort
NFI average mtShort
ng/µl

NFI has made changes

Standaards were up to 8 ng, now up to 50ng.

Switched from van 7500 to QS5 and QS7

Passive dye was mustang purple, now switched to rox

Addition of IPC, mito long removed

Conclusions

The quantification assay worked wel for the NFI, but…..

The quantification exercise resulted in large variation of measured DNA concentration, outliers and unexpected results, perhaps caused by negative effects of distribution of samples and reagents.

The results are insufficient for publication.

Thank you all for your contribution!

EDNAP collaborative exercises on DNA transfer

Progress update – September 2022

Bas Kokshoorn (Netherlands Forensic Institute)
Roland van Oorschot (Victoria Police Forensic Services Department)
Bianca Szkuta (Deakin University)

27 September 2022 | Lisbon, Portugal

Outline of series of exercises

- Exercise A: *Case file data collection*.
  Lab participation. Paper/electronically based.

- Exercise B: *Experimental data generation*.
  Lab participation. Laboratory & paper/electronically based.

- Exercise C: *Case assessment*.
  Individual participation. Paper/electronically based.

- Exercise D: *Evaluation of findings*.
  Individual participation. Paper/electronically based.

Outline of series of exercises

- **Exercise A: *Case file data collection*.**
  Lab participation. Paper/electronically based.
- Exercise B: *Experimental data generation*.
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- Exercise C: *Case assessment*.
  Individual participation. Paper/electronically based.
- Exercise D: *Evaluation of findings*.
  Individual participation. Paper/electronically based.

Proposal for Exercise A in more detail

Purpose

- First collaborative exercise on lab results to
    - Accumulate and compare data on profile types obtained from particular item types given information on item history, methods, and procedures applied to generate the profiles
    - Help assess the impacts of differences in methods and procedures
    - Help assess the appropriateness / limitation of using data from other laboratories in evaluation of findings given activity level scenarios
    - Help drive potential improvement opportunities in respect to the methodologies and procedures utilized by a lab as part of their service delivery
- The exercise will gain insight on how readily the requested information was able to be sourced within each laboratory

Timeline

- Proposal at ENAP meeting Riga (Latvia) – October 2019
- Call for expression of interest in Exercise A – Q1 2020
  - report of responses shared with EDNAP – Q2 2020

Response from laboratories

Response from 49 laboratories

- Europe	36
- Australia/New Zealand	8
- North America	4
- Asia	1

Interest in participation – exercise A

- Yes: 44
- No: 5 (reasons cited: no casework data; no interest in HVC type data; lack of detailed info on past cases)

Timeline

- Proposal at ENAP meeting Riga (Latvia) – October 2019
- Questionnaire / expression of interest Exercise A – Q1 2020
- Development of Exercise A – Started Q1 2020
  - ✓put on hold after COVID outbreak
  - ✓continued development 2021
  - ✓pilot testing VPFSD/NFI Q4 2021 - Q1 2022
  - ✓pilot testing three other labs Q2-Q3 2022
  - ❑currently addressing feedback and finalizing questionnaire and associated documentation

Proposal for Exercise A in more detail

Questions will be asked within four separate Excel sheets:

- Sheet 1: Questions relating to the sets of methodologies used
- Sheet 2: Questions relating to **Tool handles**.
- Sheet 3: Questions relating to **Gloves**.
- Sheet 4: Questions relating to Data availability and relevance

Proposal for Exercise A in more detail

Questions in sheets 2 and 3 (related to items):

- Section A: Item type
- Section B: Item history
- Section C: Packaging
- Section D: Storage
- Section E: Durations
- Section F: Prior examinations – pre DNA sampling
- Section G: Target area
- **Section H: Methodology set used (referencing set(s) detailed in Sheet 1)**
- **Section I: DNA quantitation**
- **Section J: DNA amplification**
- **Section K: DNA profile results**
- **Section L: Profile interpretation**
- Section M: Other

Screenshot of Excel document

	A	B	C	D	E	F	G	H	I	J	
1						**A: ITEM TYPE**					
2						**Item details**					
3	**Lab code**	**Sample No.**	**Exercise sample code (if desired)**	**Lab sample code** **For lab use only** *Please don't forward this info*	**Lab case number** **For lab use only** *Please don't forward this info*	1. Type of tool:	2. Handle type:	3. Handle length: (if no clear handle, please indicate length of item)	4. Handle diameter: (if no clear handle, please indicate diameter of item)	5. Handle groves: (if no clear handle, please indicate groves on item) *See information document for images*	6. Handle edges: (if no clear handle, pl *See information docume*
4	[insert text]	[insert text]	[insert text]			Screw driver - non-powered	Don't know	Don't know	Don't know	Don't know	Don't know
5	[insert text]	[insert text]	[insert text]			Screw driver - non-powered	Don't know	Don't know	Don't know	Don't know	Don't know
6	[insert text]	[insert text]	[insert text]			Screw driver - non-powered	Don't know	Don't know	Don't know	Don't know	Don't know
7	[insert text]	[insert text]	[insert text]			Screw driver - non-powered	Don't know	Don't know	Don't know	Don't know	Don't know
8	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]		1 handle typically handled by one hand (e.g. screw driver)				
9	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]		1 handle typically handled by two hands (e.g. large axe)				
10	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]		2 handles typically handled by one hand (e.g. pliers)				
11	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]		2 handles typically handled by two hands (e.g. bolt cutter)				
12	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]		No clear handle (e.g. crowbar)				
13	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]		Other (please address in comments section and Go to Q.3)				
14	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]		Don't know				
15	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
16	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
17	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
18	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
19	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
20	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
21	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
22	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
23	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
24	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
25	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
26	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
27	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
28	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
29	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
30	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						
31	[insert text]	[insert text]	[insert text]	[insert text]	[insert text]						

Sheet 1 - Methods | Sheet 2 - Tool Handles | Sheet 3 - Inside Gloves | Sheet 4 - General Questions

Timeline - tentative

- November 2022 – Reach out to labs that expressed interest
  - Gauge whether they are still interested
- January 2023 – Distribute questionnaire
- May 2023 – Return of filled out questionnaires
- Q3 2023 – Q2 2024 – Data analysis/interpretation
- Q3-Q4 2024 Communication/publication
  - EDNAP meeting
  - Publication of dataset
  - Publication of analysis/interpretation

Next exercises – Exercise B

- ReACT (ENFSI monopoly)
  - partial overlap with aims of Exercise B
  - Roland v. O. involved in both
- Exercise B on hold, pending progress of ReACT (lab based exercises planned to continue into Q2 2023)

Exercise C – Case assessment

- **Benchmark on case assessment and triage**

- Provide (mock) case
  - *Case issue*
  - *Case information*

- Purpose to compare;
  - *What info would expert use?* **(CIM)**
  - *Which scenario's would be considered relevant?*
  - *What factors impacting on DNA-TPPR are being considered?*
  - *What examination strategies would be considered?*
  - *What would be the expected outcomes for examinations?*
    -> based on which information/expertise?
  - *What would the recommended strategy be?*

Next exercises – Exercise C

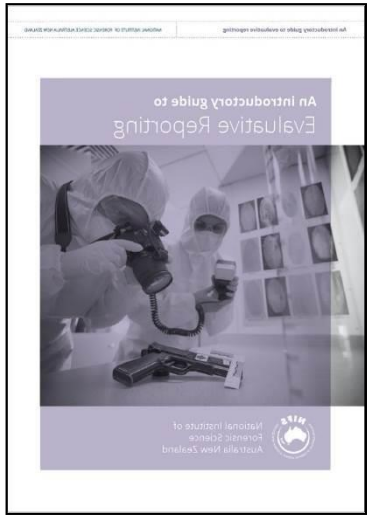
- Start TBD
- After finalizing Exercise A

Exercise D: *Evaluation of findings*

- **Benchmark on reporting given activity propositions**

- Provide (mock) case
  - *Case context*
  - *Case examination and profiling data*

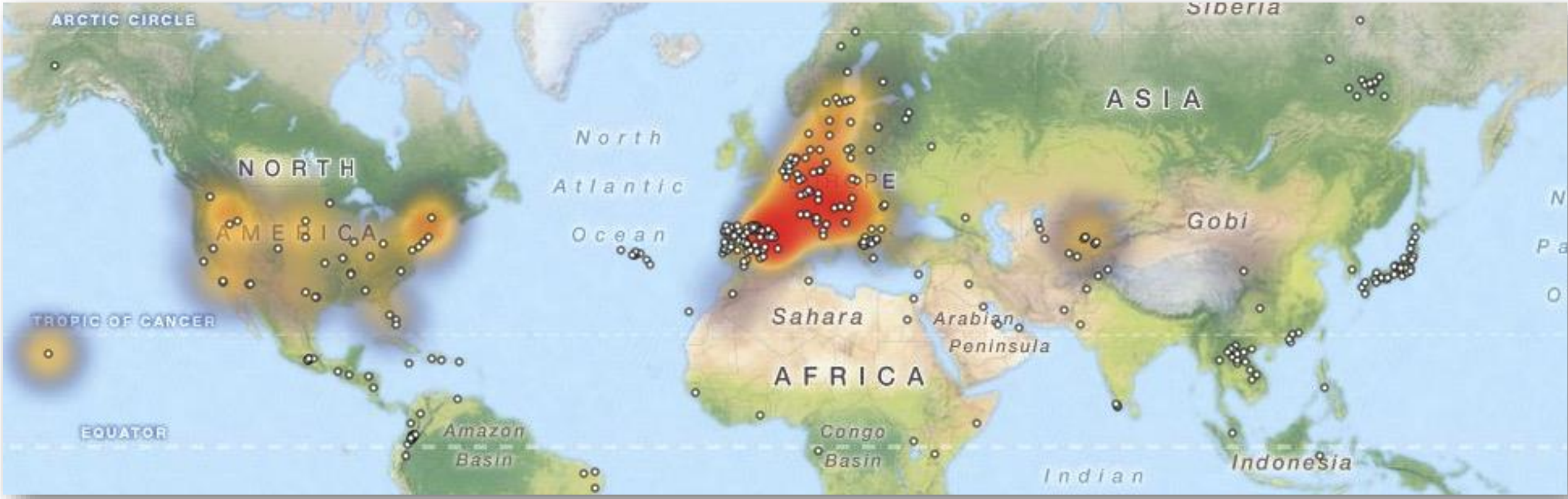
- Purpose to compare;
  - *Formulating propositions*
  - *Management of case information*
  - *Structure of argument*
  - *Data sources used*
  - *Reporting structure*



Next exercises – Exercise D

- Considering bringing this exercise forward and start planning, development and roll-out in 2023
- Project lead(s) TBD

EDNAP Meeting, Lisbon, Portugal, Sep 27 2022



mtDNA/EMPOP Update

Dr. Walther Parson
ao. Prof. Institute of Legal Medicine, Medical University of Innsbruck, Austria
adj. Prof. Forensic Science Program, Penn State University, PA, USA
walther.parson@i-med.ac.at

mtDNA publications (2019-2022)

Population studies

Bodner, M. *et al.* (2022) 'Helena's Many Daughters: More Mitogenome Diversity behind the **Most Common West Eurasian mtDNA Control Region** Haplotype in an Extended **Italian** Population Sample', *International Journal of Molecular Sciences*, 23(12), p. 6725.

Cardinali, I. *et al.* (2021) 'Mitochondrial DNA Footprints from Western Eurasia in Modern **Mongolia**', *Front Genet*, 12, p. 819337.

Bodner, M. *et al.* (2021) 'The Mitochondrial DNA Landscape of Modern **Mexico**', *Genes*, 12(9), p. 1453.

Simão, F. et al. (2021) 'The Ancestry of Eastern **Paraguay**: A Typical South American Profile with a Unique Pattern of Admixture', Genes, doi 10.3390/genes12111788

Taylor, C.R. *et al.* (2020) 'Platinum-Quality Mitogenome Haplotypes from **United States** Populations', *Genes*, 11(11), p. 1290.

Garcia, O. *et al.* (2020) 'Forensically relevant phylogeographic evaluation of mitogenome variation in the **Basque** Country', *Forensic Sci Int Genet*, 46, p. 102260.

Göbel, T.M.K. *et al.* (2020) 'Mitochondrial DNA variation in Sub-Saharan Africa: Forensic data from a mixed West African sample, **Côte d'Ivoire** (Ivory Coast), and **Rwanda**', *Forensic Science International: Genetics*, 44.

mtDNA publications (2019-2022)

Population studies - continued

Modi, A. *et al.* (2020) 'The mitogenome portrait of Umbria in **Central Italy** as depicted by contemporary inhabitants and pre-Roman remains', *Sci Rep*, 10(1), p. 10700.

Barbarić, L. *et al.* (2020) 'Maternal perspective of **Croatian** genetic diversity', *Forensic Science International. Genetics*, 44, p. 102190.

Simão, F. *et al.* (2019) 'The maternal inheritance of the Ashaninka native group from **Peru**', *Forensic Science International: Genetics Supplement Series*, 7(1), pp. 135–137.

Zimmermann, B. *et al.* (2019) 'Mitochondrial DNA control region variation in **Lebanon**, **Jordan**, and **Bahrain**', *Forensic Science International: Genetics*, 42, pp. 99–102.

Wood, M.R. *et al.* (2019) 'Resolving mitochondrial haplogroups B2 and B4 with next-generation mitogenome sequencing to distinguish **Native American** from **Asian** haplotypes', *Forensic Science International: Genetics*, 43.

Dissecting CR matches with mitogenome sequences

216 identical CR sequences 16519C 263G 315.1C (= most common CR in Europe)

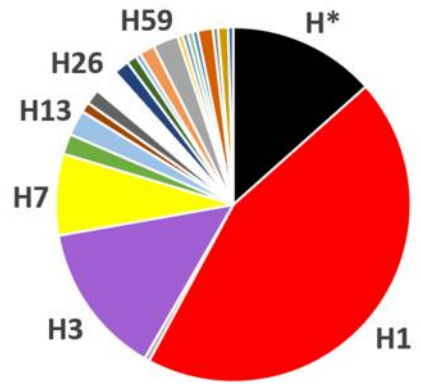
dissected into 163 different mitogenomes (131 unique)

24 different haplogroups (Phylotree b17) within hg H

Table 1. Diversity parameters of the 216 Italian mtDNAs exhibiting the most common West Eurasian control region (CR) haplotype using different sequence ranges. Percentages are rounded (see text for details).

	CR	CR + 3 codR SNPs [1]	Complete Mitogenome [2]
Haplotypes	1	4	163
Unique haplotypes	0	0	131
Discrimination capacity (DC)	–	0.019	0.755
Named haplogroups [3]	1	4	61
Random match probability (RMP)	1.000	0.342	0.009
Power of discrimination (PD) [4]	0.0%	66.1%	99.6%

[1] specific for haplogroups H1 (np 3010), H3 (np 6776), and H7 (np 4793); [2] see Table S2 for alternative scenarios; [3] including the paraphyletic group (paragroup) H*; [4] Haplotype diversity (HD).



Bodner et al 2022

mtDNA publications (2019-2022)

Archaeological studies

Cemper-Kiesslich, J. *et al.* (2021) 'aDNA Analyses of the Late Merovingian Children's Double Tomb under **Frankfurt Cathedral**, *Archaeologia Austriaca*, Band 105/2021, pp. 283–296.

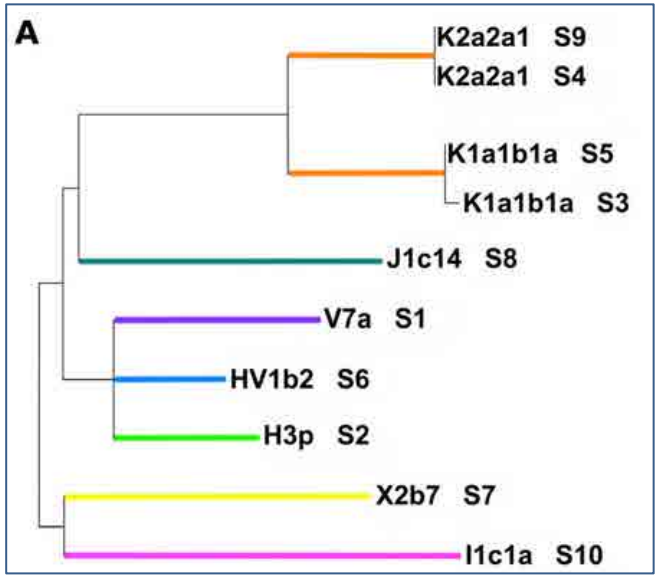
Diepenbroek, M. *et al.* (2021) 'Genetic and phylogeographic evidence for **Jewish Holocaust victims** at the Sobibór death camp', *Genome Biology*, 22(1).

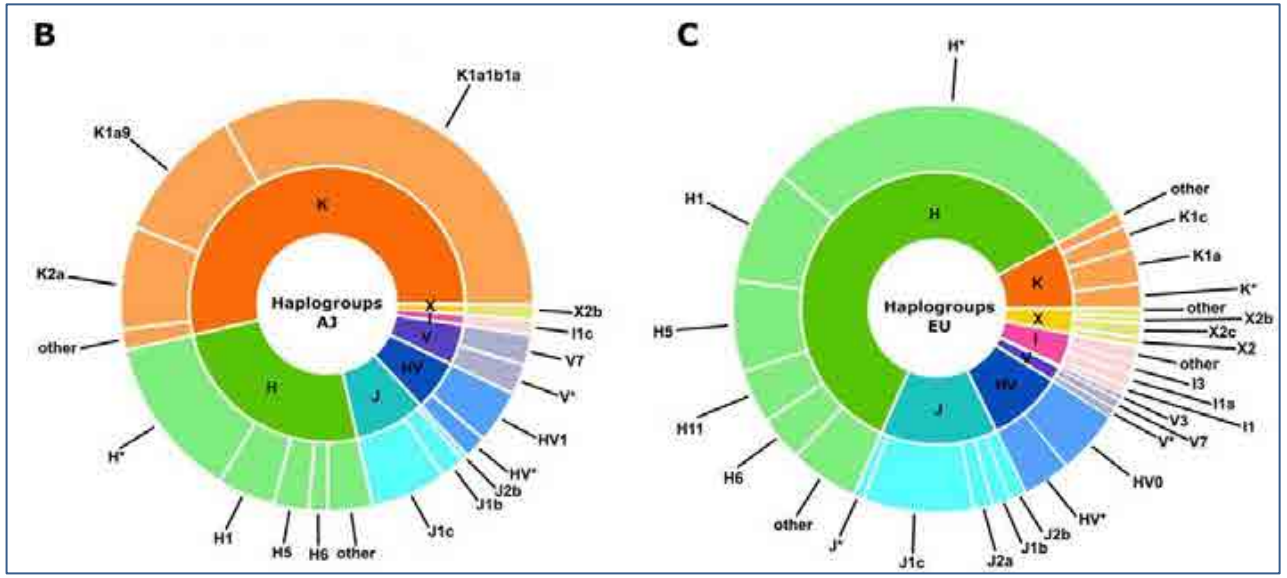
Pany-Kucera, D., *et al.* (2020) 'Social Relations, Deprivation and Violence at Schleinbach, **Lower Austria**. Insights from an Interdisciplinary Analysis of the Early Bronze Age Human Remains', *Archaeologia Austriaca*

Tobias, B. *et al.* (2020) 'House of the dead-exceptional burials of the **Avar period** (seventh century AD) in Podersdorf am See (Burgenland/A)', *Archaeological and Anthropological Sciences*, 12(8).

Bus, M.M. *et al.* (2019) 'Mitochondrial dna analysis of a **viking age mass grave in sweden**', *Forensic Science International: Genetics*, 42, pp. 268–274.

Genetic and phylogeographic evidence for Jewish Holocaust victims at the Sobibór death camp





Diepenbroek et al 2021

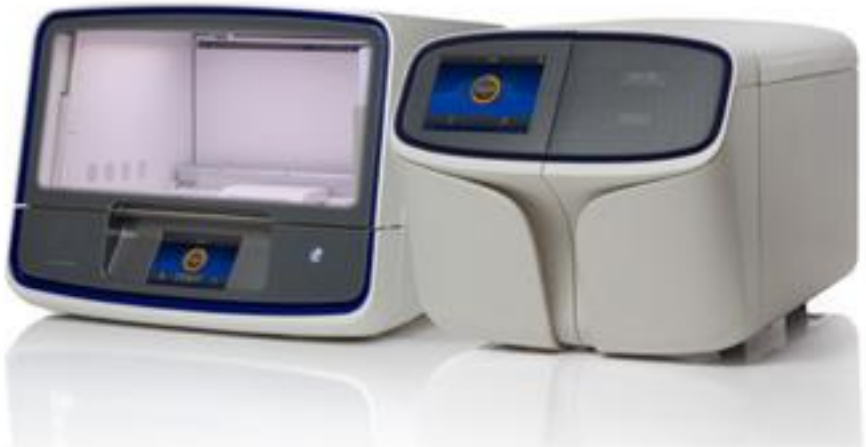
mtDNA publications (2019-2022)

Mito MPS Validation

Cihlar, Jennifer Churchill, Amory, C., *et al.* (2020) '**Developmental Validation** of a MPS Workflow with a PCR-Based Short Amplicon Whole Mitochondrial Genome Panel', *Genes*, 11(11), p. E1345.

Cihlar, J.C. *et al.* (2020) 'The lot-to-lot variability in the **mitochondrial genome of controls**', *Forensic Science International: Genetics*, 47.

Strobl, C. *et al.* (2019) 'Evaluation of **mitogenome sequence concordance, heteroplasmy detection, and haplogrouping** in a worldwide lineage study using the Precision ID mtDNA Whole Genome Panel', *Forensic Sci Int Genet*, 42, pp. 244–251.



mtDNA publications (2019-2022)

Heteroplasmy

McElhoe, J.A. *et al.* (2022) 'Exploring statistical weight estimates for mitochondrial DNA matches involving heteroplasmy', *International Journal of Legal Medicine*, 136(3), pp. 671–685.

Sturk-Andreaggi, K. *et al.* (2022) 'The Value of Whole-Genome Sequencing for Mitochondrial DNA Population Studies: Strategies and Criteria for Extracting High-Quality Mitogenome Haplotypes', *Int. Journal of Molecular Sciences*, 23(4), p. 2244.

Sturk-Andreaggi, K. *et al.* (2020) 'Impact of the sequencing method on the detection and interpretation of mitochondrial DNA length heteroplasmy', *Forensic Science International. Genetics*, 44, p. 102205.

NUMTs

Marshall, C. and Parson, W. (2021) 'Interpreting NUMTs in forensic genetics: Seeing the forest for the trees', *Forensic Science International: Genetics*, 53.

Lutz-Bonengel, S. *et al.* (2021) 'Evidence for multi-copy **Mega-NUMTs** in the human genome', *NAR*, 49(3), pp. 1517–1531

Cihlar, Jennifer Churchill, Strobl, C., *et al.* (2020) 'Distinguishing mitochondrial DNA and NUMT sequences amplified with the precision ID mtDNA whole genome panel', *Mitochondrion*, 55, pp. 122–133.

Lutz-Bonengel, S. and Parson, W. (2019) 'No further evidence for paternal leakage of mitochondrial DNA in humans yet', *Proceedings of the National Academy of Sciences*, 116(6), pp. 1821–1822.

PNAS

Biparental Inheritance of Mitochondrial DNA in Humans

Shiyu Luo[a,b], C. Alexander Valencia[a,1], Jinglan Zhang[c], Ni-Chung Lee[d], Jesse Slone[a], Baoheng Gui[a,b], Xinjian Wang[a], Zhuo Li[a,2], Sarah Dell[a], Jenice Brown[a], Stella Maris Chen[c], Yin-Hsiu Chien[d], Wuh-Liang Hwu[d], Pi-Chuan Fan[e], Lee-Jun Wong[c], Paldeep S. Atwal[f,3], and Taosheng Huang[a,3,4]

[a]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229; [b]Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Nanning, 530003 Guangxi, China; [c]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030; [d]Department of Pediatrics and Medical Genetics, National Taiwan University Hospital, 100 Taipei, Taiwan; [e]Department of Pediatrics, National Taiwan University Hospital, 100 Taipei, Taiwan; and [f]Department of Clinical Genomics, Center for Individualized Medicine, Mayo Clinic Hospital, Jacksonville, FL 32224

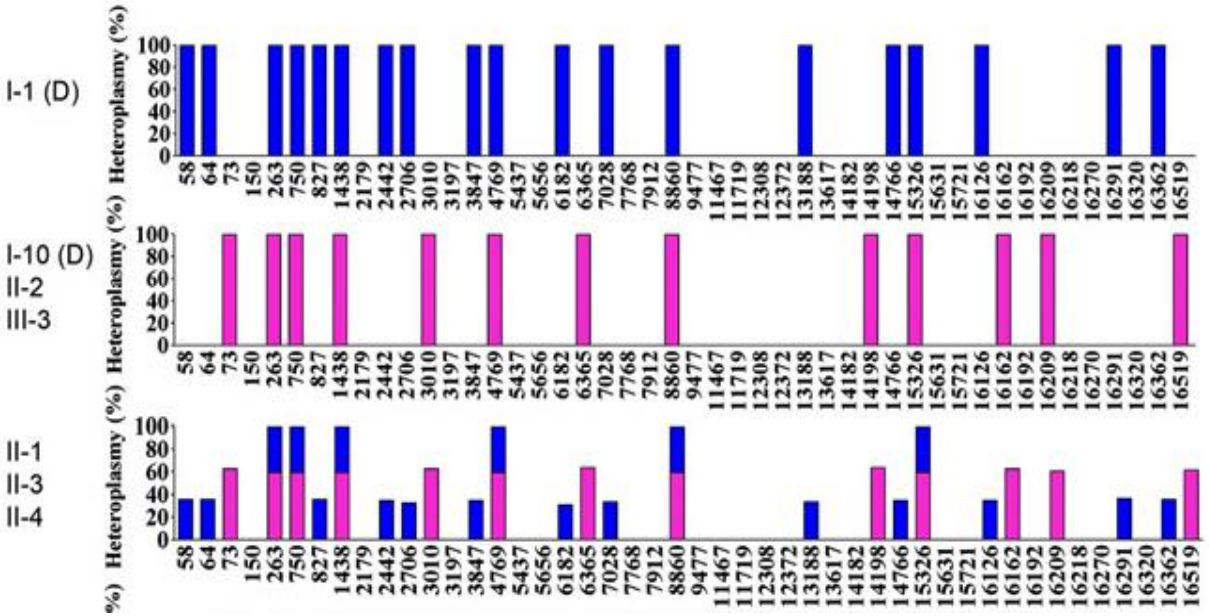
Edited by Douglas C. Wallace, Children's Hospital of Philadelphia and University of Philadelphia, Philadelphia, PA, and approved October 29, 2018 (received for review June 26, 2018)

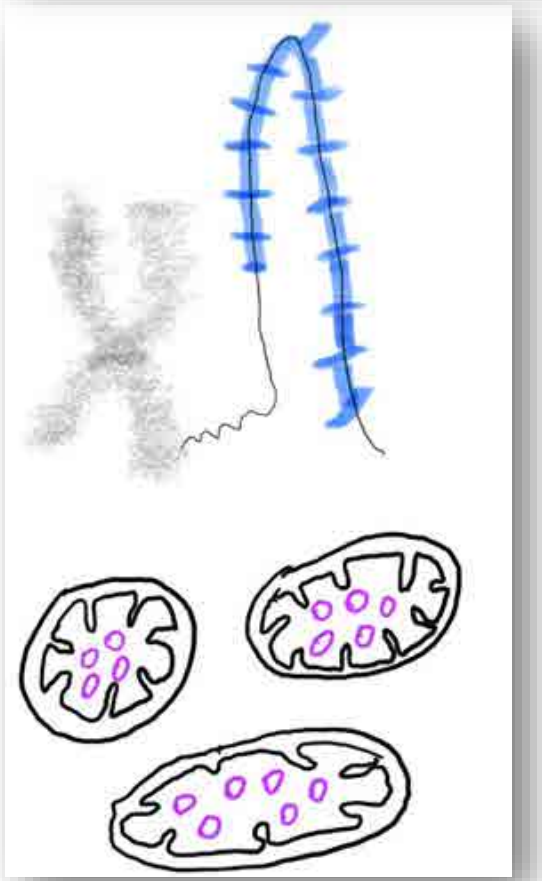
PNAS

LETTER

No further evidence for paternal leakage of mitochondrial DNA in humans yet

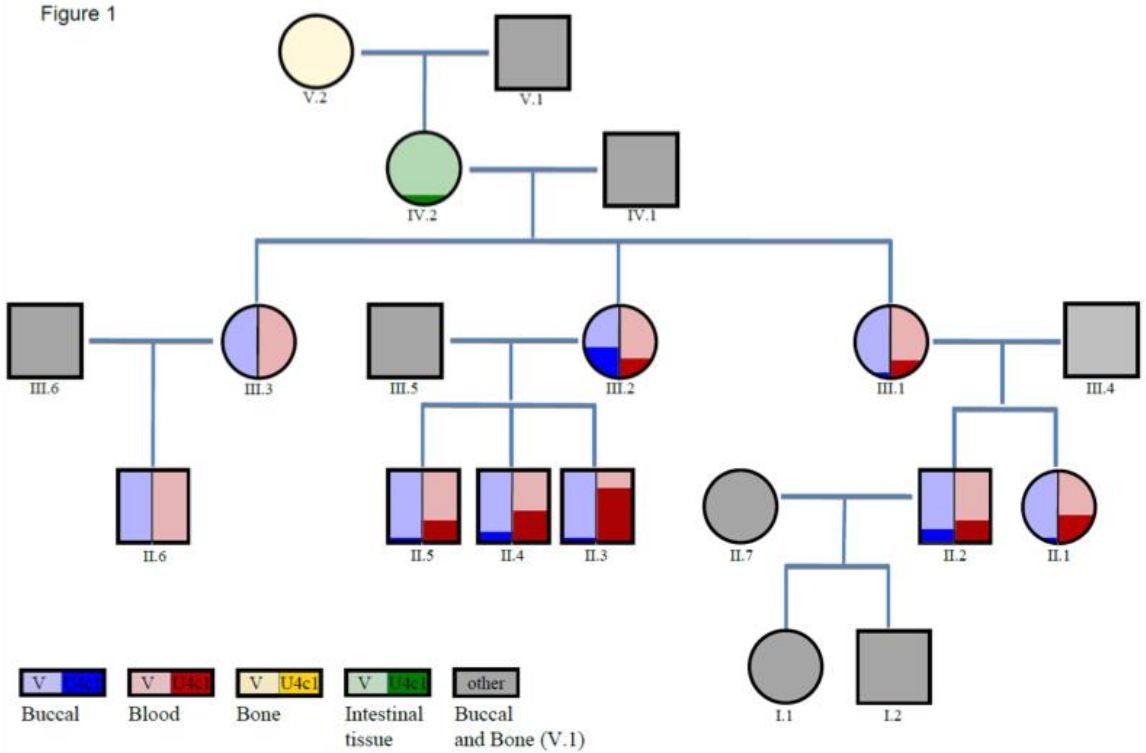
Sabine Lutz-Bonengel[a,b] and Walther Parson[c,d,1]

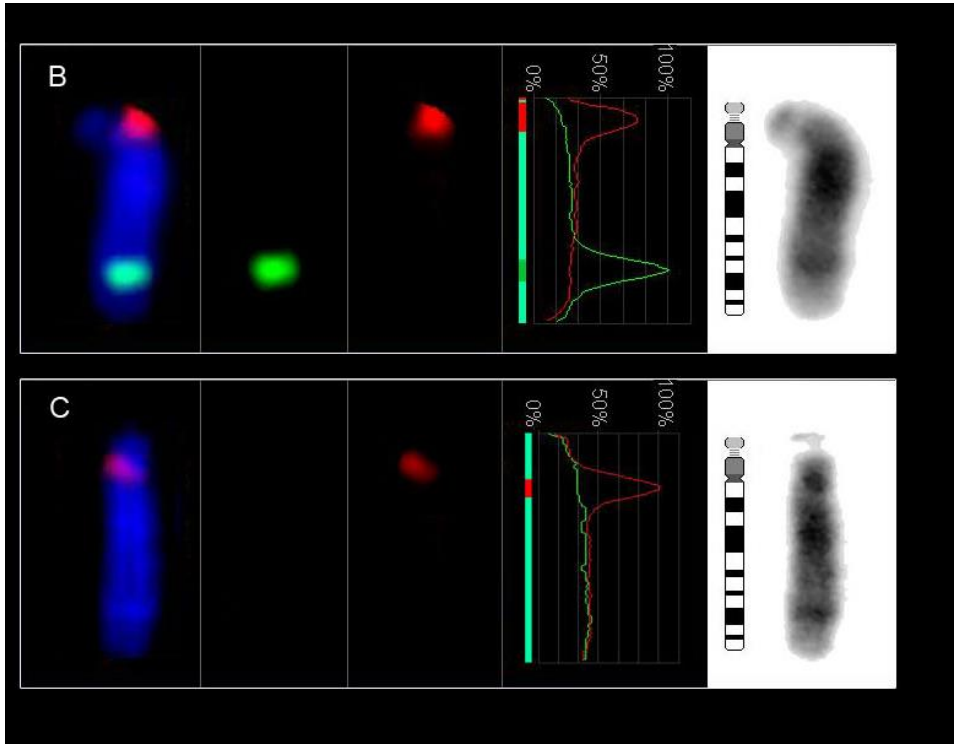


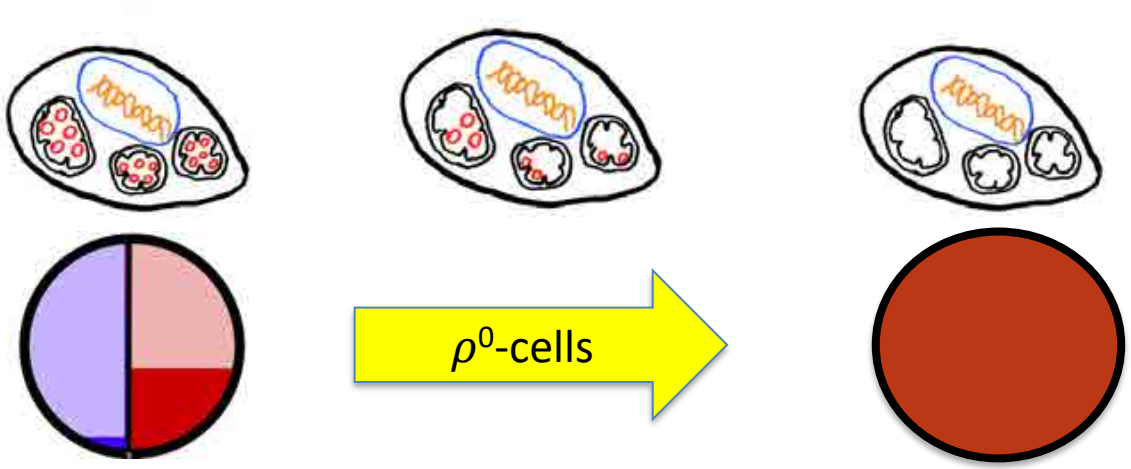


“Mega-NUMTs”

Balciuniene 2019







qPCR and ddPCR
ca. 50 mitogenome copies/cell

Lutz-Bonengel et al 2021

mtDNA publications (2019-2022)

EMPOP engine/software

Dür, A. *et al.* (2022) 'Post hoc deconvolution of human mitochondrial DNA mixtures by EMMA 2 using fine-tuned Phylotree nomenclature', *Computational and Structural Biotechnology Journal*, 20, pp. 3630–3638.

Dür, A., Huber, N. and Parson, W. (2021) 'Fine-Tuning Phylogenetic Alignment and Haplogrouping of mtDNA Sequences', *International Journal of Molecular Sciences*, 22(11), p. 5747.

Parson, W., Marshall, C., *et al.* (2020) 'Pathogenic Variant Filtering for Mitochondrial Genome Haplotype Reporting', *Genes*

Roth, C. *et al.* (2019) 'MVC: an integrated mitochondrial variant caller for forensics', *Australian Journal of Forensic Sciences*, 51(sup1), pp. S52–S55.

Deconvolution of mtDNA mixtures

Computational and Structural Biotechnology Journal 20 (2022) 3630–3638

ELSEVIER

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Post hoc deconvolution of human mitochondrial DNA mixtures by EMMA 2 using fine-tuned Phylotree nomenclature

Arne Dür [a], Nicole Huber [b,c], Alexander Röck [d], Cordula Berger [b], Christina Amory [b], Walther Parson [b,e,*]

[a] Institute of Mathematics, University of Innsbruck, Technikerstrasse 13, 6020 Innsbruck, Austria
[b] Institute of Legal Medicine, Medical University of Innsbruck, Müllerstrasse 44, 6020 Innsbruck, Austria
[c] MED-EL Elektromedizinische Geräte GmbH, Fürstenweg 77a, Innsbruck 6020 Austria
[d] synedra IT GmbH, Feldstrasse 1/13, Innsbruck 6020 Austria
[e] Forensic Science Program, The Pennsylvania State University, 13 Thomas Building, University Park, PA 16802, USA

Deconvolution of mtDNA mixtures

Splitting of sequences more complex than deconvoluting fragment sizes

Previous attempts rely on MPS data (quantitative, phased data)

Software scarce, e.g. MMDIT (Mandape et al 2021; github)

EMMA 2

Database of 6380 mitogenomes for 5435 haplogroup motifs

$Q=Q_1\&Q_2\&\ldots\&Q_k$ (currently up to 3 contributor mixtures)

Differences between Q and haplogroup motifs are quantified by costs

Costs = sum of LLRs of fluctuation rates at each mtDNA position (Röck et al 2013)

Output is graded by clustering costs and corresponding haplogroups

Dür et al 2022

Deconvolution of mtDNA mixtures

Random mitogenome mixtures (1000 two contributors; 100 three contributors)

Table 2
Artificial mixtures deconvolved.

	2 components	3 components
covered by rank 1 combinations	997/1000 (99.7%)	95/100 (95%)
covered by rank 2 combinations	3/3 (100%)	4/5 (80%)

GEDNAP mixtures (with know contributors)

Dür et al 2022

Deconvolution of mtDNA mixtures

Q **GEDNAP 36 S4**
CR: 16093Y 16224Y 16256Y 16311Y 16352Y 16519Y 73R 152Y 263G 309.1C 315.1C 497Y

# contributors	Costs	Haplogroups (MRCA)
1	3.12-3.61	R
2	0.80-1.29	H&K1a
3	1.20-1.70	R&R0&K1a

True components
Q1: 16093C 16224C 16311C 16519C 73G 263G 315.1C 497T (hg K1a)
Q2: 16256T 16352C 152C 263G 309.1C 315.1C (hg H14a)

mod. from Dür et al 2022

Deconvolution of mtDNA mixtures with EMMA 2

Splitting is fast and does not require raw data (Sanger, MPS)

Identify up to 3 contributor mixtures in less than an hour (conventional PC)

Can also be used to identify NUMTs (Dür et al in preparation)

Limitations

Private mutations may be diagnostic for other haplogroups

Dür et al 2022

EMPOP training

ISFG Summer School, Online, Jul 20-30 2021

EMPOP training

Dublin, IRE, Mar 29-31 2022

EMPOP training

ISFG pre-congress workshop, Aug 29 2022

Co-funded by the Internal Security Fund of the European Union

MONOPOLY 2016 - STEFA - WP G7

Empowering forensic genetic DNA databases for the interpretation of next generation sequencing profiles (**dna.bases**)

STRidER & EmPOP

Jan 2018 - Dec 2019

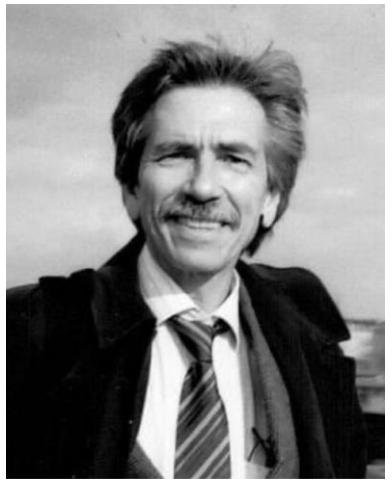
Sequence alignments
Increase sample size
Increase markers/regions
Further develop QC tools
User-friendly access

STRidER dna.bases EMPOP

ISFG Update

EDNAP Innsbruck 2018

EDNAP Santiago 2015



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Treasurer: Marielle Vennemann, Münster • **Representative of the Working Parties:** Leonor Gusmão, Rio de Janeiro

Achievements and Activities 2019-2022

- Moving to Regular Virtual Executive Board Meetings
- Newsletters and Website
- Conference Proceedings from Prague
- Virtual Summer School 2021
- DNA Commission publications
- *FSI Genetics* Impact Factor (Loss and Restoration)
- Forensic Databasing Advisory Board (FDAB)
- Prize Winners
- Future Meetings

Executive Board Meetings:
Mostly Virtual Now and More Often

GoToMeeting

September 14, 2021

WebEx

August 2, 2022

Virtual Meetings since ISFG 2019 in Prague:

2019 (1): November 21

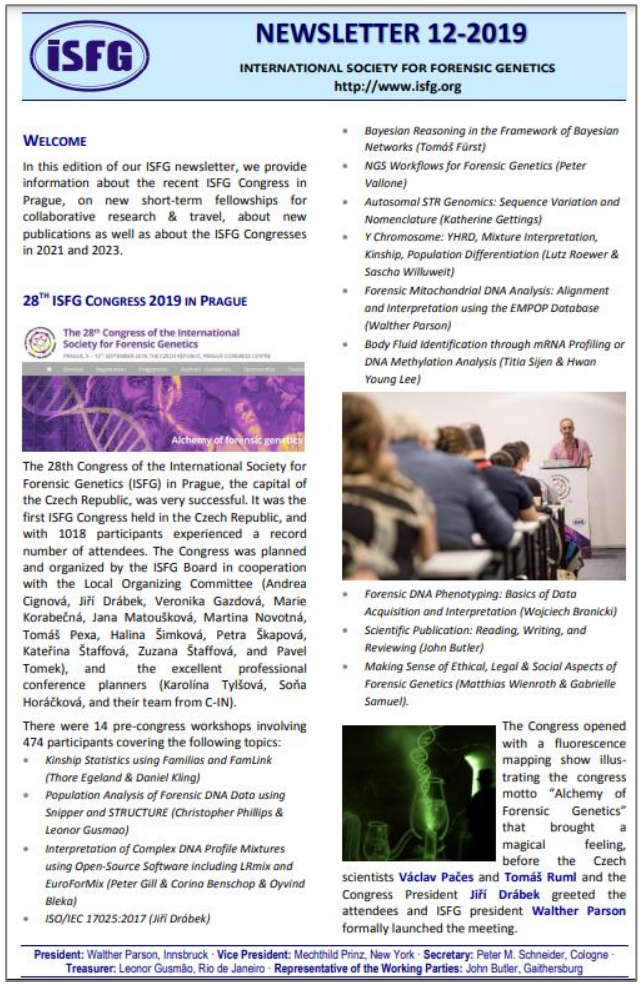
2020 (5): May 13, July 10, September 3, October 15, November 11

2021 (10): January 20, February 2, February 15, March 10, May 5, July 9, September 14, October 6 (FDAB), November 10, December 9 (FDAB), December 15

2022 (8): March 2, April 27, April 28, June 22, July 12, July 21 (FDAB), August 2, August 28

cc JM Butler

Newsletters Published to Inform ISFG Members



NEWSLETTER 12-2019

INTERNATIONAL SOCIETY FOR FORENSIC GENETICS
http://www.isfg.org

WELCOME

In this edition of our ISFG newsletter, we provide information about the recent ISFG Congress in Prague, on new short-term fellowships for collaborative research & travel, about new publications as well as about the ISFG Congresses in 2021 and 2023.

28TH ISFG CONGRESS 2019 IN PRAGUE

The 28th Congress of the International Society for Forensic Genetics

Alchemy of forensic genetics

The 28th Congress of the International Society for Forensic Genetics (ISFG) in Prague, the capital of the Czech Republic, was very successful. It was the first ISFG Congress held in the Czech Republic, and with 1018 participants experienced a record number of attendees. The Congress was planned and organized by the ISFG Board in cooperation with the Local Organizing Committee (Andrea Cignová, Jiří Drábek, Veronika Gazdová, Marie Korabečná, Jana Matoušková, Martina Novotná, Tomáš Pexa, Halina Šimková, Petra Škapová, Kateřina Šťastná, Zuzana Šťastná, and Pavel Tomek), and the excellent professional conference planners (Karolina Tylšová, Soňa Horáčková, and their team from C-IN).

There were 14 pre-congress workshops involving 474 participants covering the following topics:

- *Kinship Statistics using Familias and FamLink (Thore Egeland & Daniel Kling)*
- *Population Analysis of Forensic DNA Data using Snipper and STRUCTURE (Christopher Phillips & Leonor Gusmao)*
- *Interpretation of Complex DNA Profile Mixtures using Open-Source Software including LRmix and EuroForMix (Peter Gill & Corina Benschop & Oyvind Bleka)*
- *ISO/IEC 17025:2017 (Jiří Drábek)*
- *Bayesian Reasoning in the Framework of Bayesian Networks (Tomáš Fürst)*
- *NGS Workflows for Forensic Genetics (Peter Vallone)*
- *Autosomal STR Genomics: Sequence Variation and Nomenclature (Katherine Gettings)*
- *Y Chromosome: YHRD, Mixture Interpretation, Kinship, Population Differentiation (Lutz Roewer & Sascha Willuweit)*
- *Forensic Mitochondrial DNA Analysis: Alignment and Interpretation using the EMPOP Database (Walther Parson)*
- *Body Fluid Identification through mRNA Profiling or DNA Methylation Analysis (Titia Sijen & Hwan Young Lee)*

- *Forensic DNA Phenotyping: Basics of Data Acquisition and Interpretation (Wojciech Branicki)*
- *Scientific Publication: Reading, Writing, and Reviewing (John Butler)*
- *Making Sense of Ethical, Legal & Social Aspects of Forensic Genetics (Matthias Wienroth & Gabrielle Samuel).*

The Congress opened with a fluorescence mapping show illustrating the congress motto "Alchemy of Forensic Genetics" that brought a magical feeling, before the Czech scientists **Václav Pačes** and **Tomáš Ruml** and the Congress President **Jiří Drábek** greeted the attendees and ISFG president **Walther Parson** formally launched the meeting.

President: Walther Parson, Innsbruck · **Vice President:** Mechthild Prinz, New York · **Secretary:** Peter M. Schneider, Cologne · **Treasurer:** Leonor Gusmão, Rio de Janeiro · **Representative of the Working Parties:** John Butler, Gaithersburg

NEWSLETTER 09-2020

INTERNATIONAL SOCIETY FOR FORENSIC GENETICS
http://www.isfg.org

WELCOME

In this edition of our ISFG newsletter, we provide information about recent developments regarding our journal *FSI Genetics*, the impact of COVID-19 pandemic on various activities in our field, about new publications, and an important update on the ISFG 2021 Congress.

ISFG CONGRESS 2021 DELAYED TO 2022

WASHINGTON, DC ISFG 2021 INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

In discussions with Christian Westring (ISFG 2021 Congress President) and the local organizing committee, **we have decided to delay the 29th International Congress for one year** due to the ongoing COVID-19 pandemic. International travel will likely be uncertain well into next year and large indoor gatherings are currently prohibited in the United States. This difficult decision has been made as we have considered the health and wellbeing of our membership as well as the viability of holding a large scientific gathering during these uncertain times. While we hope that conditions improve before August 2021, we must go forward with the meeting planning. We do not feel that replacing the ISFG 2021 meeting with a virtual conference would be a viable option given that ISFG membership spans time zones worldwide.

However, we are considering the possibility of **virtually conducting a series of educational workshops next summer**. These could be pre-recorded and thus be conveniently accessible to our membership. Presenters for 16 pre-Congress workshops have been recruited for ISFG 2021 prior to our recent decision to move the meeting to 2022, and we will discuss with these presenters the possibility of contributing to some 2021 educational workshops.

The 29th International Congress will still take place in Washington, D.C., in the **Marriott Marquis Hotel** located only a few blocks from the city center. The local organizing committee is working with the hotel to finalize new dates. **We expect the ISFG 2022 meeting to occur August 29 to September 2, 2022.** As always, the congress will cover the latest research and discuss legal and ethical concerns in forensic genetics. Abstract submission and early registration will open December 2021, please monitor the website http://www.isfg2021.org for information.

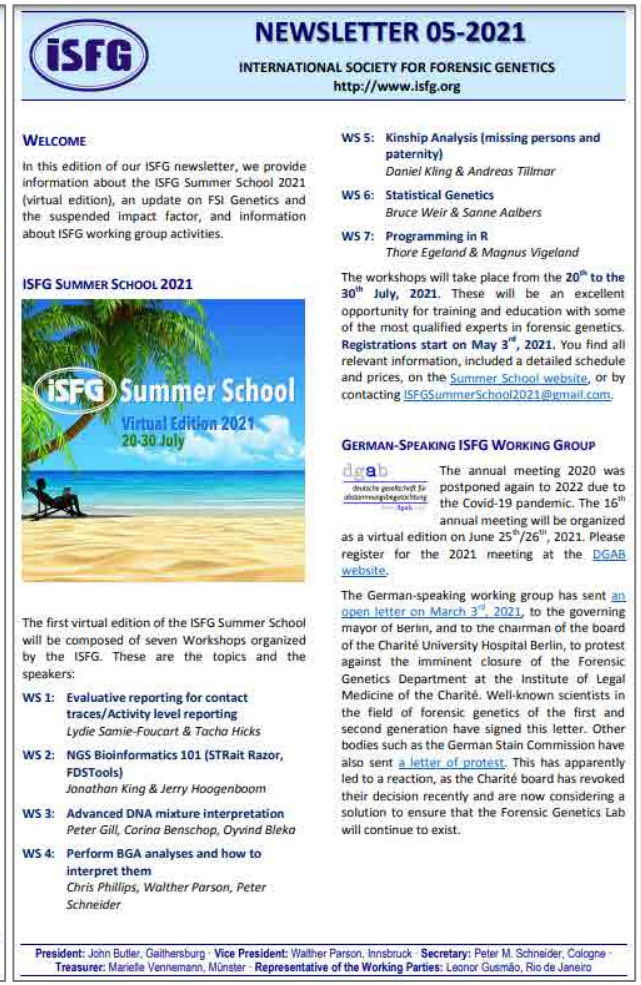
An impressive list of invited speakers has been recruited as follows:

- Genomics/Ancestry: **Chris Phillips** (Scientific Prize Winner)
- Biostatistics: **Thore Egeland** (Scientific Prize Winner)
- Population Genetics: **Noah Rosenberg** (Stanford)
- Legal Issues: **David Kaye** (Penn State law professor)
- Interpretation: **Tacha Hicks** (University of Lausanne)
- Genetic Genealogy **Debbie Kennett** (University College London)

In addition, a **discussion panel** to review the "lessons learned" from the high profile **O.J. Simpson case** in 1994-1995 has been organized with the following participants, who all played major roles during the criminal proceedings:

- **Robin Cotton** (forensic DNA expert) , **Bruce Weir** (statistics expert), **Rockne Harmon** (prosecutor), **Barry Scheck** (defense counsel)

President: John Butler, Gaithersburg · **Vice President:** Walther Parson, Innsbruck · **Secretary:** Peter M. Schneider, Cologne · **Treasurer:** Marielle Vennemann, Münster · **Representative of the Working Parties:** Leonor Gusmão, Rio de Janeiro



NEWSLETTER 05-2021

INTERNATIONAL SOCIETY FOR FORENSIC GENETICS
http://www.isfg.org

WELCOME

In this edition of our ISFG newsletter, we provide information about the ISFG Summer School 2021 (virtual edition), an update on FSI Genetics and the suspended impact factor, and information about ISFG working group activities.

ISFG SUMMER SCHOOL 2021

The first virtual edition of the ISFG Summer School will be composed of seven Workshops organized by the ISFG. These are the topics and the speakers:

- **WS 1: Evaluative reporting for contact traces/Activity level reporting** *Lydie Samie-Foucart & Tacha Hicks*
- **WS 2: NGS Bioinformatics 101 (STRait Razor, FDSTools)** *Jonathan King & Jerry Hoogenboom*
- **WS 3: Advanced DNA mixture interpretation** *Peter Gill, Corina Benschop, Oyvind Bleka*
- **WS 4: Perform BGA analyses and how to interpret them** *Chris Phillips, Walther Parson, Peter Schneider*
- **WS 5: Kinship Analysis (missing persons and paternity)** *Daniel Kling & Andreas Tillmar*
- **WS 6: Statistical Genetics** *Bruce Weir & Sanne Aalbers*
- **WS 7: Programming in R** *Thore Egeland & Magnus Vigeland*

The workshops will take place from the **20th to the 30th July, 2021**. These will be an excellent opportunity for training and education with some of the most qualified experts in forensic genetics. **Registrations start on May 3rd, 2021.** You find all relevant information, included a detailed schedule and prices, on the Summer School website, or by contacting ISFGSummerSchool2021@gmail.com.

GERMAN-SPEAKING ISFG WORKING GROUP

dgab deutsche gesellschaft für abstammungsbegutachtung

The annual meeting 2020 was postponed again to 2022 due to the Covid-19 pandemic. The 16th annual meeting will be organized as a virtual edition on June 25th/26th, 2021. Please register for the 2021 meeting at the DGAB website.

The German-speaking working group has sent an open letter on March 3rd, 2021, to the governing mayor of Berlin, and to the chairman of the board of the Charité University Hospital Berlin, to protest against the imminent closure of the Forensic Genetics Department at the Institute of Legal Medicine of the Charité. Well-known scientists in the field of forensic genetics of the first and second generation have signed this letter. Other bodies such as the German Stain Commission have also sent a letter of protest. This has apparently led to a reaction, as the Charité board has revoked their decision recently and are now considering a solution to ensure that the Forensic Genetics Lab will continue to exist.

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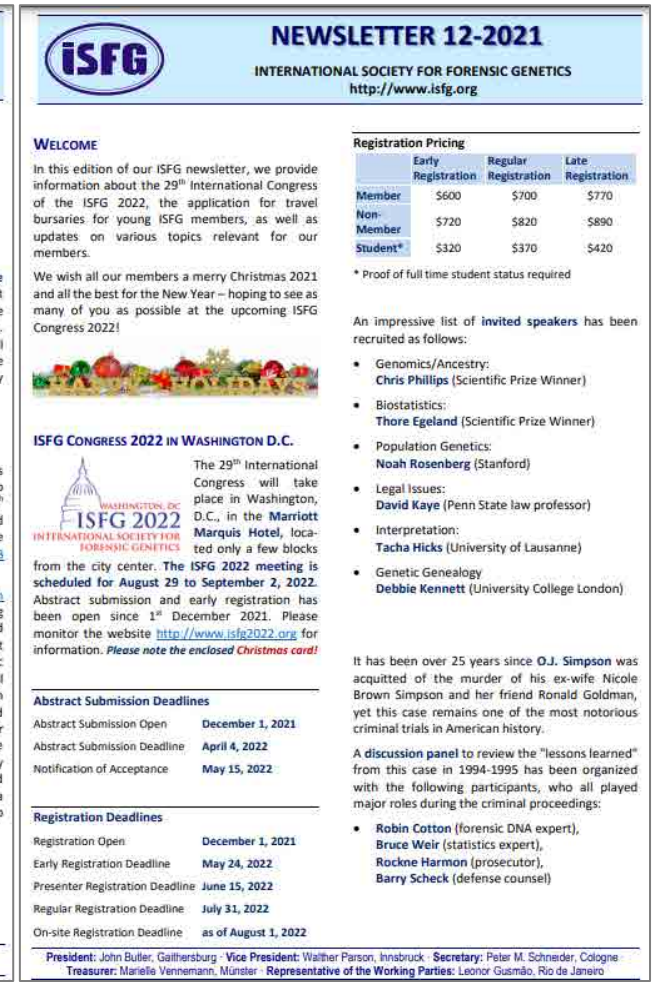

NEWSLETTER 12-2021

INTERNATIONAL SOCIETY FOR FORENSIC GENETICS
http://www.isfg.org

WELCOME

In this edition of our ISFG newsletter, we provide information about the 29th International Congress of the ISFG 2022, the application for travel bursaries for young ISFG members, as well as updates on various topics relevant for our members.

We wish all our members a merry Christmas 2021 and all the best for the New Year – hoping to see as many of you as possible at the upcoming ISFG Congress 2022!

ISFG CONGRESS 2022 IN WASHINGTON D.C.

WASHINGTON, DC ISFG 2022 INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

The 29th International Congress will take place in Washington, D.C., in the **Marriott Marquis Hotel**, located only a few blocks from the city center. **The ISFG 2022 meeting is scheduled for August 29 to September 2, 2022.** Abstract submission and early registration has been open since 1st December 2021. Please monitor the website http://www.isfg2022.org for information. ***Please note the enclosed Christmas card!***

Abstract Submission Deadlines	
Abstract Submission Open	December 1, 2021
Abstract Submission Deadline	April 4, 2022
Notification of Acceptance	May 15, 2022

Registration Deadlines	
Registration Open	December 1, 2021
Early Registration Deadline	May 24, 2022
Presenter Registration Deadline	June 15, 2022
Regular Registration Deadline	July 31, 2022
On-site Registration Deadline	as of August 1, 2022

Registration Pricing

	Early Registration	Regular Registration	Late Registration
Member	$600	$700	$770
Non-Member	$720	$820	$890
Student*	$320	$370	$420

* Proof of full time student status required

An impressive list of **invited speakers** has been recruited as follows:

- Genomics/Ancestry: **Chris Phillips** (Scientific Prize Winner)
- Biostatistics: **Thore Egeland** (Scientific Prize Winner)
- Population Genetics: **Noah Rosenberg** (Stanford)
- Legal Issues: **David Kaye** (Penn State law professor)
- Interpretation: **Tacha Hicks** (University of Lausanne)
- Genetic Genealogy **Debbie Kennett** (University College London)

It has been over 25 years since **O.J. Simpson** was acquitted of the murder of his ex-wife Nicole Brown Simpson and her friend Ronald Goldman, yet this case remains one of the most notorious criminal trials in American history.

A **discussion panel** to review the "lessons learned" from this case in 1994-1995 has been organized with the following participants, who all played major roles during the criminal proceedings:

- **Robin Cotton** (forensic DNA expert), **Bruce Weir** (statistics expert), **Rockne Harmon** (prosecutor), **Barry Scheck** (defense counsel)

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https://www.isfg.org/files/News1219.pdf (13 pages)

https://www.isfg.org/files/News0920.pdf (7 pages)

https://www.isfg.org/files/News0521.pdf (5 pages)

https://www.isfg.org/files/News1221.pdf (6 pages)

cc JM Butler

ISFG Update Published in *FSI Genetics*

Forensic Science International: Genetics 50 (2021) 102394

ISFG update for FSI genetics

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

1. President's message
2. ISFG 2021 moved to 2022
3. Virtual ISFG Summer School in 2021
4. DNA-TrAC - keeping track of DNA transfer
5. Forensic Practitioner's Guide to the Interpretation of Complex DNA Profiles
6. Recommendations published from Italian Working Group

ISFG 2019 Proceedings

- **Published in December 2019**
- FSI Genetics Supplement Series, Volume 7
- **914 pages freely available online**
- https://www.fsigeneticssup.com/current
- **347 articles** + 1 editorial + 1 corrigendum

Abstract Selection Meeting – April 27-28, 2022

- Reviewed **415 abstracts**

Selected:

- 49 orals
- 12 session chairs
- 307 posters

- *We rejected 73 due to multiple submissions from the same author*

An additional 45 did not register and therefore were removed

cc JM Butler

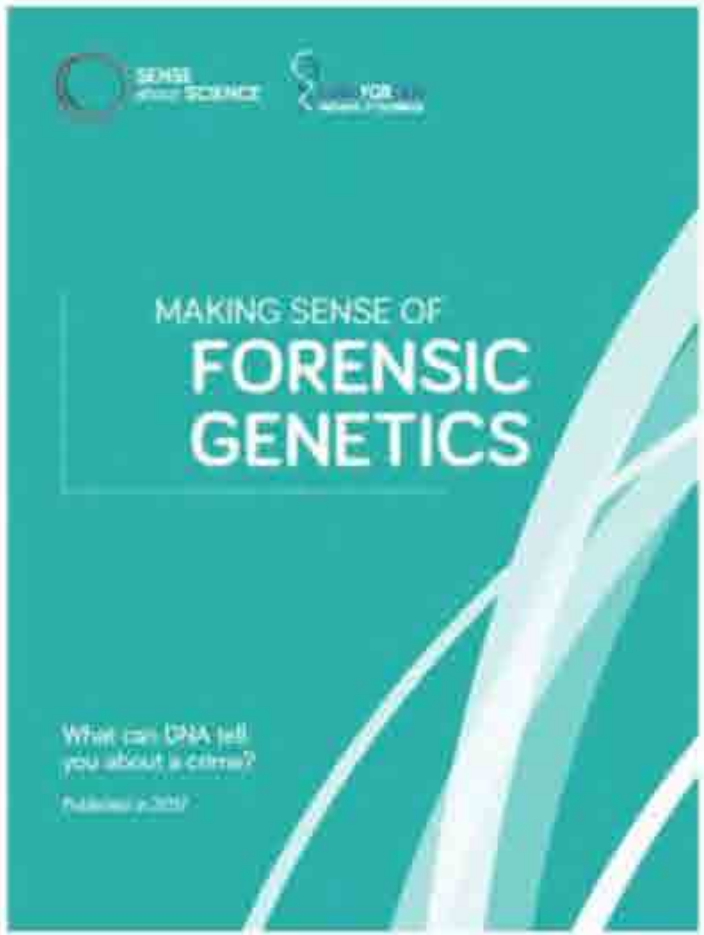
ISFG Virtual Summer School 2021

WS	Title	Speakers
WS 1	Evaluative reporting for contact traces/Activity level reporting	Lydie Samie-Foucart & Tacha Hicks
WS 2	NGS Bioinformatics 101 (STRait Razor, FDSTools)	Jonathan King & Jerry Hoogenboom
WS 3	Advanced DNA mixture interpretation	Peter Gill, Corina Benschop, Oyvind Bleka
WS 4	Perform BGA analyses and how to interpret them	Chris Phillips, Walther Parson, Peter Schneider
WS 5	Inference of relationships – from Basic to Advanced Kinship Statistics	Daniel Kling & Andreas Tillmar
WS 6	Statistical Genetics	Bruce Weir & Sanne Aalbers
WS 7	Pedigree Analysis in R	Thore Egeland & Magnus Vigeland

- Organized by Cíntia Alves
- **279 people registered for 532 participations** from all over the world for these seven courses
- Recordings were later watched by 68 people from August to December 2021 for a total of 137 workshop view requests

cc JM Butler

Educational Materials in Multiple Languages



German

Italian

Spanish

Portuguese

translation in preparation
approx. EUR 10,000/translation

other languages to be considered?

Polish and Spanish versions completed

German, Italian, and Portuguese versions in preparation

https://senseaboutscience.org/activities/making-sense-of-forensic-genetics/

ISFG DNA Commissions

Publications since 2019

1. Activity level propositions (Gill & Hicks et al. 2020)
2. Y-STR interpretation (Roewer et al. 2020)

On-Going Efforts (meeting virtually)

- **STR Nomenclature** (Chair: Katherine Gettings, NIST)
- **Phenotyping** (Chair: Manfred Kayser, Erasmus Medical University)

Discussion on Impact Factor Suppression for *FSI Genetics* in 2020

Forensic Science International: Genetics 48 (2020) 102357

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Editorial

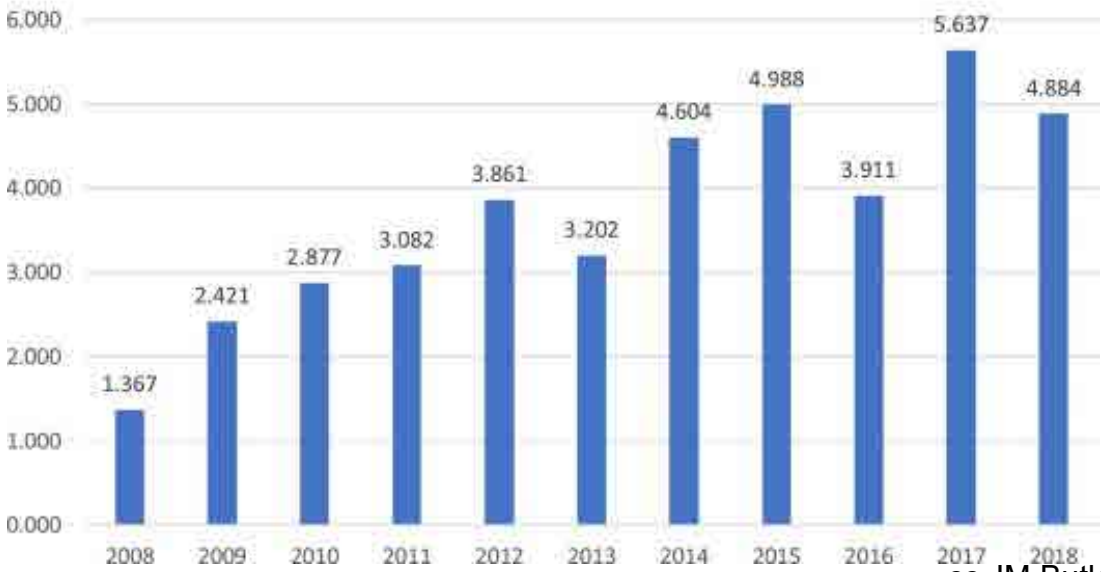
On the suppression of Forensic Science International: Genetics from the 2019 Journal Citations Report

Angel Carracedo (Editor-in-Chief, Forensic Science International: Genetics), John M. Butler (President, International Society for Forensic Genetics), Leonor Gusmao (Associate Editor, Forensic Science International: Genetics and Representative for All Working Parties, International Society for Forensic Genetics), Adrian Linacre (Associate Editor, Forensic Science International: Genetics), Walther Parson (Associate Editor, Forensic Science International: Genetics and Vice President, International Society for Forensic Genetics), Peter M. Schneider (Associate Editor, Forensic Science International: Genetics and Secretary, International Society for Forensic Genetics), Peter M. Vallone (Associate Editor, Forensic Science International: Genetics), Marielle Vennemann (Treasurer, International Society for Forensic Genetics)

Table 1
Self citations vs. total citations, *FSI Genetics* 2014-2019.

Year	IF	Self cites	Total cites	% Self
2014	4.604	552	999	55.3%
2015	4.988	779	1272	61.2%
2016	3.911	616	1232	50.0%
2017	5.637	781	1629	47.9%
2018	4.884	654	1348	48.5%
2019	n/a	n/a	n/a	45.0 %

*Data from JCR 2018 [7] and 2019 suppression list [2].



cc JM Butler

Response to *FSI Genetics* Impact Factor Clarivate Suppression in 2020

- Thank you to members of the ISFG Working Groups who provided letters of support
  - **GHEP-ISFG manifest and petition** (August 26, 2020)
  - Korean Speaking Working Group (August 2020)
  - Polish Speaking Working Group (August 25, 2020)
  - German Speaking Working Group (August 31, 2020)
  - Italian Speaking Working Group (September 2, 2020)
  - Spanish & Portuguese Speaking Working Group (September 3, 2020)
  - French Speaking Working Group (September 9, 2020)
- German Society of Legal Medicine (September 15, 2020)
- ENFSI DNA Working Group (October 26, 2020)

Journal Impact Factor was restored in June 2021:

4.882

https://www.isfg.org/Clarivate+suppression

cc JM Butler

Forensic Science International: Genetics 48 (2020) 102299

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Ethical publication of research on genetics and genomics of biological material: guidelines and recommendations

Maria Eugenia D'Amato[a,*], Martin Bodner[b], John M. Butler[c], Leonor Gusmão[d], Adrian Linacre[e], Walther Parson[b,f], Peter M. Schneider[g], Peter Vallone[c], Angel Carracedo[h]

[a] Forensic DNA Laboratory, Department of Biotechnology, Faculty of Natural Sciences, University of the Western Cape, South Africa
[b] Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria
[c] National Institute of Standards and Technology, Gaithersburg, MD, USA
[d] DNA Diagnostic Laboratory, State University of Rio de Janeiro, Brazil
[e] Flinders University, College of Science & Engineering, Adelaide, Australia
[f] Forensic Science Program, The Pennsylvania State University, University Park, PA, USA
[g] Institute of Legal Medicine, University Clinic and Faculty of Medicine, University of Cologne, Cologne, Germany
[h] Institute of Forensic Sciences, Genomic Medicine Group-CIBERER, University of Santiago de Compostela, Spain

CRIME-SOLVING DNA DATABASE FACES ETHICAL SCRUTINY

Geneticists say a global Y-chromosome database holds profiles from men who are unlikely to have given free informed consent. **By Quirin Schiermeier**

"Judges anywhere in the world rely on robust forensic data. Excluding data from minority groups could bias statistical evaluations in forensic reports – to their disadvantage."

the inhabitants of Kollum, a small village in the Netherlands. A local 16-year-old girl was found raped and murdered in a field nearby, and some people said that Iraqi or Afghan residents at an asylum seekers' centre in the village could be to blame. Tensions rose: a fight broke out at

case unsolved, the public prosecutor turned to a newly launched research database containing Y-chromosome profiles from men across the world. When forensic scientists compared DNA from semen collected at the crime scene with profiles stored in this Y-chromosome Haplotype Reference Database (YHRD) and

elsewhere, they found that the murderer was very probably of northwestern European descent, showing that the villagers' assumptions were unfounded. The discovery helped to calm social tensions – although the case was not solved for many years until, with the aid of more DNA work, a local farmer was found guilty.

The YHRD, which was first released online in 2000, is now widely used across the world to help solve sex crimes and settle paternity cases. Holding more than 300,000 anonymous Y-chromosome profiles, it shows how particular genetic markers are fingerprints of male lineages in more than 1,300 distinct global populations. It can point to the likely geographic origin of mystery males, as in the Kollum case, but is now more often relied on to calculate the weight of evidence against a male suspect whose Y-chromosome DNA profile matches traces found at a crime scene. Although the YHRD is a research database, scientists both from academia and crime laboratories have uploaded data to it, and it has become a key tool for prosecutors and defence lawyers.

"The YHRD is absolutely essential for suspects anywhere in the world to get a fair chance in court," says Walther Parson, a forensic geneticist at Innsbruck Medical University in Austria, and the vice-president of the International Society for Forensic Genetics (ISFG).

But some European geneticists say that the

An article in *Nature* June 2021

Y-chromosome
Haplotype
Reference
Database
https://yhrd.org/

…it asks for, but doesn't verify, consent or ethical approval

Concerns have been raised about **DNA samples taken from Chinese ethnic minorities** without informed consent

The **ISFG** is now setting up an oversight board to examine cases in which consent is unclear

cc JM Butler

Supporting Forensic Population Databases

- The Board has met several times with Sascha Willuweit and Lutz Roewer regarding YHRD as well as with EMPOP (Walther Parson) and STRidER (Martin Bodner) database managers

- A **Forensic Databasing Advisory Board (FDAB)** has been created (as described in our latest newsletter) and the Board has reviewed their initial draft of recommendations (Eugenia D'Amato will speak later in this program)

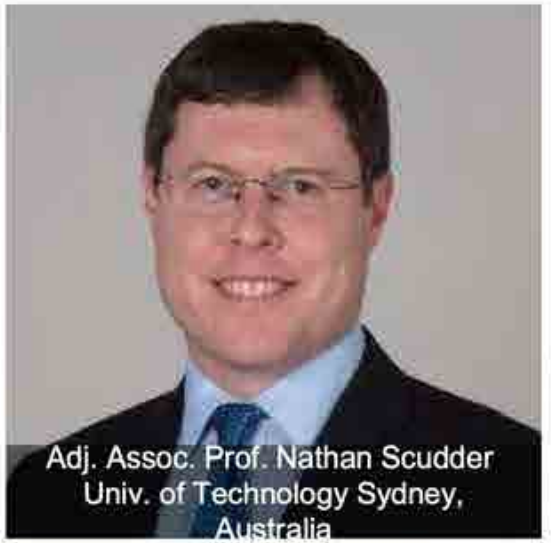
- An LLC (Limited Liability Company) is a required instrument for ISFG to be able to carry the database work forward, and the Board has met with a legal consultant to explore how this action might be pursued

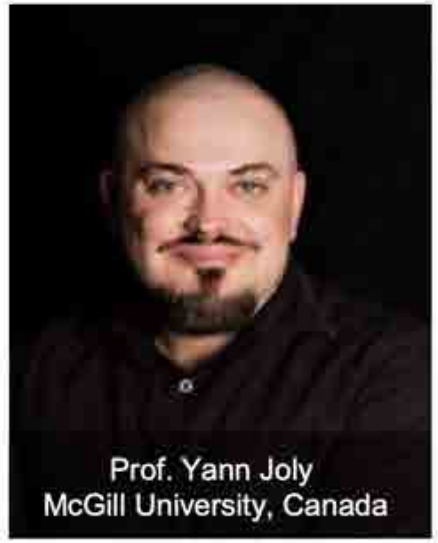

John Butler
Peter M. Schneider
Walther Parson
Marielle Vennemann
Leonor Gusmão
Helena Machado
Maria Eugenia D'Amato
Nature article (15 June 2021): **Forensic database challenged over ethics of DNA holdings**
https://www.nature.com/articles/d41586-021-01584-w
9 July 2021 Board meeting discussing creating the **Forensic Databasing Advisory Board** (FDAB)

cc JM Butler

Forensic Database Advisory Board (FDAB)

FDAB BOARD MEMBERS





cc E D'Amato

Forensic Database Advisory Board (FDAB)

FDAB MANDATE

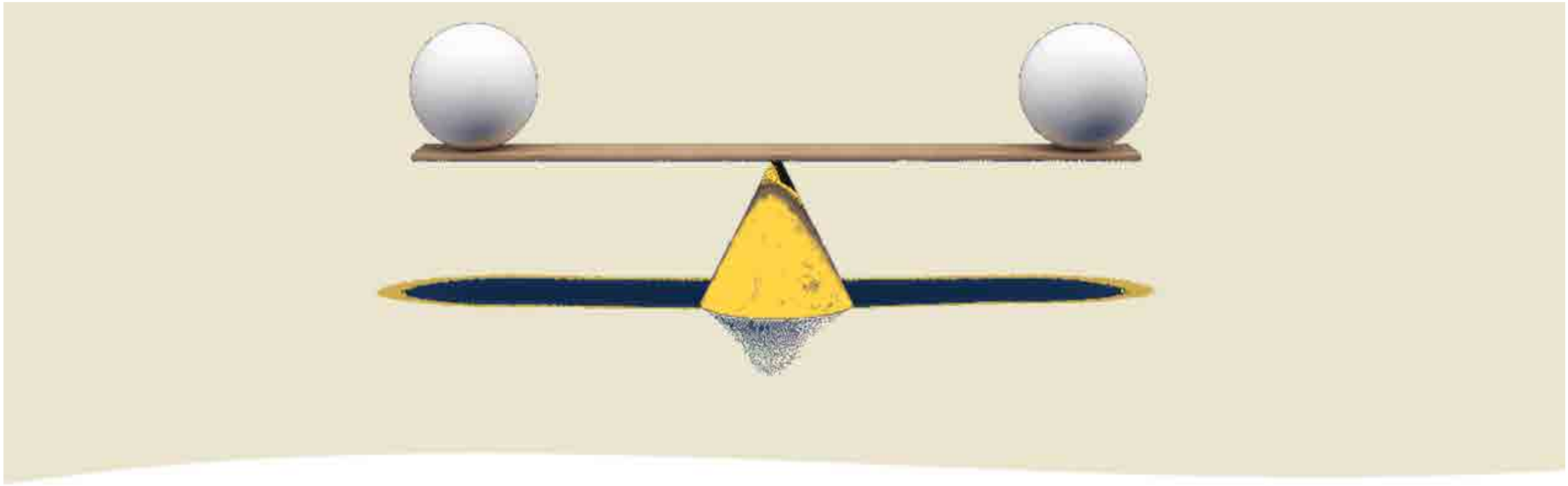
- **Draft guidelines for the forensic community and curators of the Forensic Genetic Frequency Databases* ('FGFD')**

- **Assessment of:**
  - Law enforcement processes and ethics
  - Minorities/vulnerable populations
  - Legacy data/samples
  - Data sensitivity/privacy
  - Data protection
  - Custody of the FGFD*

*YHRD, EMPOP and STRidER

cc E D'Amato

Forensic Database Advisory Board (FDAB)



Risk-Benefit Evaluation

The evaluation of the content of the FGFD was categorized in terms of **High**, **Medium** or **Low** risk of having contravened ethical principles

- **Criteria:**
  - Submitter categories
  - Sample categories
  - Temporal categories

Forensic Database Advisory Board (FDAB)

NEXT STEPS

- Dissemination of first report: online ISFG site
- Online workshop-meeting: feedback (2022-23?)
- Publication of the first report (submission 2022)
- Work in progress: ISFG meeting 2024

cc E D'Amato

Expand the ISFG Executive Board

Currently

1. President
2. Past-president (VP)
3. Secretary
4. Treasurer
5. Representative of the Working Parties
6. Representative for Training and Education
7. Operational Manager for ISFG Interests (future LLC)

- Increase Executive Board by 2 members
  - Representative for Training and Education – **Corina Benschop**
  - Operational Manager for ISFG Interests (LLC) – **PM Schneider**
- New Representative WP – **Lourdes Prieto**
- Use committees to accomplish more
  - Scientific Prize Committee (organized by VP Walther Parson)
  - Best Oral Presentation Review Committee
  - Best Poster Presentation Review Committee
- Need to change society statutes to expand the Board
  - will be discussed and voted on later in this meeting

cc JM Butler

ISFG Membership

1217 members from 77 countries (as of 8 July 2022)


Argentina (41)


Armenia (1)


Australia (57)


Austria (23)


Bahrain (1)


Belgium (21)


Bolivia (2)


Brazil (28)


Canada (8)


Cayman Islands (1)


Chile (8)


China (27)


Colombia (10)


Costa Rica (2)


Croatia (2)


Cyprus (2)


Czech Republic (4)


Denmark (42)


Dominican Republic (3)


Ecuador (4)


Egypt (1)


El Salvador (2)


Estonia (1)


Finland (3)


France (16)


Georgia (1)


Germany (137)


Ghana (2)


Greece (4)


Guatemala (1)


Hong Kong (5)


Hungary (6)


Iceland (1)


India (6)


Iraq (2)


Ireland (1)


Israel (2)


Italy (56)


Japan (15)


Kazakhstan (1)

Korea (11)

Kuwait (4)

Lithuania (2)

Luxembourg (2)

Macedonia (2)

Malaysia (2)

Malta (1)

Mauritius (1)

Mexico (17)

Mongolia (1)

Netherlands (24)

New Zealand (15)

Norway (16)

Oman (1)

Panama (1)

Peru (9)

Philippines (2)

Poland (55)

Portugal (17)

Qatar (1)

Romania (2)

Russian Federation (8)

Saudi Arabia (3)

Serbia (7)

Singapore (3)

Slovakia (2)

Slovenia (2)

South Africa (2)

Spain (84)

Sweden (11)

Switzerland (39)

Thailand (2)

United Arab Emirates (14)

United Kingdom (57)

United States (244)

Venezuela (1)

Zimbabwe (1)

ISFG Membership Ranked by Country (Top 25)

1217 members from 77 countries (as of 8 July 2022)

United States
(244)

Germany
(137)

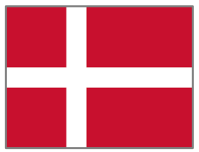
Spain
(84)

Australia
(57)

United Kingdom
(57)

Italy
(56)

Poland
(55)



Denmark
(42)

Argentina
(41)

Switzerland
(39)

Brazil
(28)

China
(27)

Netherlands
(24)

Austria
(23)

Belgium
(21)

Mexico
(17)

Portugal
(17)

France
(16)

Norway
(16)

Japan
(15)

New Zealand
(15)

United Arab
Emirates (14)

Korea
(11)

Sweden
(11)

Colombia
(10)

Working Group Report (by Leonor Gusmão)

Due to the pandemic, most activities slow down and scientific meeting were cancelled during 2022

Working Group	Working Group Chair (Location)	Recent Activities
German	Uta-Dorothee Immel (Mainz)	Virtual Meeting in June 2021; Casework Workshop ISFG/UFG in May/June (virtual); Meeting in June 2022 (Halle an der Saale)
English (ESWG-ISFG)	Andreas Tillmar (Linköping)	Virtual Meeting in October 2021; Meeting in Washington 2022; active proficiency testing program on relationship testing
French	Christel Roudaut (Bordeaux)	Virtual Meeting in May 2021 and in June 2022; Meeting in Washington 2022
Italian (Ge.F.I.)	Loredana Buscemi (Ancona)	Virtual workshops in October 2020 and in April/May 2022; Meeting in Washington 2022
Spanish & Portuguese (GHEP-ISFG)	Leonor Gusmão (Rio de Janeiro)	Virtual Meeting in December 2020 and in October 2021; Meeting in Washington 2022; published 2 articles since 2019; active proficiency test program
Chinese	Yiping Hou (Sichuan)	Meeting in Washington 2022
Korean	Kyoung-Jin Shin (Seoul)	Virtual scientific meetings in May 2020 and in November 2021; Meeting in Washington 2022
Polish	Tomasz Kupiec (Krakow)	Virtual Meeting in November 2021; Meeting in Washington 2022; published 2 articles since 2019
Arabian	Rashed Alghafri (Dubai)	Virtual Meeting in April 2021; Meeting in Washington 2022
CaDNAP	Cordula Berger (Innsbruck)	Organizing bi-annual proficiency tests for canine DNA genotyping

Short-Term Fellowship Awardees

- Purpose: **To support transnational exchange visits between collaborating research groups for specific projects related to forensic genetics**
- For Terms of Reference, see
  - https://www.isfg.org/files/ISFG_Fellowships_Nov2016.pdf
- Announcement was made via the November 2016 ISFG newsletter
- Financial support for travel and accommodations for **up to 1000 euros (within continent)** and **2000 euros (between continents)**
- Selection committee include the Working Group chairs and is chaired by the representative of the ISFG Working Groups
- Application rounds: (1) April 2017, (2) October 2017, (3) April 2018, (4) October 2018, (5) April 2019, (6) October 2019 – see
  - https://www.isfg.org/Members+Area/Short+Term+Fellowships

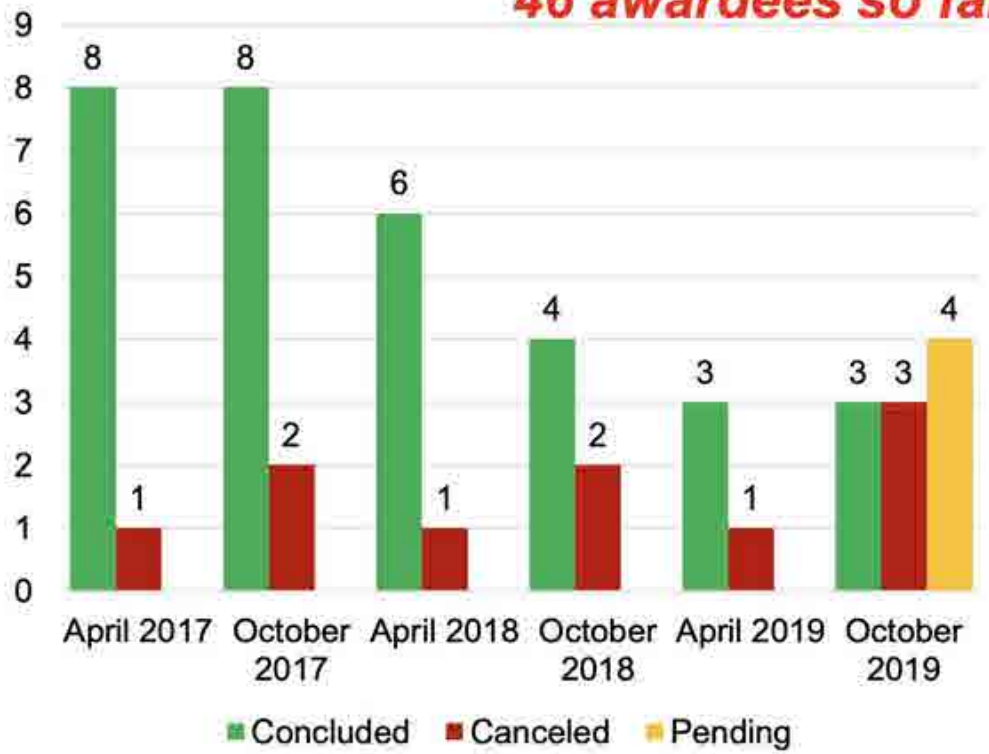
Due to the COVID-19 pandemic, the fellowship program has been suspended

cc JM Butler

ISFG Short-Term Fellowship Awardees

Can fund up to 10 awardees per competition

46 awardees so far



32 concluded so far

*	Vania Pereira	University of Copenhagen (Denmark)	DNA Diagnostic Laboratory, State University of Rio de Janeiro (Brazil)	Assessing differences between ancestry estimates with different marker sets and exploring Y-chromosomal diversity in South America	
	Torben Tvedebrink	Aalborg University (Denmark)	University of Santiago de Compostela (Spain)	Work on ancestry related research questions involving both statistical models and informative markers	Canceled
*	Margherita Colucci	University of Leicester (UK)	Norwegian University of Life Sciences (Norway)	Exploring the potential of massively parallel sequencing (MPS) forensic multiplexes and genome-wide SNP data in relationship estimation with simulations and with real-world data	
*	Kimberly Sturk-Andreaggi	Armed Forces DNA Identification Laboratory (USA)	Medical University of Innsbruck (Austria)	Perform mtDNA phylogenetic analyses on approximately 2000 mtGenomes using EMPOP database and software	
	Byron Freire Paspuel	Universidad de Las Américas, Quito (Ecuador)	University of Copenhagen (Denmark)	Gain experience regarding human identification techniques, sample and data management	Canceled
	Franco Marsico	Banco Nacional de Datos Genéticos (Argentina)	Norwegian University of Life Sciences (Norway)	Evaluation of the statistical power of DNA-based identification of family groups in their database, which was developed to find the missing grandchildren of Argentina, and to take a course being taught in Oslo during his visit	Report
*	Masinda Nguidi	DNA Diagnostic Laboratory, State University of Rio de Janeiro (Brazil)	Medical University of Innsbruck (Austria)	Acquire knowledge and training in MPS technology and analyze mtDNA data from three Nigerian population groups	
	Isabela Brunelli Ambrosio	Universidade Estadual Júlio de Mesquita Filho (Brazil)	IPATIMUP (Portugal)	Study Y-STR mutations in father-son duos	Canceled

*** Postponed to 2023**

ISFG Short Term Fellowhips awarded in 2021

Recipient	Coming from	Visiting at	Topic	Report
Jorge Ruiz Ramirez	Universidade de Santiago de Compostela	International Commision on Missing Persons	Analysis of tri-allelic markers for the identification of missing persons	Report
Julyana da Silva Varela Ribeiro	Universidade do Estado do Rio de Janeiro	IPATIMUP (Portugal)	Y chromosomal lineages in South America	Report

cc JM Butler

ISFG Short-Term Fellowship Awardees

The board decided to resume the fellowship program

Call for the next year travels:

- ✓ Applications to be submitted between **October 1 to November 15, 2022**
- ✓ 10 fellowships – up to **EUR 1,000 for visits within the same continent**, and up to **EUR 2,000 for visits from continent to continent**
- ✓ Deadline for use (travels in 2023)

cc JM Butler

WASHINGTON, DC
ISFG 2022
INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

Comparison to Previous ISFG Meetings

	Washington DC (29th Congress)	Prague (28th Congress)	Seoul (27th Congress)	Krakow (26th Congress)
Registered Participants	783	1017	705	750
Countries	49	64	68	69
Top Country (# Participating)	United States (365 attended)	Germany (105 attended)	South Korea (>100 attended)	United States (~115 attended)
Submitted Abstracts	415	753	535	480
Oral Presentations	62	67	57	57
Poster Presentations	262	637	478	423
Workshops	**16**	14	11	10
Conference Proceedings *FSI Genetics Suppl Ser*	v8 (??? articles) <560 pages	v7 (347 articles) 914 pages	v6 (236 articles) 612 pages	v5 (265 articles) 679 pages

Only 1 per presenter accepted

Thank you to all workshop, oral, and poster presenters!

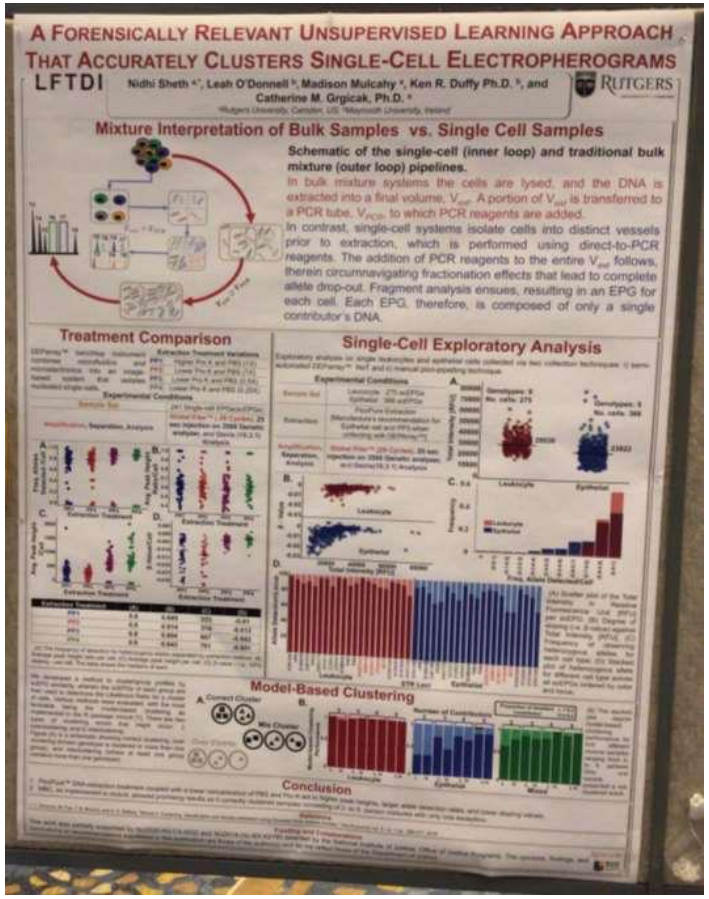
You are the "giants" on whose shoulders we stand to see further

cc JM Butler

ISFG 2022 Travel Award Winners

cc JM Butler

ISFG Prize for best poster presentation



P194 **A forensically relevant unsupervised learning approach that accurately clusters single-cell electropherograms**

Nidhi C. Sheth*, Leah O'Donnell, Madison M. Mulcahy, Ken R. Duffy, Catherine M. Grgicak

Rutgers University

ISFG Prize for best oral presentation

O-06 **A novel rotationally-driven microfluidic approach for forensic epigenetic sample preparation for human chronological age determination**

Rachelle Turiello*, Leah M. Dignan, Logan Cunningham, Soumil Madhiwala, James P. Landers

**University of Virginia*

Previous Honorary ISFG Members

1. E. Essen-Möller (Germany)
2. P. Dahr (Germany)
3. E. Krah (Germany)
4. M. Krüpe (Germany)
5. W. Zimmermann (Germany)
6. J.-J. van Loghem (The Netherlands)
7. F. Levine (USA)
8. R.R. Race (UK)
9. R. Sanger (UK)
10. O. Prokop (Germany)
11. H. Leithoff (Germany)
12. K. Hummel (Germany)
13. B. Dodd (UK)
14. E. van Loghem (The Netherlands)
15. M. Pereira (UK)
16. E. Schwarzfischer (Germany)
17. C.P. Engelfriet (The Netherlands)
18. K. Henningsen (Denmark)
19. A.G. Gathof (Germany)
20. H.H. Hoppe (Germany)
21. W. Spielmann (Germany)
22. D.A. Hopkinson (UK)
23. H. Matsumoto (Japan)
24. A. Arndt-Hanser (Germany)
25. R. Bütler (Germany)
26. Alec Jeffreys (UK)
27. A. Fiori (Italy)
28. E. Villanueva (Spain)
29. P.J. Lincoln (UK)
30. C. Rittner (Germany)
31. B. Brinkmann (Germany)
32. B. Olaisen (Norway)
33. W. Bär (Switzerland)
34. J. Gómez Fernández (Spain)
35. Wolfgang Mayr (Austria)
36. George Sensabaugh (USA)
37. Liu Yacheng (China)
38. Ate Kloosterman (The Netherlands)
39. Hermann Schmitter (Germany)

Proposed 2022 Additions

Antonio Amorim (Portugal)
Bruce Budowle (USA)
Daniel Corach (Argentina)
Ken Kidd (USA)
Niels Morling (Denmark)

Previous Honorary ISFG Members

Proposed 2022 Additions

Antonio Amorim (Portugal)
Bruce Budowle (USA)
Daniel Corach (Argentina)
Ken Kidd (USA)
Niels Morling (Denmark)

Previous ISFG Scientific Prize Winners

- 1987 - **Wolfgang Dahr** (Germany)
- 1989 - **Manfred Hochmeister** (Switzerland)
- 1997 – **Antti Sajantila** (Finland)
- 1997 – **Colin Kimpton & UK National DNA Database Group** (England)
- 1999 – **Lutz Roewer** (Germany)
- 2003 – **John Butler** (USA)
- 2005 – **Walther Parson** (Austria)
- 2007 – **Reinhard Szibor** (Germany)
- 2009 – **Antonio Salas** (Spain)
- 2013 – **Peter Gill** (Norway)
- 2015 – **Thomas Parsons** (Bosnia & Hercegovina)
- 2017 – **Manfred Kayser** (The Netherlands)
- 2019 – **Thore Egeland** (Norway)
- 2019 – **Chris Phillips** (Spain)
- 2022 – **Charla Marshall** (USA)

ISFG Prize for Scientific Excellence

INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

ISFG

The Biennial Scientific Prize 2022
for outstanding contributions
(Scientific Excellence)
has been awarded to

Charla Marshall

for development of forensically-motivated capture-based massively parallel sequencing to aid missing persons identifications with particularly challenging samples.

The President
(John M. Butler)

ISFG 2022

The Secretary
(Peter M. Schneider)

Washington DC, 1st September 2022

ISFG Award for Lifetime Achievement

INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

ISFG

The Biennial Scientific Prize 2022
for outstanding contributions
(Lifetime Achievement)
has been awarded to

Bruce Budowle

for being a leading contributor to our field for the past 40 years, for pioneering many aspects of DNA analysis, and for sharing his knowledge and enthusiasm with the forensic genetics community from fellow experts to those just starting their careers

The President
(John M. Butler)

ISFG 2022

The Secretary
(Peter M. Schneider)

Washington DC, 1st September 2022

30 ISFG Conference 2024

31 ISFG Conference 2026

https://www.isfg.org/files/ISFG2026_Montreal_Bid_2022.pdf

Your Research and Efforts Benefit the World

Gracias

Obrigado

Vielen Dank

Merci

Grazie

Dziękuję Ci

ありがとうございました

謝謝

مرکشتم

ہری ش

مكل ا ركش

תודה

Баярлала

ขอบคุ

ਤੁਹਾਡਾ ਧੰਨਵਾਦ

Thank you!

धन्यवाद

감사합니다

teşekkür ederim

Dank je

tak skal du have

Tack

Děkuji

Dankie

Eskerrik asko

Takk skal du ha

Terima kasih

Paldies

Mulțumesc

Köszönöm

Salamat

Kiitos

Ευχαριστώ

Cảm ơn bạn

Hvala vam

Ačiū

Спасибо

Update ENFSI DNA Expert Working Group activities

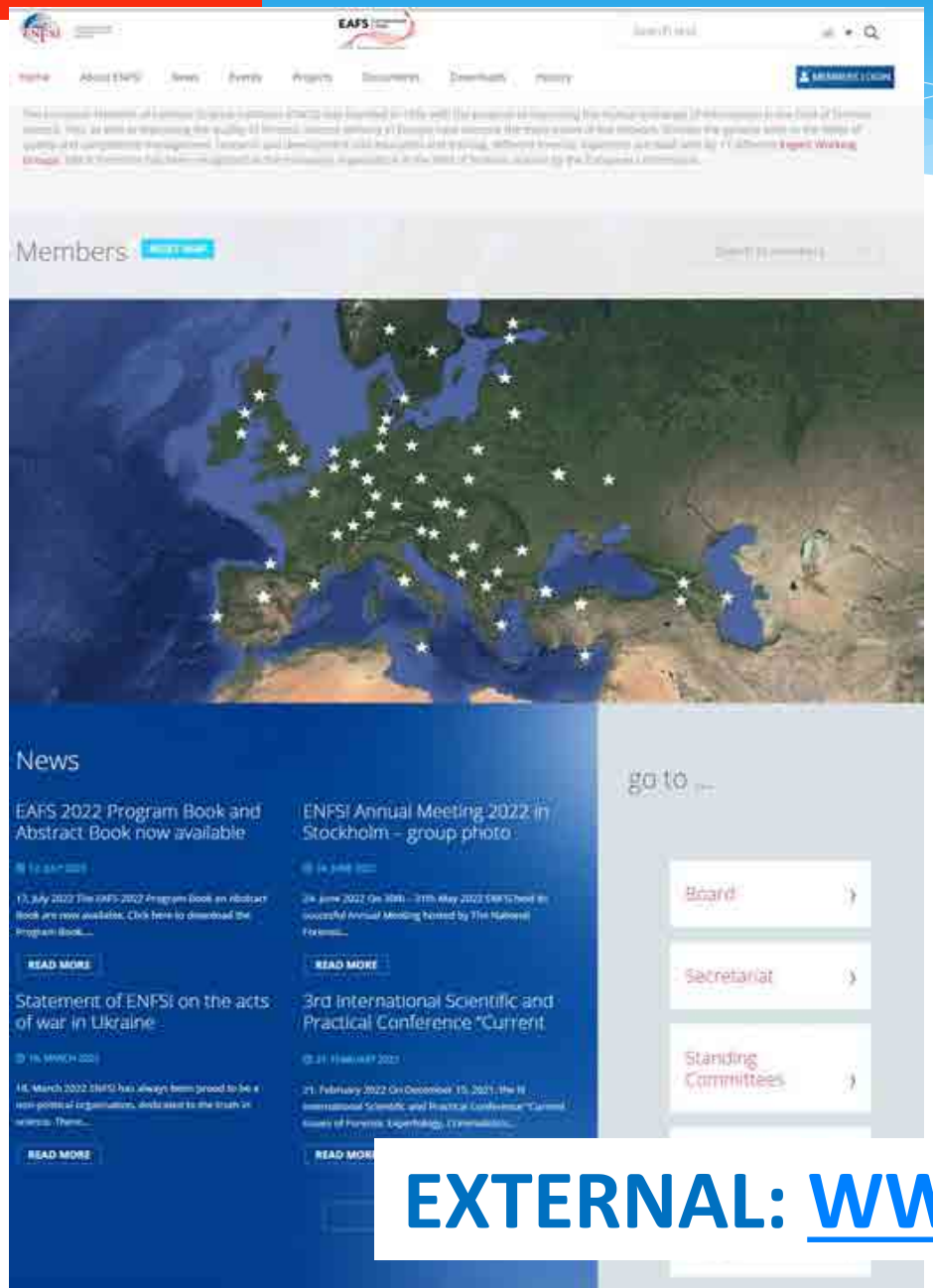
Sander Kneppers
Chair ENFSI DNA Expert Working Group

Netherlands Forensic Institute
Division Biological Traces

ENFSI IS RECOGNIZED AS A PROMINENT VOICE IN FORENSIC SCIENCE WORLDWIDE BY ENSURING THE QUALITY OF DEVELOPMENT AND DELIVERY OF FORENSIC SCIENCE SERVICES THROUGHOUT EUROPE

71 MEMBERS IN 38 COUNTRIES

COMMUNICATION



EXTERNAL: WWW.ENFSI.EU

ENFSI Permanent Members

REPRESENTATION

Secretariat

Board

EXECUTIVE

QCC

R&D

ADVISORY

Working Groups

Task Forces

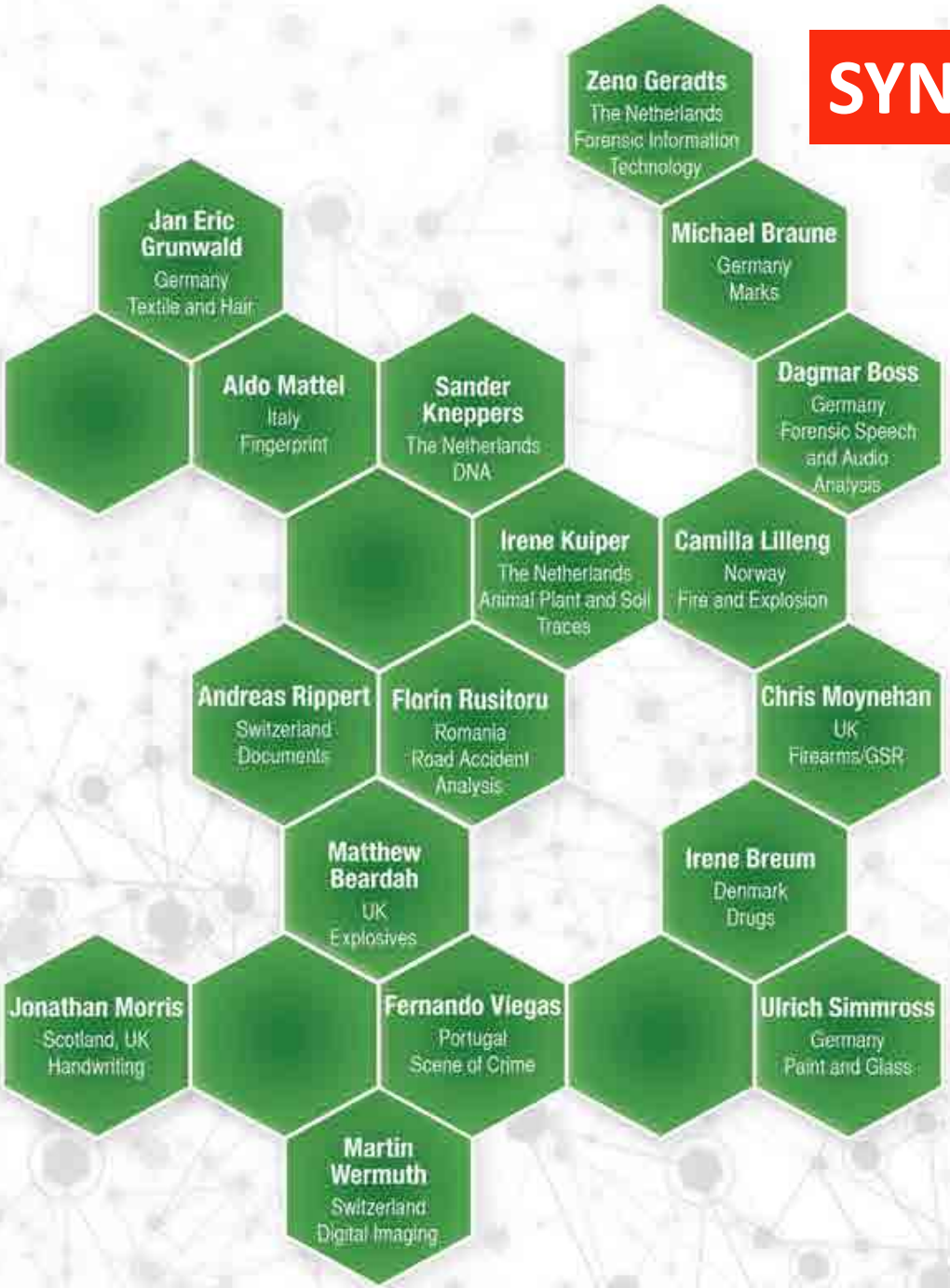
SCIENTIF. & OPER.

EAFS

OOSs

EVENTS

SYNERGY IN NETWORKING

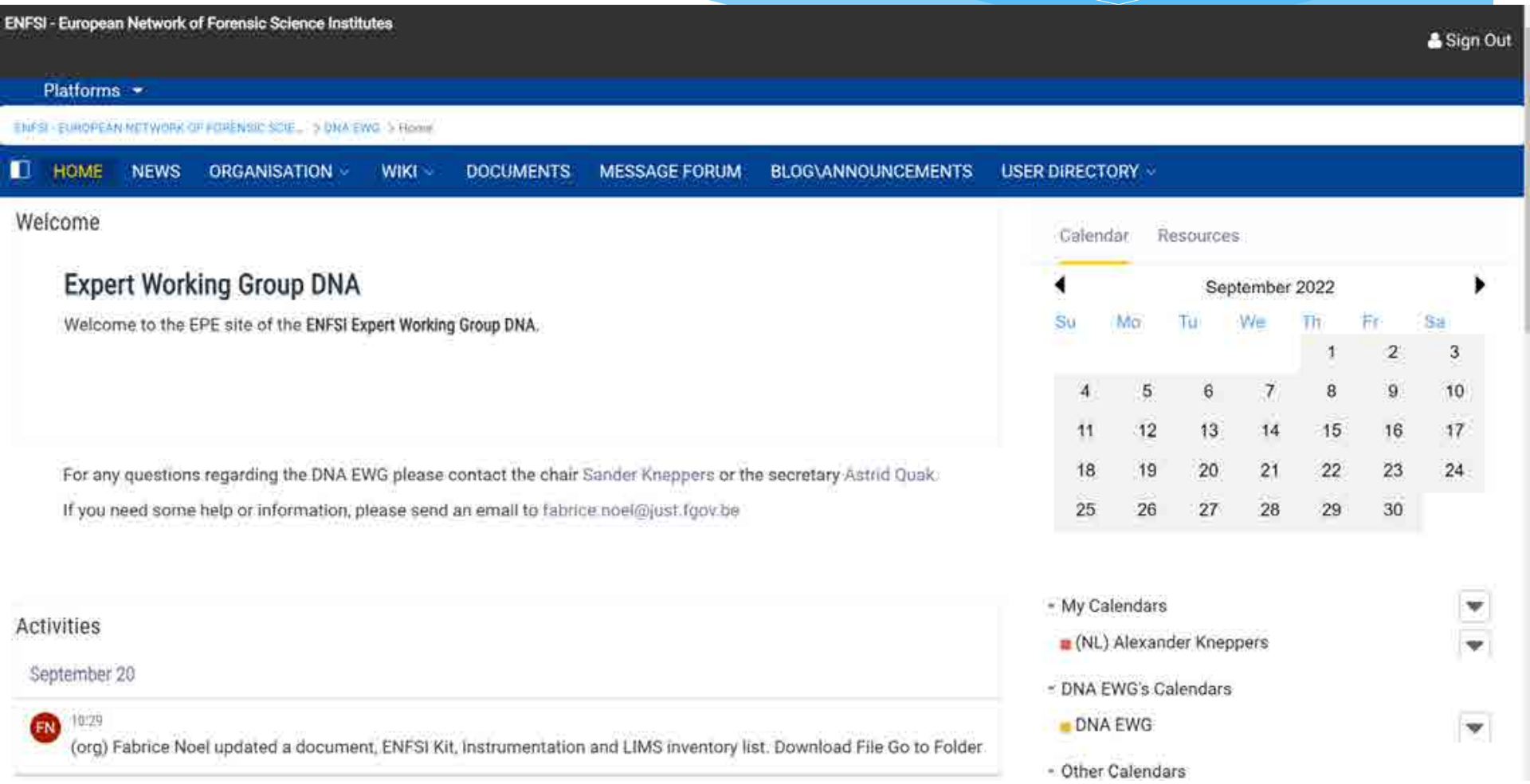


> 1000

Forensic Experts

17 WORKING GROUPS

COMMUNICATION

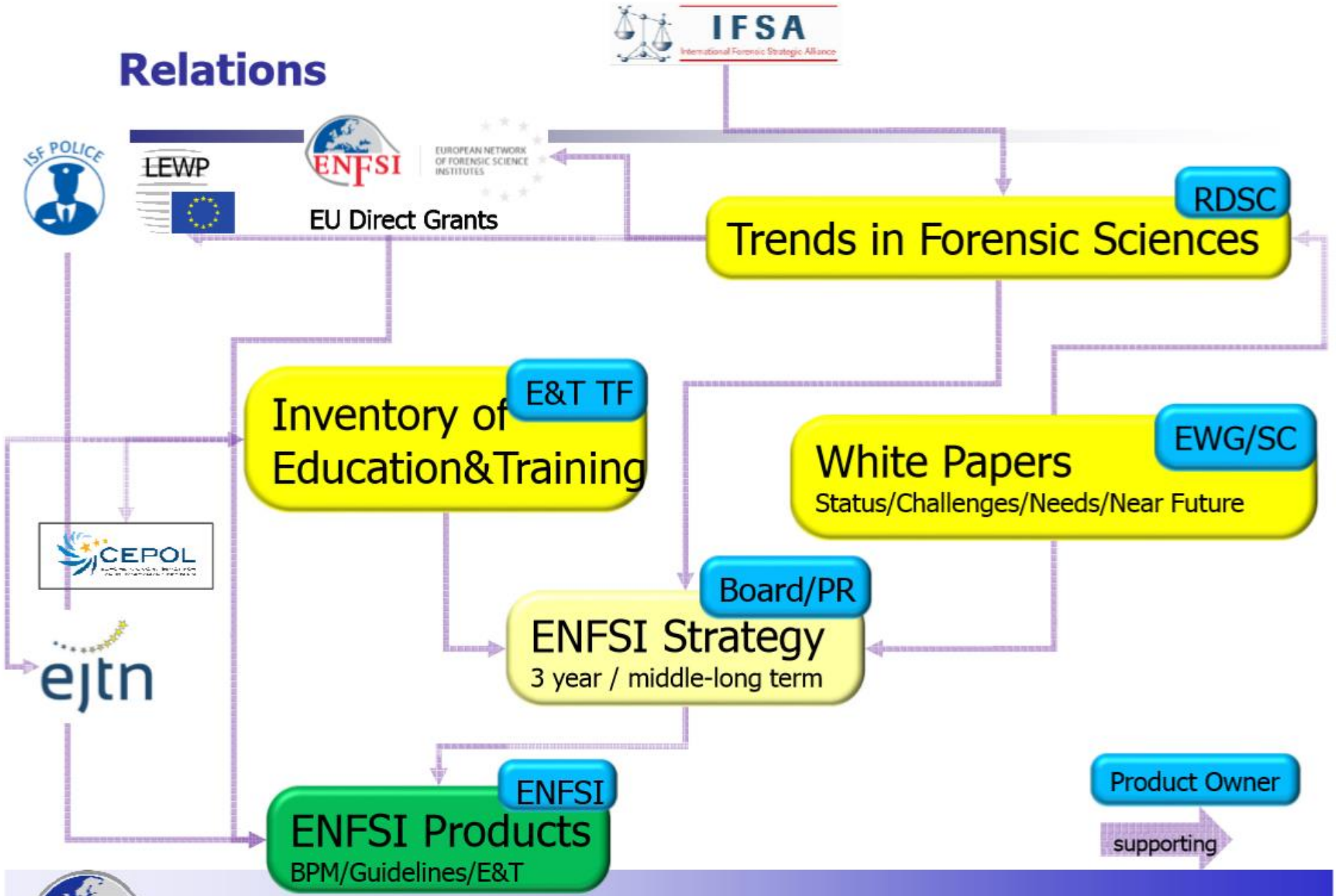


INTERNAL: EPE.EUROPOL.EUROPA.EU

Board Members ENFSI

* Christina Bertler Edlund (Chairperson)
* Dorijan Kerzan (Treasurer)
* Agnieszka Łukomska (Member)
* Alexandra André (Member)
* Chris Porter (Member)
* Aleksandar Ivanovic (Member)
* Attila Kuczmann (Member)

Relations


IFSA
International Forensic Strategic Alliance
ISF POLICE
LEWP
ENFSI
EUROPEAN NETWORK OF FORENSIC SCIENCE INSTITUTES
EU Direct Grants
RDSC
Trends in Forensic Sciences
E&T TF
Inventory of Education&Training
EWG/SC
White Papers
Status/Challenges/Needs/Near Future
CEPOL
ejtn
Board/PR
ENFSI Strategy
3 year / middle-long term
ENFSI
ENFSI Products
BPM/Guidelines/E&T
Product Owner
supporting

Strategic Plan 2020-2023

I - The medium- and long-term trends in forensic science are recognized and a process for development is defined

II - Consolidate the interaction with the stakeholders and partners

III- Strengthening the network through professionalization

Summary of the report 2021

ENFSI has focused on activities as follows;

* Summary of White Papers
* Dissemination of project results/information at EAFS 2022
* CERTAIN-FORS (EU-funded projects) has started
* ENFSI Vision of European Forensic Science Area 2030
  * "Improving the Reliability and Validity of Forensic Science and Fostering the Implementation of Emerging Technologies"
* Implementation of GDPR-procedure

Membership DNA working group

* To have a structure of a working group
  * One person per laboratory
  * Unless active in working group, than an additional person is allowed to attend meetings.

Number of members in mailing list	101
Number of full members (institutes):	51
Number of associated members	50

ENFSI DNA Working group Steering Committee

* Chair	Sander Kneppers NFI, the Netherlands
* Vice chair	Livia Zatkalikova, Ministry of Interior, Slovakia
* Secretary	Astrid Quak, NFI, the Netherlands
* Treasurer	Ingo Bastisch, BKA, Germany
* QCLG	Heli Autere, Nat. bureau of investigation, Finland
* R&D	vacant position
* E&T	Paula di Simone, Italian National Police
* Webmaster	Fabrice Noël, NICC Belgium
* EDNAP	Niels Morling, Univ. Copenhagen, Denmark

DNA working group subgroups

* Group A: Quality Assurance
  * Stavroulla Xenophontos
  * Heli Autere
* Group B: DNA Analysis Methods & Interpretation
  * Antonio Alonso
  * Walther Parson
* Group C: DNA Database and Legislation
  * Izanda Puncule
  * Emilia Lindberg
* Group D: Automation & Expert Systems
  * Christina Forsberg
  * Shazia Khan
* Group E: Forensic Biology and casework
  * Ricky Ansell
  * Arnoud Kal

Public review of ENFSI documents

* proper, balanced and agreed content of these documents for the target groups (forensic community)
* a transparent and documented, public reviewing process is needed > practicable procedure for public review of ENFSI documents

* OSAC requirement that only documents which went through an SDO assessment (standardizing body like ASTM or ISO) will be listed in the OSAC registry

Documents DNA EWG

Best Practice
BPM:Human DNA Analysis (concept)
BPM: DNA pattern recognition and comparison
Guidance
Quality Assurance Program for DNA Laboratories
Recommended Minimum Criteria for the Validation of Various Aspects of theDNA Profiling Process
Validation of mixture interpretation software
Training of staff
Contamination prevention guidelines
Document on DNA Database Management
Surveys and inventory lists:
ENFSI Kit, Instrumentation and LIMS inventory list
Inventory list test pre-examination in use
R&D inventory list
Survey regarding DNA DBs

Education and Training

E&T Liaison Paola Di Simone

Online ENFSI training course 2020

* DNA Mixture Analysis and Statistical Interpretation
* Corina Benschop, Øyvind Bleka and Peter Gill

Online ENFSI training course 2021

* Kinship statistics using Familias
* Teacher: Thore Egeland
* December 2021

Education and Training

ANNEX 3 - TRAINING CATALOGUE OF GRANTED ACTIVITIES 2022

86/2022: Analysis of Complex DNA Profiles	**Cat. 9**	**Forensics**

Duration	4 days
Minimum number of participants	26
Maximum budget	EUR 25,000

In cooperation with ENFSI - DNA Working Group which may provide experts for the course development.

Trainers: Corina Benschop, Øyvind Bleka and Peter Gill
Organizer: Izanda Puncule
October 2022 (17^{th} to 21^{st}) in Riga

Education and Training

Online ENFSI training course 2022

* Kinship statistics using Familias
* Teacher: Thore Egeland
* 17th and 18th of November 2022
* Information and registration after the meeting

Monopoly 2018 AFORE
(Accreditation of Forensic Laboratories in Europe)

- "Accreditation of Forensic Laboratories in Europe" (AFORE)
  - Accreditation of Scene of Crime Services
  - Training of Forensic Personnel in Accreditation Matters
  - Training of Technical Experts
  - Production of New and/or Updated Best Practice Manuals
    - BPM on Digital Image Authentication
    - BPM on Forensic Examination on Fibres
    - BPM on Forensic Examination of Gunshot Residues
    - BPM on Forensic Handwriting Examination
    - BPM on Forensic Voice Comparison
    - BPM on Human DNA Analysis (Application for funding (40K EUR))
    - BPM on Glass or BPM on Paint

ENFSI direct grant 2020
ISF-Police

* **CERTAIN-FORS:**
- **Competency**
- **Education**
- **Research**
- **Testing**
- **Accreditation**
- **Innovation**
- **…… ……In Forensic Science**

Monopoly Projects 2020

Selected proposals

Work Package	Title
#1	Project management, finance management & administration
#2	UNLOCK - fUNdamentaL fOrensiC Knowledge
#3	Development of E Learning Concept Phase 2 – Evaluative Reporting and Interpretation, Textile Damage and Raman Spectroscopy Courses
#4	Training and competence assessment for Forensic Handwriting Experts
#5	Establishment of a Trace DNA Transfer Rate Repository & Bayes Net to Calculate LRs
#6	App Analyses and Reference Database Solution
#7	Forensic Multilingual Voices Database
#8	Development of a New PT on the Interpretation of GSR Findings according to ISO 17043 and ISO13528 Demands
#9	Multidisciplinary Proficiency Test and Collaborative Exercises in Forensics
#10	Benchmarking of Proficiency Tests for the Fingerprint Domain
#11	Fingerprint WG Best Practice Manual 2nd Edition
#12	European Day - Dissemination Event

Horizon 2020

ENFSI MP2020 Project

Establishment of a Trace DNA Transfer Rate Repository & Bayes Net(s) to Calculate LRs

ReAct

(Recovery; Activity)

Horizon 2020 ReAct

* Ingo Bastisch (project lead) with core team
* 34 participating laboratories
* Budget € 295.000
* Period January 2022-December 2023

Monopoly Project 2020 – WP9

Multidisciplinary Collaborative Exercises
Project Leader: Francesco Zampa (RaCIS, Italy)

- EFP-WG (Fingerprints): Helen Bandey (DSTL, UK), Aldo Mattei (RaCIS, Italy) and Andy Becue/Alexandre Anthonioz (UNIL, Switzerland)
- DNA-WG: Livia Zatkalikova (IFS, Slovakia) and Sander Kneppers (NFI ,The Netherlands)
- EDEWG (Documents): Kairi Kriiska-Maivali (FSI, Estonia) and Juergen Bugler (LKA Munich, Germany)
- ENFHEX (Handwriting): Maria Joao Branco (University of Porto, Portugal)
- ETHG (Textile and Hair): Maria Kambosos (BKA, Germany) and Eric Bouzaid (SNPS, France)
- FINEX (Explosives): Matthew Beardah (DSTL, UK)

Multidisciplinary Collaborative Exercise

- **Multidisciplinary Collaborative Exercise 2022**

  Documents, DNA, Fingerprints and Handwriting

As a follow up of the MP2016 STEFA project

54 laboratories participated

Currently result laboratories under review, report by December 2022

- **Multidisciplinary Collaborative Exercise 2023**

  DNA, Fingerprint, Explosives, Textile/Hair

FBI Rapid DNA multi-laboratory study

The FBI is planning a multi-laboratory to test Rapid DNA enhancements outlined in the Joint Letter to the Editor in Forensic Science International – Genetics titled :

Rapid DNA for crime scene use: Enhancements and data needed to consider use on forensic evidence for State and National DNA Databasing - An agreed position statement by ENFSI, SWGDAM and the Rapid DNA Crime Scene Technology Advancement Task Group (FSI-Genetics 48 (2020) 102349).

FBI Rapid DNA multi-laboratory study

* main objectives of the study
  * to determine the variability between the instruments of the same manufacturer
  * to determine the limitations of the enhanced technology through sensitivity and mixture studies
* two current manufacturers of the Rapid technology
  * Thermo Fisher Applied Biosystems
  * ANDE
* The FBI will provide the test samples at no cost.
* 6 USA labs and 3 ENFSI labs
* Topic for the Automation and Expert Systems subgroup on Wednesday

Future grant possibilities

- ISF-P funding program 2021 – 2025 Direct Grants options for ENFSI
- “Horizon Europe” which is operational 2021-2030

DNA EWG meetings

Two annual meetings per year

* One virtual meeting
* One in person meeting

Local organizers 2022

* Sandra Cristina Costa & Paolo Miguel Ferreira
* *Biology and DNA Laboratory*
* *Laboratório de Polícia Científica | Portuguese Forensic Science Laboratory*

Next meetings

- DNA EWG Steering committee online meetings every two months
- 48th annual DNA working group meeting and CODIS/EDNAP meetings
  - **Lisbon week 27th September– 30th September 2022**
    - **16th European CODIS meeting 27th September 2022**
    - **57th EDNAP meeting 27th September 2022**
    - **48th ENFSI DNA EWG meeting 28th to 30th September 2022**
- Annual ENFSI joint meeting (board/EWG chairs/Standing Committees)
  - 29th November – 1st December 2022, Bratislava
- Annual ENFSI meeting with directors
  - 23rd May – 26th May 2023, the Hague
- Next candidates to host the annual DNA working group meeting (and EDNAP/European CODIS meeting)
  - 2023 – ?
  - 2024 –?
- EAFS
  - 2024/2025?


43rd DNA Working Group Meeting
2019
ENFSI
EUROPEAN NETWORK
OF FORENSIC SCIENCE
INSTITUTES
7th-10th May.

MPSproto: A tool to interpret STR-MPS mixtures with artefacts

An **extension of EuroForMix** for
modelling MPS stutters with complex structure

Øyvind Bleka(1) , Maria Martin Agudo(1), Peter Gill(1,2),

1) Forensic Genetics Research Group, Oslo University Hospital, Oslo, Norway
2) Department of Clinical Medicine, University of Oslo, Oslo, Norway

Oslo University Hospital

Part I: The MPSproto model(s)

Used to interpret mixtures where analytical threshold (AT) is reduced

Requires following calibrations:

1. **Locus specific amplification efficiency** (LSAE)
   - Constant and (optionally) Distribution
2. **Stutter proportions** (for each stutter type per locus)
   - Supports many kinds of stutter types
3. **Noise model**
   - Sequences not explained as stutters
   - Modelled per locus

Utilizes lusSTR to convert sequences into block lengths (**bracket format**)

- Reimplemented as LUSstrR available at https://github.com/oyvble/LUSstrR

- Example for D3S1358
  - '**TCTATCTGTCTGTCTATCTA…. TCTA**'
  - Bracket format='**TCTA [TCTG]2 [TCTA]13**'

- Block lengths are easy to extract using bracket format

Great thanks to

Rebecca Mitchell

Daniel Standage

Rebecca Just

Challenging STR-MPS stutters

- Some markers exhibit comprehensive stutters
- Example of D12 with structure [AGAT]n [AGAC]m

BW: Backward stutter
DBW: Double backward stutter
FW: Forward stutter

Stutter type: LUS coding

MPSproto coding

Donor allele

[AGAT]11 [AGAC]6

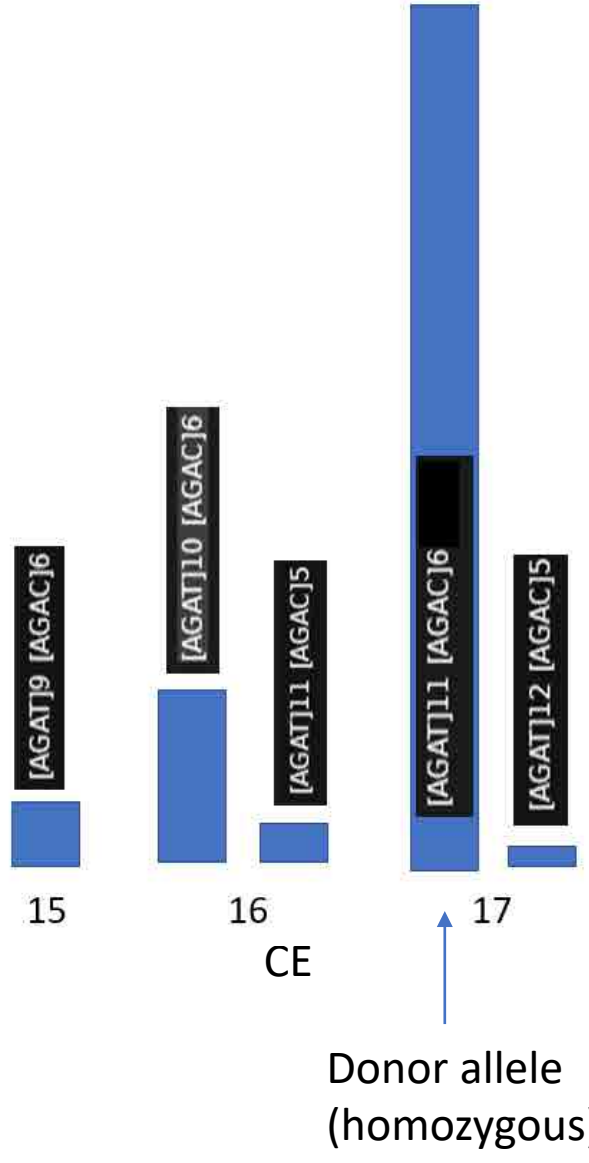
Coding:
LUS='1'
Non-LUS='2'

BW: '1' → [AGAT]10 [AGAC]6 — BW1

DBW: '1' → [AGAT]9 [AGAC]6 — DBW1

BW: '2' → [AGAT]11 [AGAC]5 — BW2

"n0"
'FW: 1' + 'BW: 2' → [AGAT]12 [AGAC]5 — FWBW



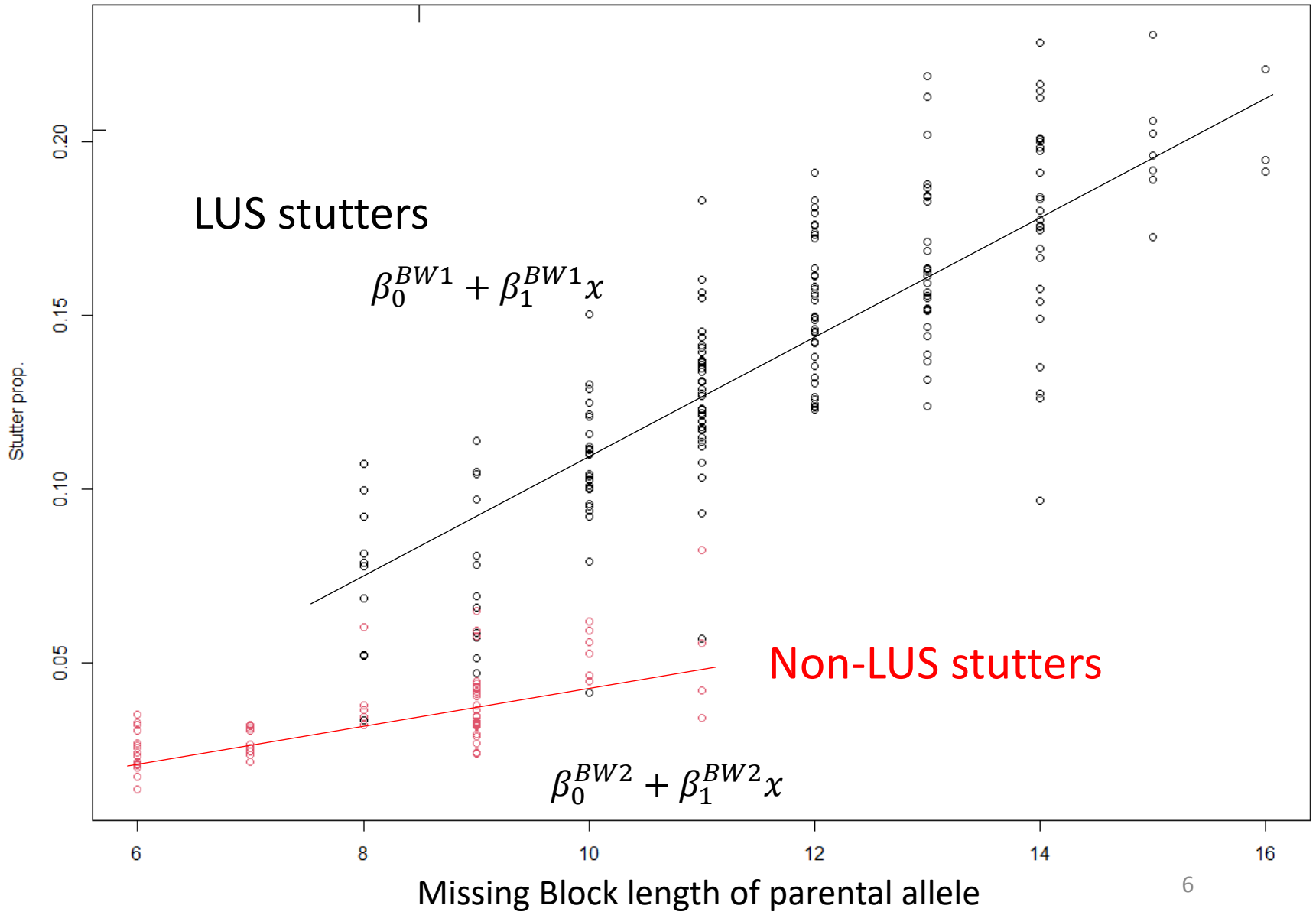
Modeling stutter proportions with block lengths

Example parental allele:
[AGAT]12 [AGAC]8 AGAT

LUS Non-LUS

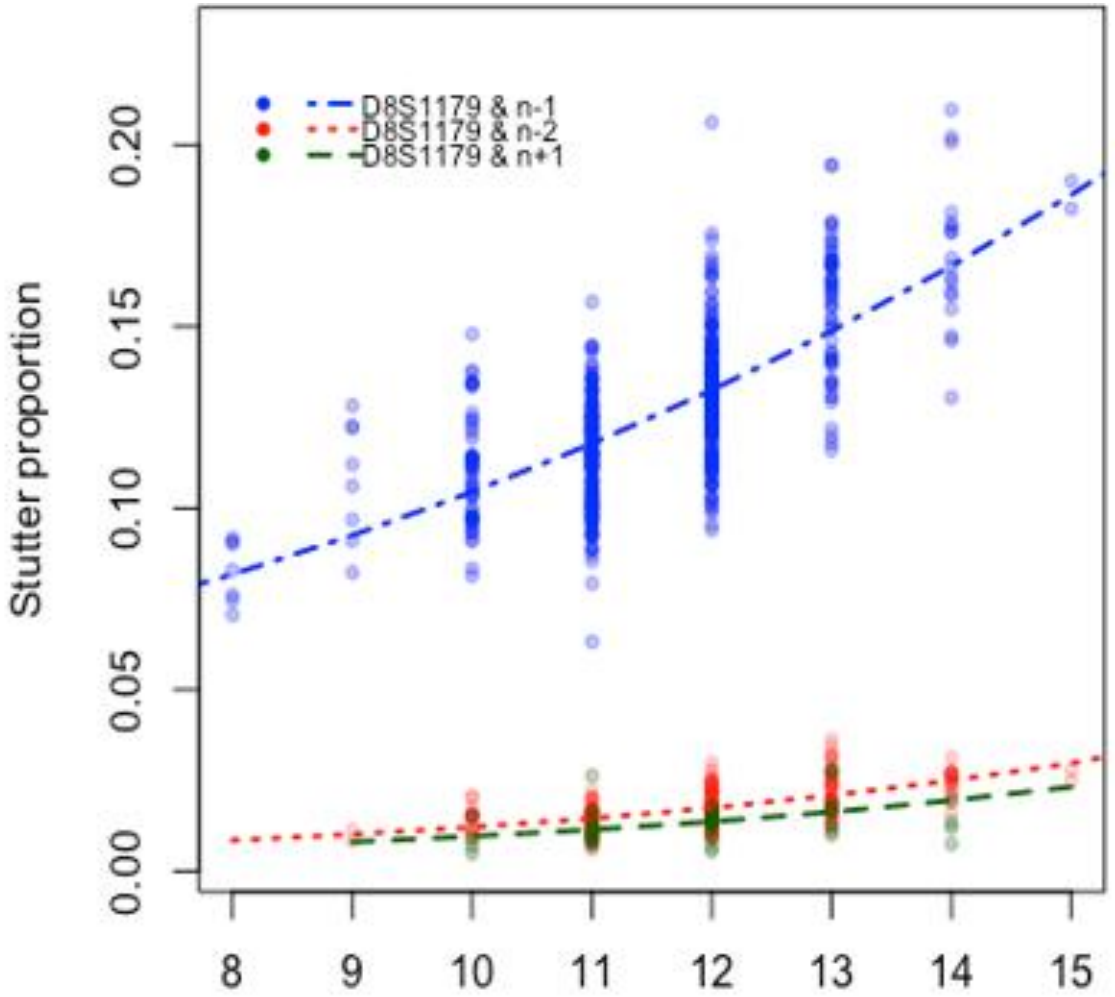
Missing block length
of parental allele

BW1: x=12
BW2: x=8



Expected stutter proportions can be fitted using beta-regression models

Part of calibration step



$e^{BW1}_{D8S1179,a}$

$e^{BW2}_{D8S1179,a}$

$e^{FW1}_{D8S1179,a}$

Expected stutter proportions for different model types

- Backward stutters (BW)
- Forward stutters (FW)
- Double BW

....

Research paper Forensic Science International: Genetics 60 (2022) 102728

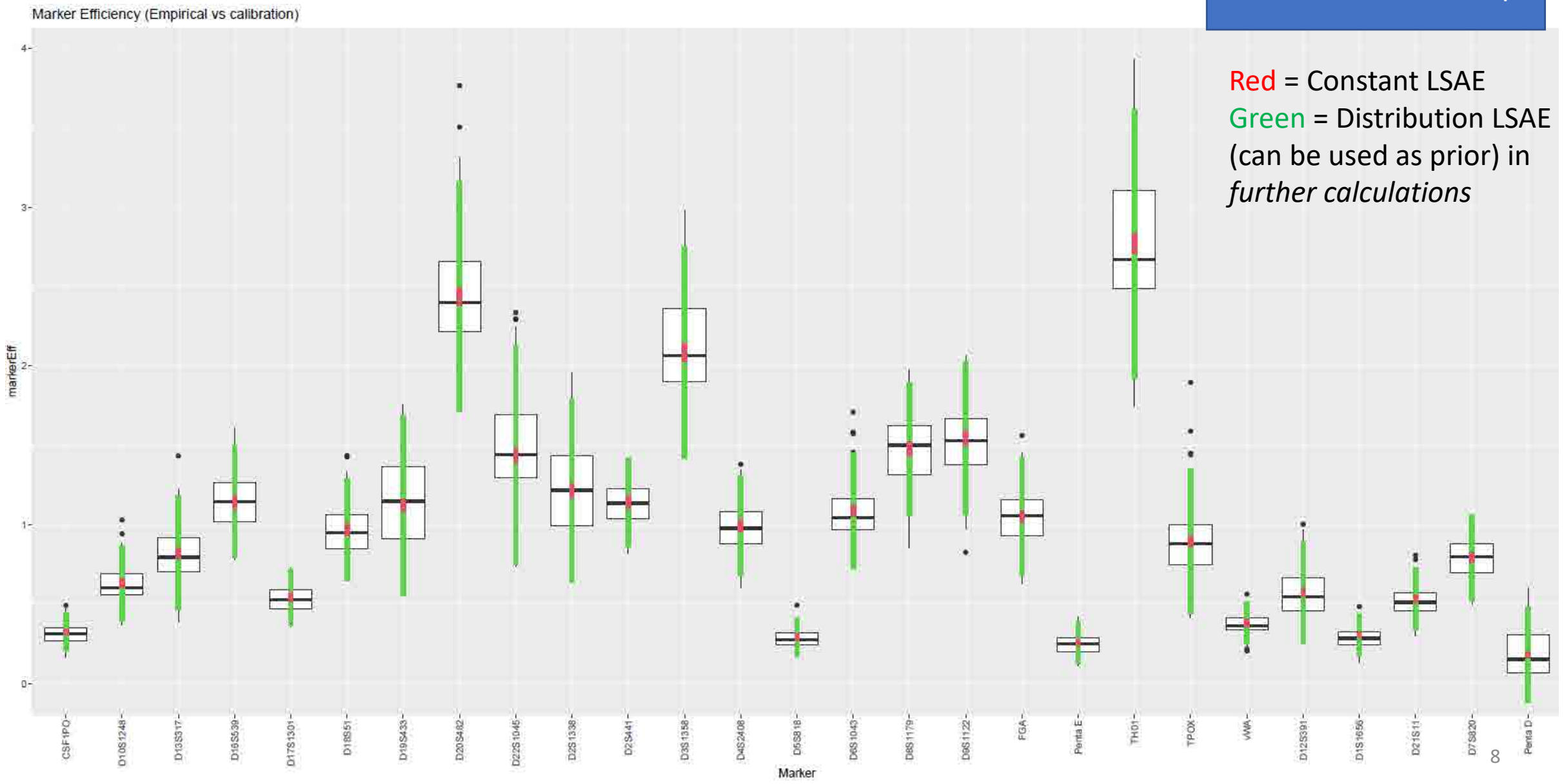
A comprehensive characterization of MPS-STR stutter artefacts

Maria Martin Agudo [a,b,*], Håvard Aanes [a], Arne Roseth [a], Michel Albert [a], Peter Gill [a,b], Øyvind Bleka [a]

Inference of locus specific amplification efficiency

LSAE

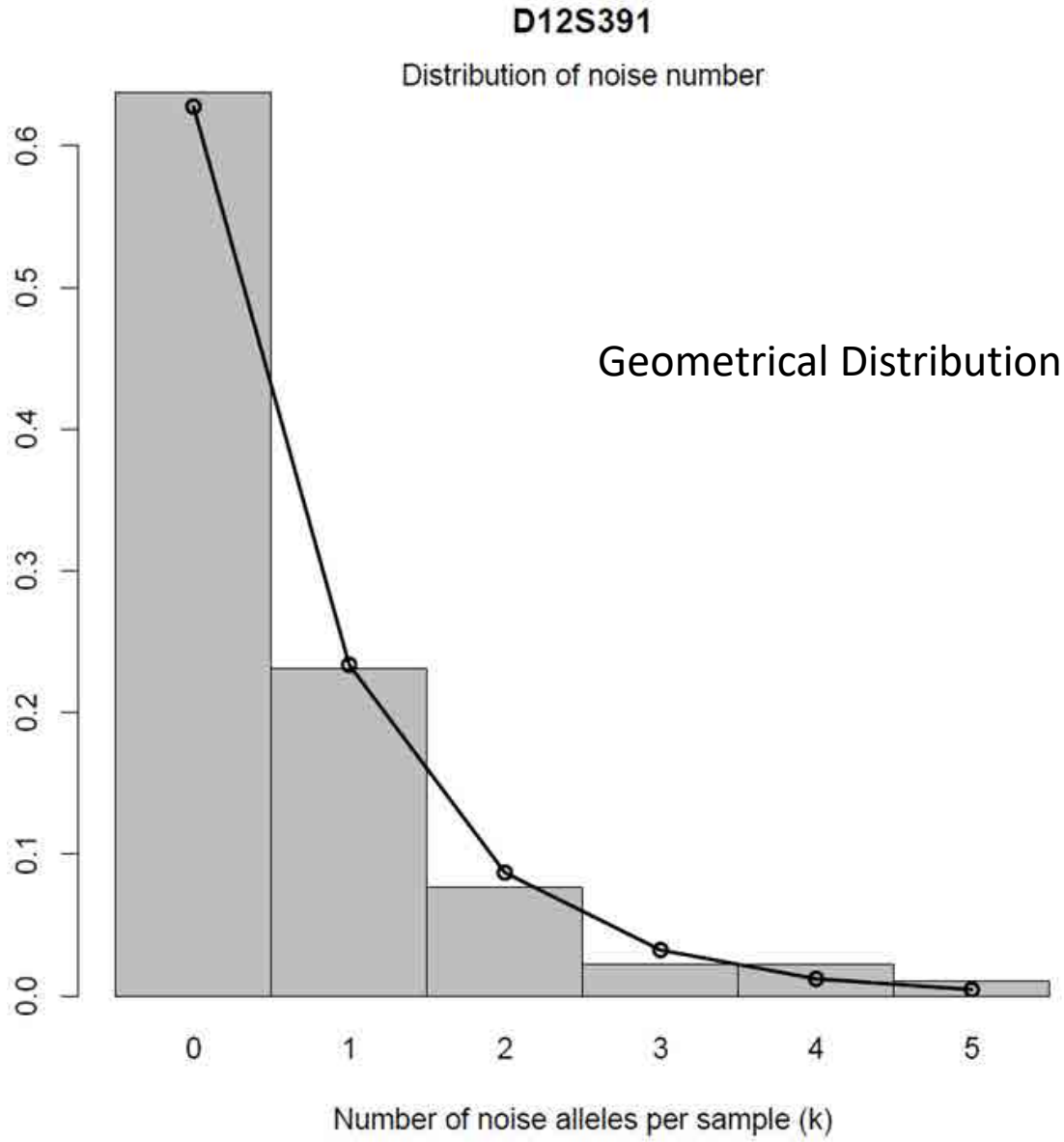
Part of calibration step



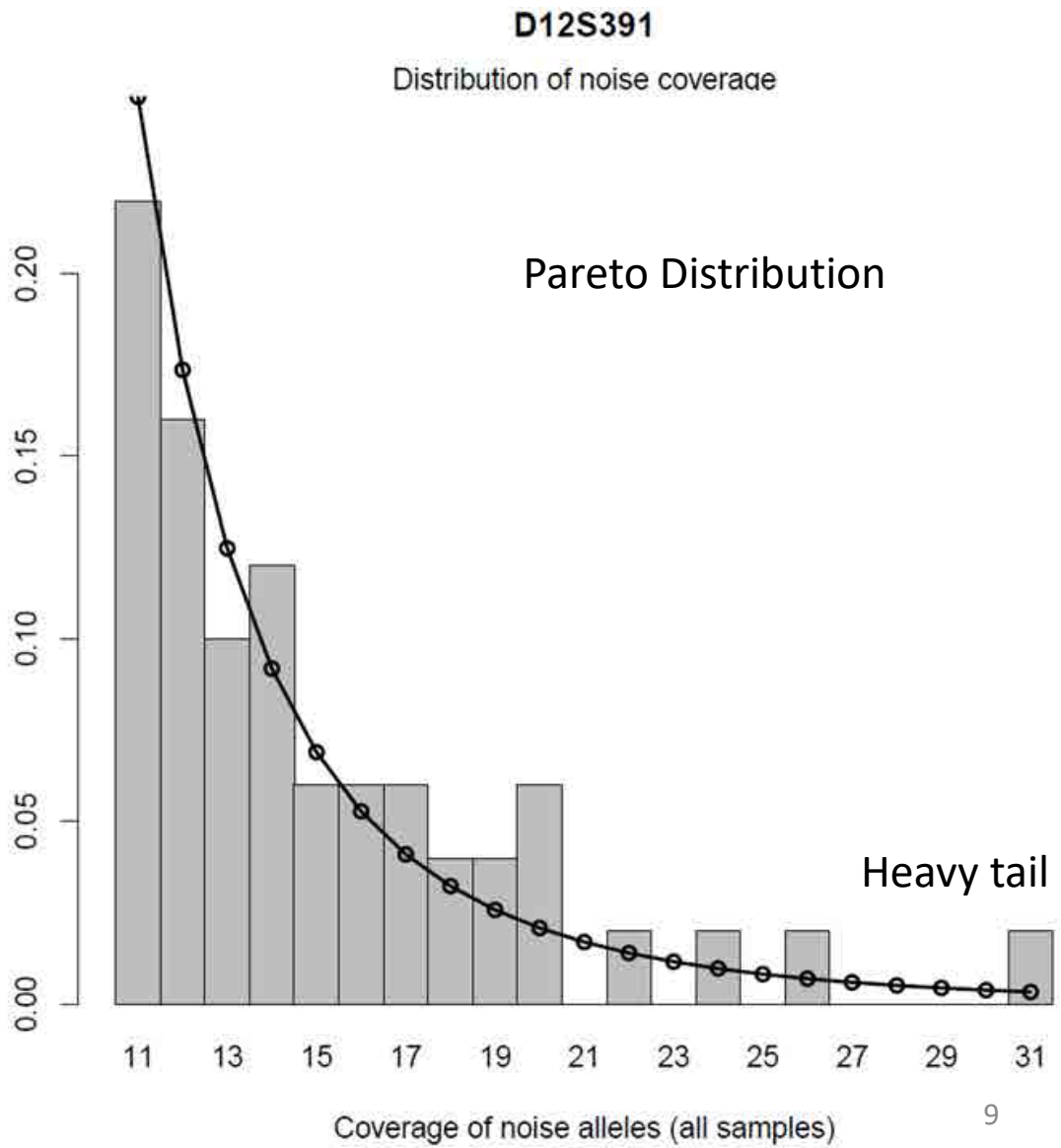
Red = Constant LSAE
Green = Distribution LSAE (can be used as prior) in *further calculations*

The “Noise model”

Part of calibration step



Geometrical Distribution



Pareto Distribution

Heavy tail

The MPSproto model(s) for **read depths (coverage)**

- Model 1: **The GA model**: Extending the EuroForMix model

$$\mathbf{Gamma}(\text{shape} = A * \omega^{-2}, \text{scale} = \mu\omega^{2})$$

μ=P.H.expectation
ω=P.H.variability

where A is a LSAE parameter

- **Model 2: The NB model**: The model as described by Vilsen et al (2016)

$$\mathbf{Negative-Binomial}(\text{mu} = A * \mu, size = \mu/(\mu\omega^{2}-1))$$

μ=P.H.expectation
ω=P.H.variability

- MPSproto **optimizes** the parameters μ,ω per hypothesis

An LR-comparison between EuroForMix and MPSproto models (GA vs NB vs EFM)

Revisiting the 2-4 person mixtures from paper

Based on the ForenSeq kit

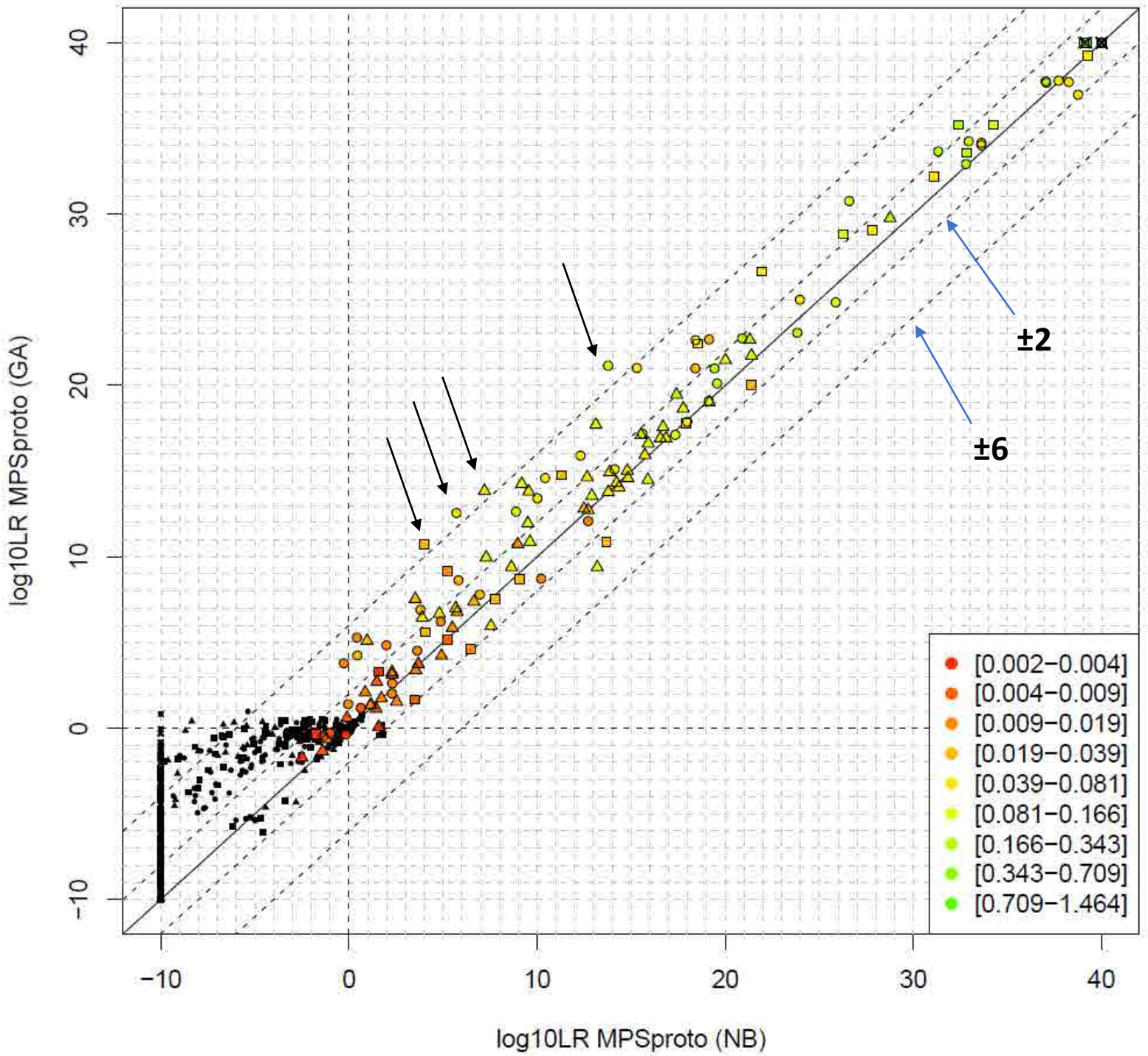
Research paper Forensic Science International: Genetics 48 (2020) 102319

An examination of STR nomenclatures, filters and models for MPS mixture interpretation

Øyvind Bleka[a,*], Rebecca Just[b,d], Jennifer Le[b], Peter Gill[a,c]

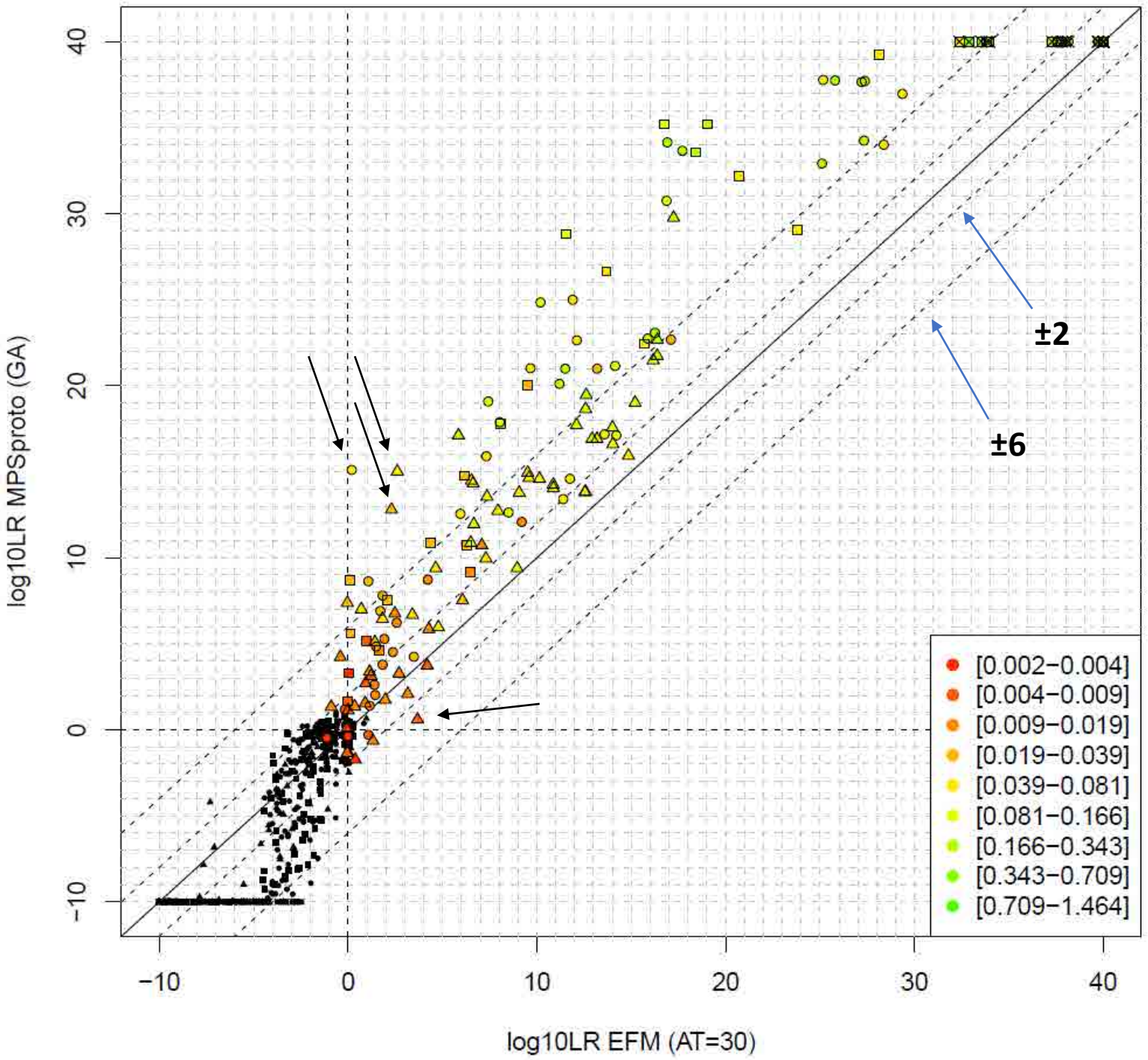
AT=11 reads for MPSproto

AT=30 reads for EuroForMix



MPSproto: GA vs NB

- *Quite* similar performance
- GA obtained higher LRs (both for Hp true and Hd true)
- Some few situations with more than log10LR=6 in difference.
- Could be explained by situations with dropouts where GA penalized less than NB
- Both models were adequate

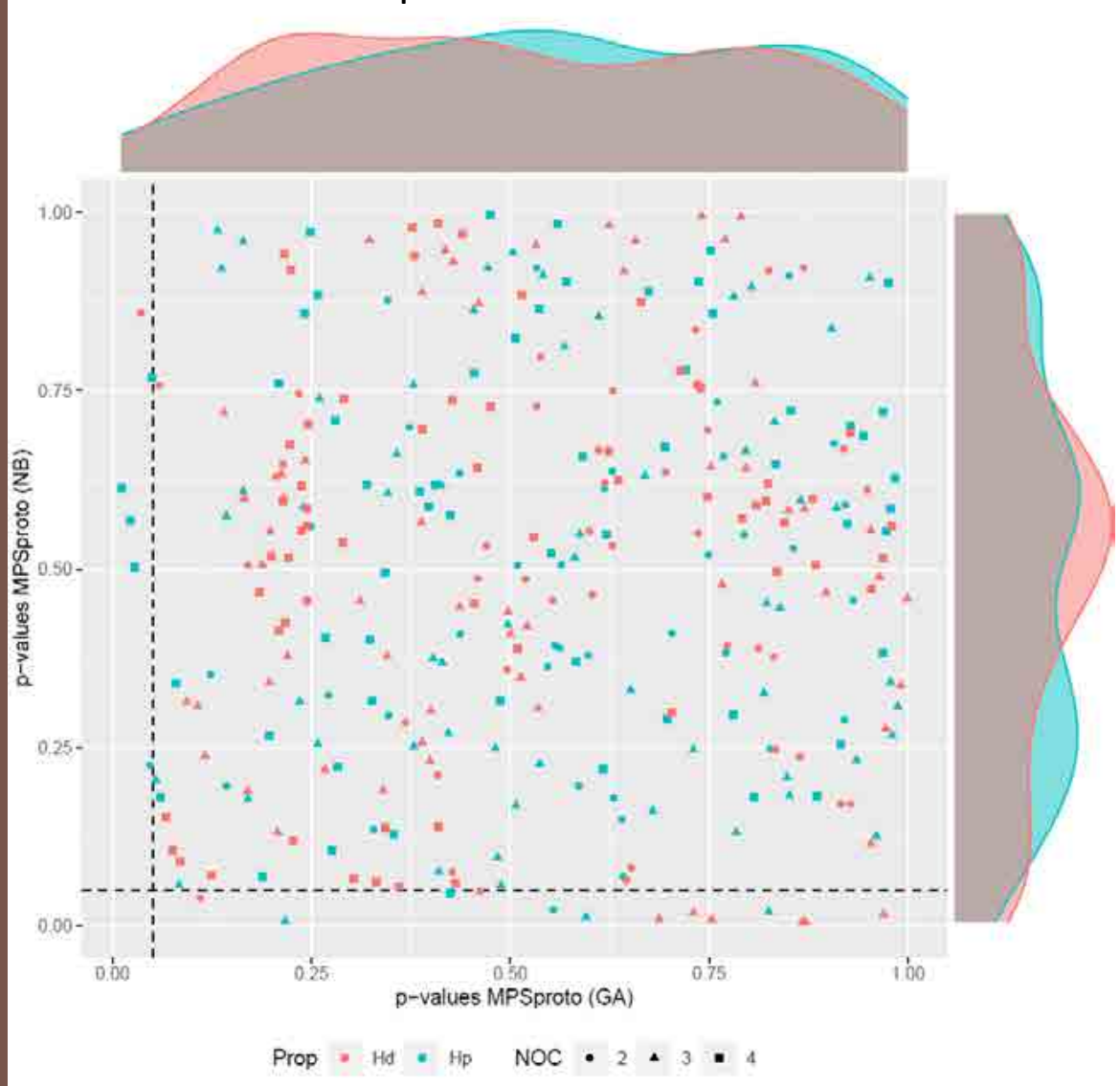


MPSproto (GA) vs EFM

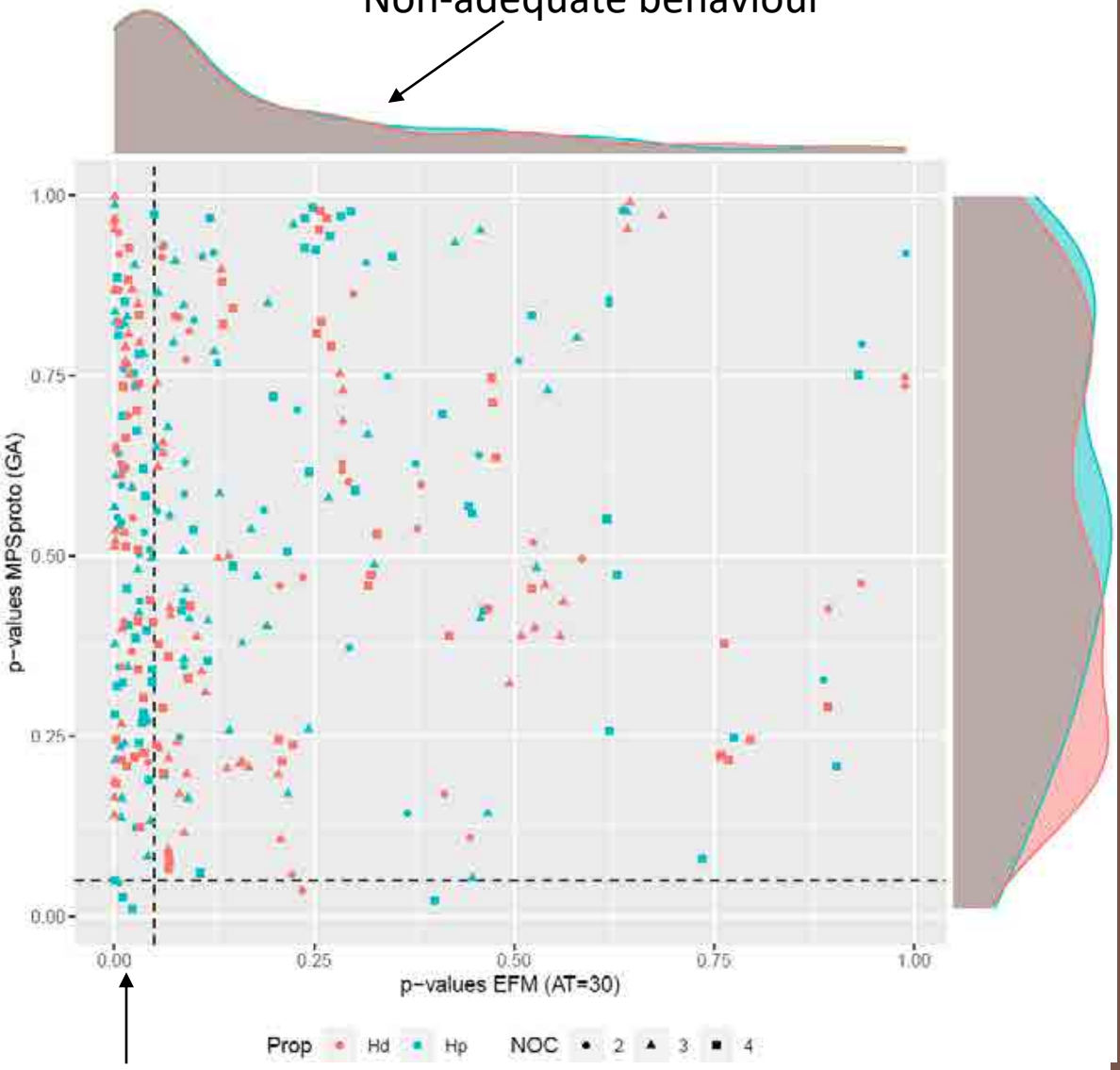
- GA obtained considerably higher LRs than EFM
- Many situations with more than log10LR=6 in difference.
- Could be explained by situations where alleles of POI fell below AT=30 threshold used for EFM
- Lowering AT for EFM gave smaller differences (AT=20)
- The use of a low AT leads to a less adequate model for EFM

Kolmogorov-Smirnov p-values

Adequate behaviour



Non-adequate behaviour

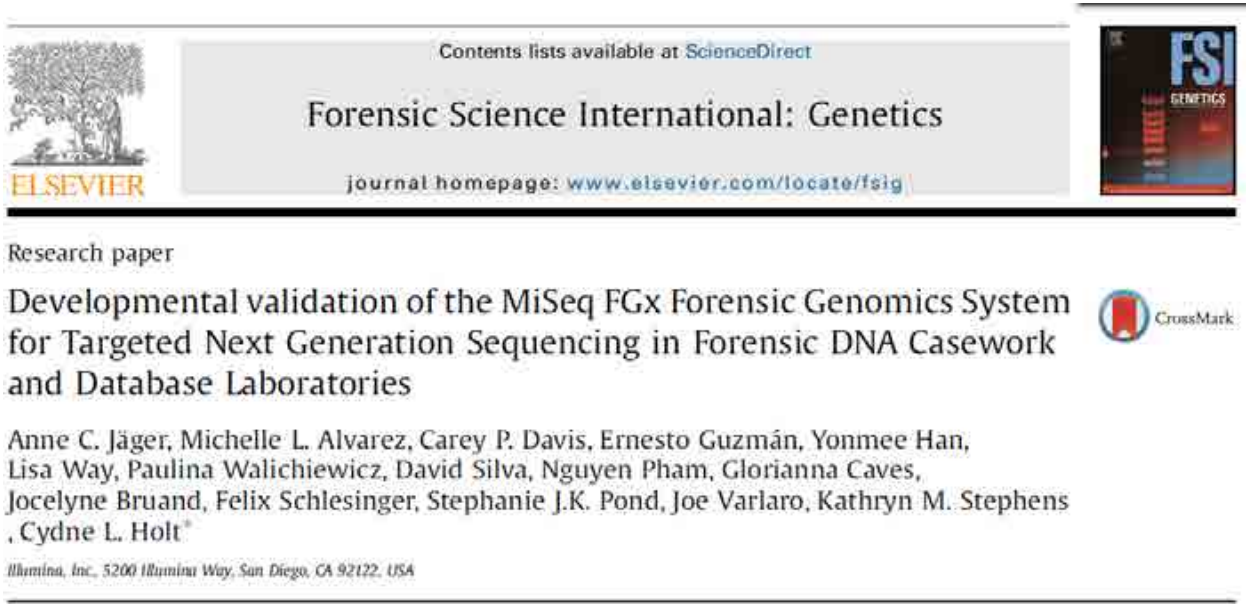


Conclusion

- MPSproto is an important contribution to the interpretation of MPS-STR profiles since the analytical threshold (AT) can be reduced
  - This is important for increased sensitivity
  - Can be used for both mixtures and non-mixtures
- Utilizes the “bracket format” to enhance the STR-stutter model
- The MPSproto models were adequate for the read depths when using AT=11, whereas the EuroForMix model was not using AT=30 or lower (most of the times)
- The two models of MPSproto behaved similarly overall, but different for some comparisons
  - ➢ Gamma model more robust to drop-outs (lead to higher dropout probabilities)
  - ➢ This also leads to higher LR for non-contributors
- Implemented as the R-package MPSproto
  - Details available at https://github.com/oyvble/MPSproto

Part II: Why the current use of thresholds limits usefulness of MPS

- The paper of Jager et al. outlines an interpretation method that is based on two thresholds, which will be discussed next



Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

Developmental validation of the MiSeq FGx Forensic Genomics System for Targeted Next Generation Sequencing in Forensic DNA Casework and Database Laboratories

Anne C. Jäger, Michelle L. Alvarez, Carey P. Davis, Ernesto Guzmán, Yonmee Han, Lisa Way, Paulina Walichiewicz, David Silva, Nguyen Pham, Glorianna Caves, Jocelyne Bruand, Felix Schlesinger, Stephanie J.K. Pond, Joe Varlaro, Kathryn M. Stephens , Cydne L. Holt*

Illumina, Inc., 5200 Illumina Way, San Diego, CA 92122, USA

Threshold based interpretation guidelines

- Two thresholds used: Analytical Threshold (AT) and Interpretation Threshold (IT)
- AT and IT values are determined for a locus by multiplying the analysis parameter percentage value (from table) by the sum of read counts
- In cases of low coverage, a minimum coverage of 650 reads was used for the locus in determination of the threshold values.
  - Common parameter percentage value are AT=1.5% and IT=4.5%
  - So this is a minimum AT=10 and minimum HT=30
- Default stutter filter percentages for autosomal STR, Y-STR, and X-STR markers are documented and range from 7.5% (D2S441, D4S2408, PentaD) to 50% (DYS481).

Thresholds from Jager et al

Loci	% Stutter	% Analytical	% Interpretation
DYS19	< 15	> 1.5	> 4.5
DYS385a-b	< 20	> 1.5	> 4.5
DYF387S1	< 20	> 1.5	> 4.5
DYS389I	< 20	> 1.5	> 4.5
DYS389II	< 35	> 5	> 15
DYS390	< 15	> 1.5	> 4.5
DYS391	< 20	> 1.5	> 4.5
DYS392	< 30	> 1.5	> 4.5
DYS437	< 45	> 1.5	> 4.5
DYS438	< 15	> 1.5	> 4.5
DYS439	< 15	> 1.5	> 4.5
DYS448	< 15	> 3.3	> 10
DYS460	< 15	> 1.5	> 4.5
DYS481	< 50	> 1.5	> 4.5
DYS505	< 15	> 1.5	> 4.5
DYS522	< 15	> 1.5	> 4.5
DYS533	< 15	> 1.5	> 4.5
DYS549	< 22	> 1.5	> 4.5
DYS570	< 22	> 1.5	> 4.5
DYS576	< 15	> 1.5	> 4.5
DYS6121	< 35	> 1.5	> 4.5
DYS635	< 15	> 3.3	> 10
DYS643	< 20	> 1.5	> 4.5
Y-GATA-H4	< 35	> 1.5	> 4.5

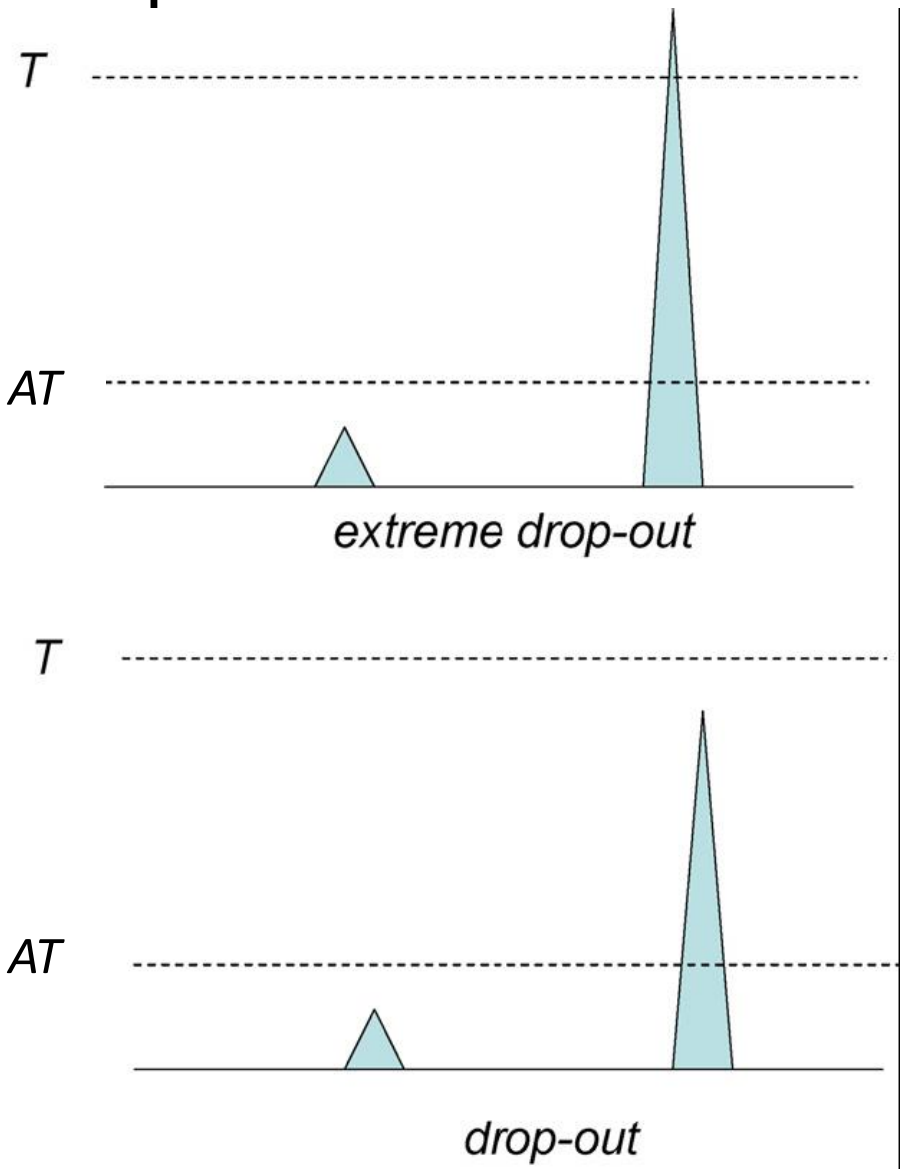
Rule based interpretation from Jager et al

- If a single autosomal allele was greater than the interpretation threshold (IT), it was called as a homozygote e.g., (12,12)
- whereas if reads for a single allele were detected between the AT and IT, then was designated as an “Ambiguous Genotype” (e.g., (13,*)), to account for possible non-detection of a sister allele.
- In cases where the highest signal (read counts) was less than the AT an allele was not called.
- But this raises issues about how to interpret – in particular what LR to apply
- However, exactly the same issues have been addressed in relation to CE based applications

Ten years ago: consequences of threshold based interpretation were outlined

- Although the rules are designed to be ‘conservative’ this is not always the case
- Application of filters for stutters will also remove ‘true’ alleles, which can be anti-conservative

Example

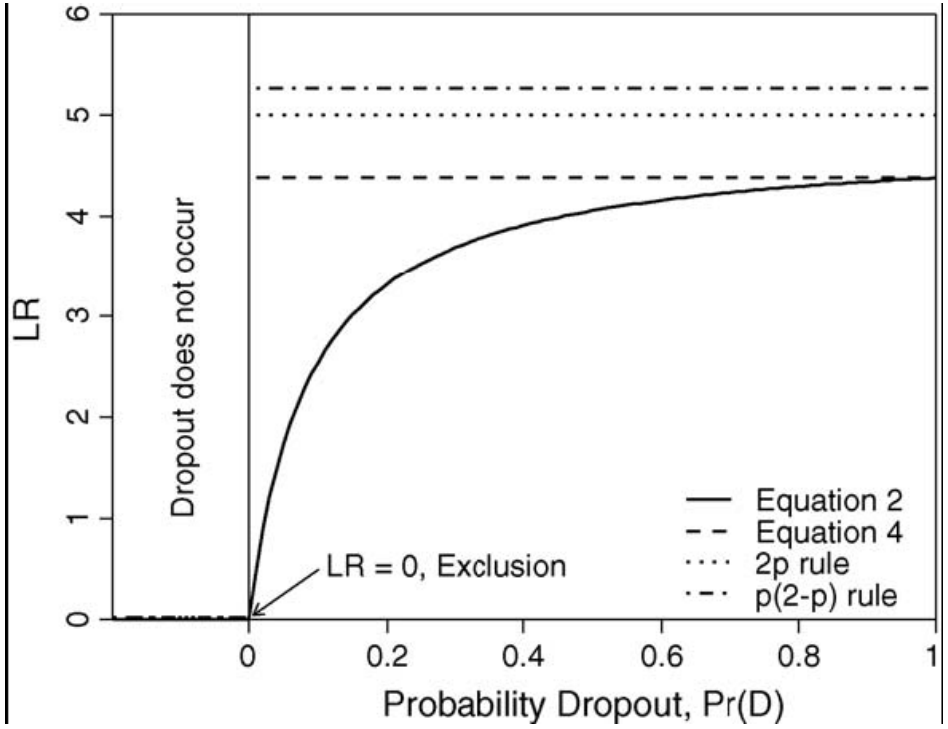


- T is the stochastic threshold used to signify PrD≈0
- It is designed to capture the event S=ab C=aa.
- If allele<T then it is given the F designation
- If allele>T it is designated as a homozygote
- The threshold won't capture all events (unless set to infinity)
- If it's too high then too many samples are rejected to make it feasible
- So all thresholds will be subject to some error
- How much error can be tolerated
- Who decides this?

The 2p rule

- Suppose S=ab and C=aa and a>T
- This cannot be viewed as neutral evidence

(Buckleton) – can be very anticonservative



Thresholds

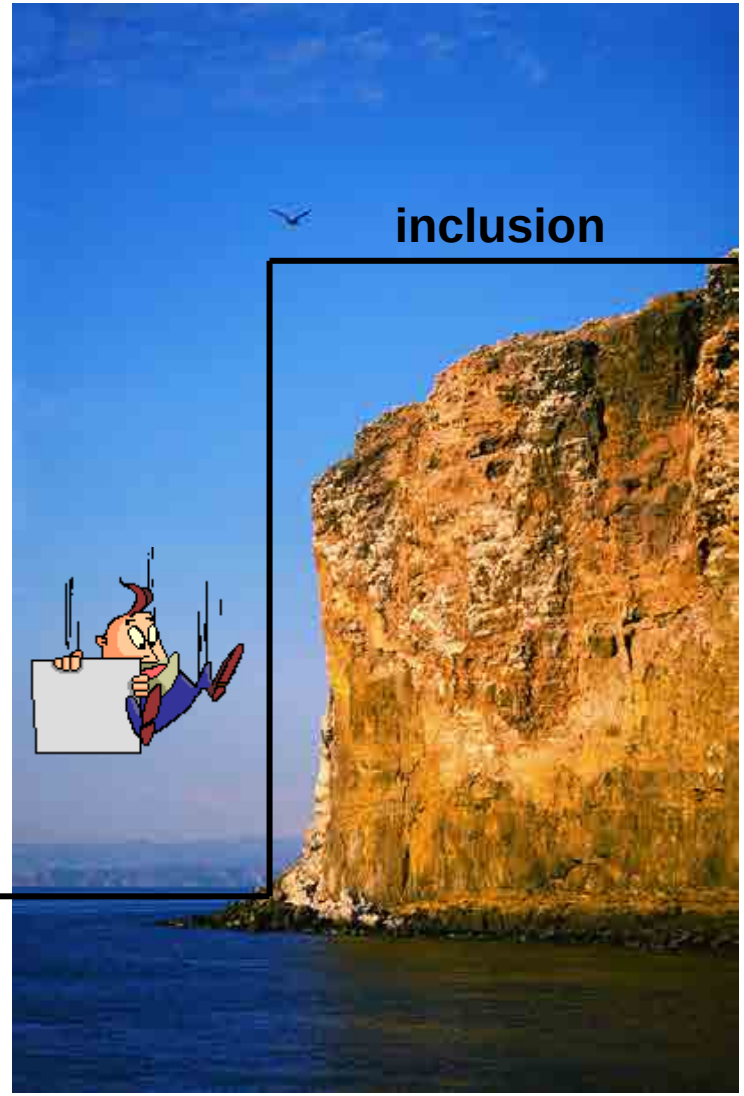
- Falling off the cliff
- E.g. if we have a

Rule that states:

150rfu – no dropout is possible

V. 149rfu – dropout is possible

- There is nothing in between



In reality it’s a gentle ride downhill

What does this mean?

- It is very difficult to define the meaning of the following words:
- match, inclusion, exclusion, inconclusive
- This is because the context of the words carries a meaning that is definitive
- We always encounter the ‘threshold dilemma’

included	inconclusive	exclusion
match	Cannot be excluded	Non-match

The underlying model is continuous

- Thresholds are difficult to apply and cannot be used in a definitive way unless associated with an estimate of (acceptable) risk.
- It is tempting to use the ‘inconclusive’ category and to use statements like ‘ the suspect cannot be excluded’.
- But this kind of statement may be prosecution biased – especially if a proper analysis favours the defence hypothesis.
- Therefore, it is not possible to demonstrate that such guidelines are always more conservative, simply by increasing the number of inconclusive calls.

A different calculation is needed

- If the profile is unambiguous (ie matches suspect then the numerator =1
- If the profile is ambiguous (ie does not match suspect completely) then the numerator is less than one
- i.e. we are used to calculating

$$\frac{1}{2ab}$$

The bottom line:
If this is less than one then the strength of evidence decreases

AND

If there is any uncertainty about
The prosecution hypothesis then
This must be less than one (not neutral)

Removing thresholds and filters by using continuous models

- Continuous models model both numerator and denominator
- Modelling stutters and noise greatly facilitates interpretation of evidence, not only for mixtures, **but also for non-mixtures too!**
- Interpretation is much more robust because we do not remove information.
- We get rid of ad-hoc guidelines that waste information and can be greatly anti-conservative
- The threshold of 11 reads used by MPS-proto, universally applied, is a considerable improvement, which will greatly increase the number of cases that can be reported
- MPS-STRs are much more complex than CE based interpretation, primarily because of the modelling of multiple stutter-types that need to be taken into account.

Further developments: EFMrep

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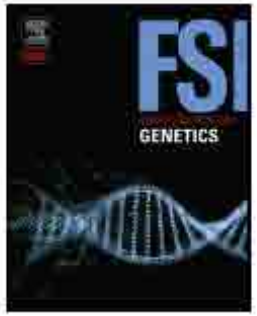


EFMrep: An extension of EuroForMix for improved combination of STR DNA mixture profiles

Øyvind Bleka [a,*], Lourdes Prieto [b,c], Peter Gill [a,d]

[a] Forensic Genetics Research Group, Oslo University Hospital, Oslo, Norway
[b] Instituto de Ciencias Forenses, Grupo de Medicina Xenómica, Universidade de Santiago de Compostela, Santiago de Compostela, Spain
[c] Comisaría General de Policía Científica, Laboratorio de ADN, Madrid, Spain
[d] Department of Forensic Medicine, University of Oslo, Oslo, Norway

EFMrep

- Enables combination of STR DNA mixture samples from different multiplexes by allowing different model parameters to be assigned to each DNA profile in the analysis
- Also allows related individuals to be specified
- Enables combination of profiles from the same or different extracts

Combining mRNA and DNA tests in sexual assault cases

Forensic Science International: Genetics 60 (2022) 102750

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Transfer, persistence and recovery of DNA and mRNA vaginal mucosa markers after intimate and social contact with Bayesian network analysis for activity level reporting

Helen Johannessen [a,*], Peter Gill [a,b], Gnanagowry Shanthan [b], Ane Elida Fonneløp [b]

[a] Department of Forensic Medicine, University of Oslo, Norway
[b] Department of Forensic Sciences, Oslo University Hospital, Norway

The problem

- In many cases of sexual assault, the source of the body fluid is often in question especially if there is some evidence of potential social contact between victim and the suspect
- As an example, recall the case *R v Weller* in the appeal court of England and Wales

R v Weller

- The case circumstances
  - The victim claimed that the defendant had sexually assaulted her by digital penetration
  - The defendant claimed only social contact occurred when he helped her to bed after she became intoxicated at a party. He touched her hair
- The evidence
  - DNA mixture underneath the fingernails of the left hand of the suspect where he was the major contributor and the victim was a minor contributor
  - Sub-source inference was uncontested
- Activity level propositions
  - Either the suspect sexually assaulted the victim by digital penetration
  - Or he only had social contact with her, helping her to bed and touching her hair

Activity level in R v Weller

- Clearly, under the prosecution proposition digital penetration occurred, hence the origin of the DNA would be from vaginal mucosa
- Under the defence proposition, the DNA came from skin cells
- Note that no test for vaginal mucosa was carried out
- In court it was argued that the high levels of victim DNA was more likely to arise from sexual assault rather than from social contact.
- The conviction was upheld

mRNA markers for vaginal mucosa (VM)

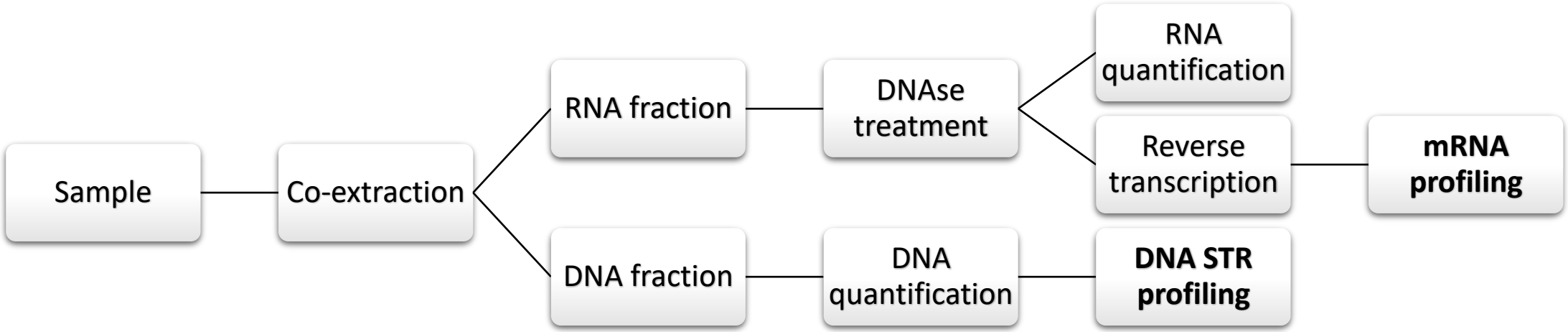
- Most common mRNA markers are:
  - Mucin 4 (MUC4)
  - Human beta-defensin (HBD1)
  - Myozenin (MYOZ1)
  - Cytochrome P450,
  - Family 2 Subfamily B Polypeptide 7 Pseudogene 1 (CYP2B7P1)
- MUC4 and the HBD1 markers are less specific as they often cross react with other body fluids, especially saliva and nasal mucosa
- MYOZ1 and CYP2B7P1 are more specific
- But there is no specific (confirmatory) test
- To assign whether VM was present/absent the NFI method (Lindenburgh et al. was followed) where >50% of markers must be observed to be classed as present

The experimental design

- Twenty four participants (12 couples) volunteered
- DNA reference samples collected from each
- Fingernail and penile swabs taken at five different time points post intimate contact
- Boxershorts worn by the male were also collected both before and after intimate contact
- Non-intimate samples were collected from same locations to monitor prevalence and background

Sample processing

- Tips of cotton swabs were extrace
- Boxershorts sampled with mini-tape
- Samples co-extracted with QIAamp DNA mini kit (QIAGEN) and mirVANA^{TM} miRNA isolation kit (invitrogen by Thermo Fisher Scientific).
- Quantification with Powerquant^{R} and amplified by Powerplex Fusion 6C aiming for 1ng input of DNA



Sub source propositions

- H_p: The DNA is from the person of interest (POI)
- H_d: The DNA is from an unknown individual, unrelated to POI
- The donor was conditioned under both propositions as per the standard procedure in case work
- EuroForMix was used to calculate subsource LRs, and mixture proportions (Mx) for the individual contributors were used to calculate the RFU contribution for the POI which is adjusted by a factor (dl) to compensate the effect of dilution (otherwise the values would be too low)

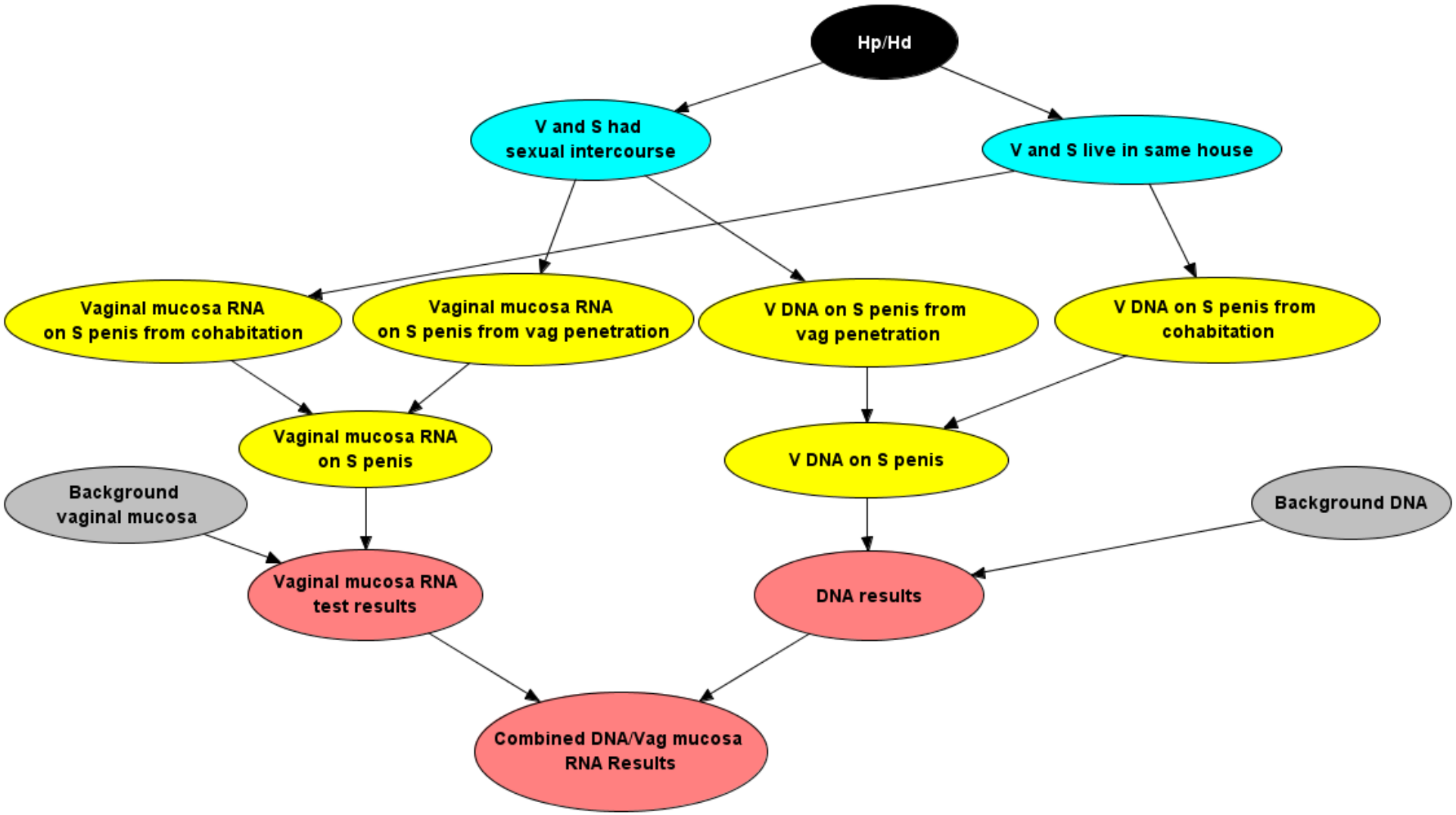
$$\overline{RFU}_{POI} = M_X \times \frac{RFU_{tot}}{m} \times d_l$$

Activity level

- Case circumstances simulated are generic and representative of majority of casework for this kind of offence

1) A victim claims to be sexually assaulted by a suspect and alleges that vaginal penetration occurred.
2) The victim and the suspect have had previous non-intimate contact. They may co-habit or share facilities in an apartment, for example.
3) The suspect denies the allegations stating that he only had social contact with the victim.
4) There is no allegation that the assault was committed by an unknown individual

Bayesian network



What is different about this BN?

- Note that we do not carry out a specific source level evaluation
- i.e. we do not calculate the LR that evaluates the strength of evidence if vaginal fluid is/is not present
- Rather, we ask a different question at activity level:
  - What is the probability of the combined findings if Hp/Hd are true?
- We argue that this approach is better because there is no requirement to ask the court to make a definitive decision about the presence/ absence of vaginal mucosa before we move to the activity level
- Also, we are not so concerned by the necessity to provide RNA systems that are completely body fluid specific, because the efficacy of the system is reflected by value of the activity level LR itself.

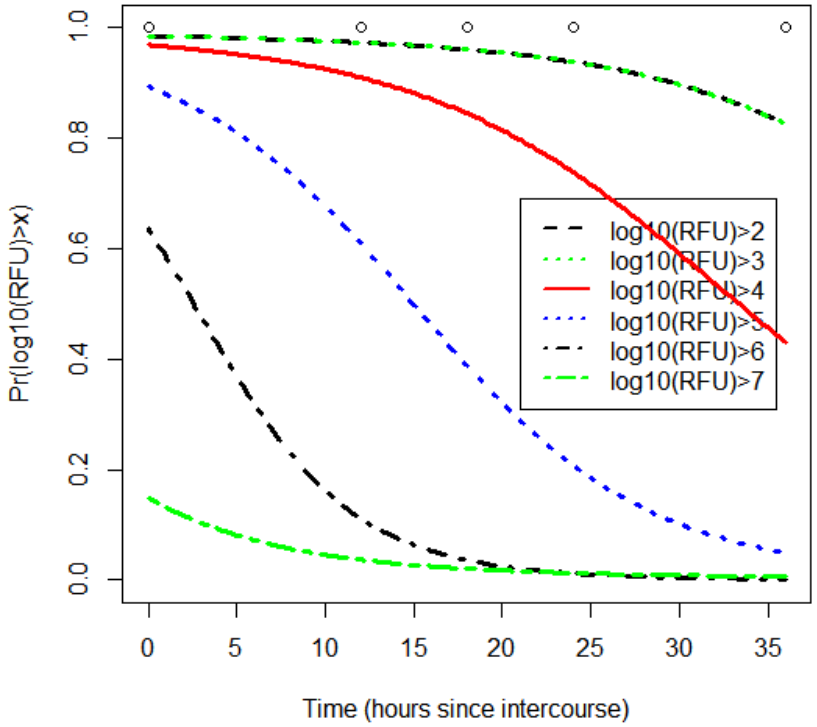
Background and prevalent body fluid markers

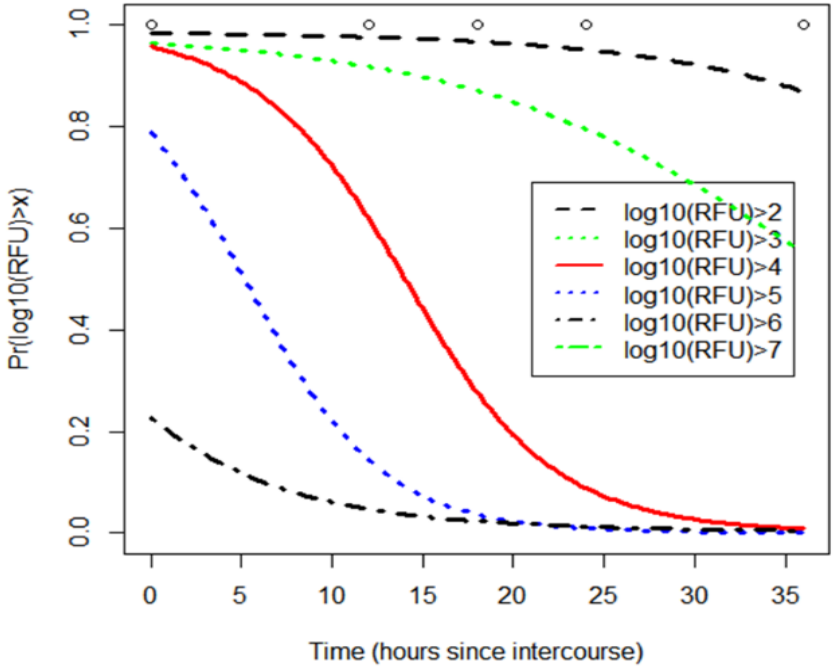
- In order to assess probability of evidence if social contact occurred, it is necessary to have information about the prevalence of VM from known individuals and the levels of background i.e. from unknown individuals
- Whereas we can distinguish between known and unknown DNA contributors, we cannot do the same for body fluids, hence we have to use the same probability for both
- From observations of penile swabs, where no sexual activity occurred,

this probability was assigned as 1/23

Probability of direct transfer given time since intercourse

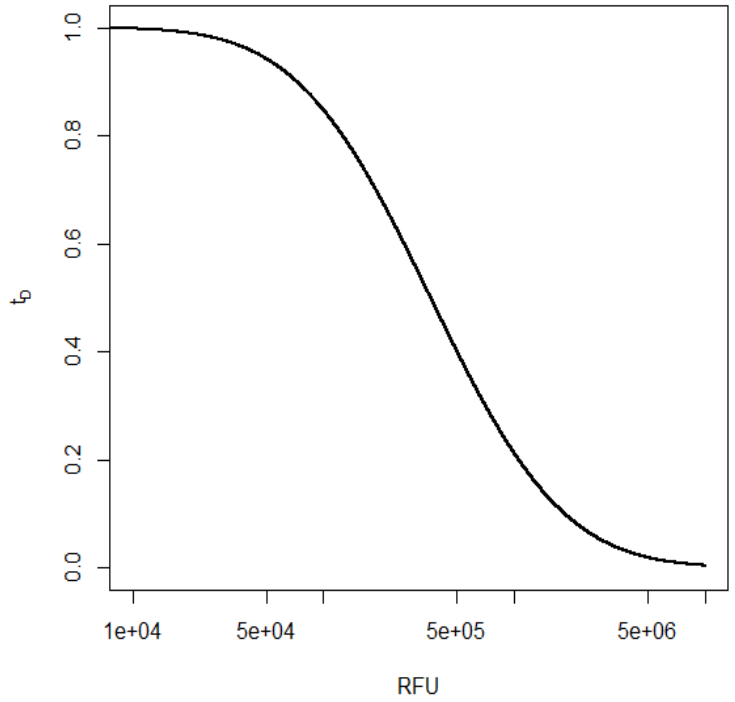
- Logistic regressions of a) penile swabs (left), b) fingernails swabs (right). Time since intercourse vs $Pr(log10(\overline{RFU}_{POI}) > x)$, showing probability of DNA transfer, persistence and recovery for a range of threshold values x.





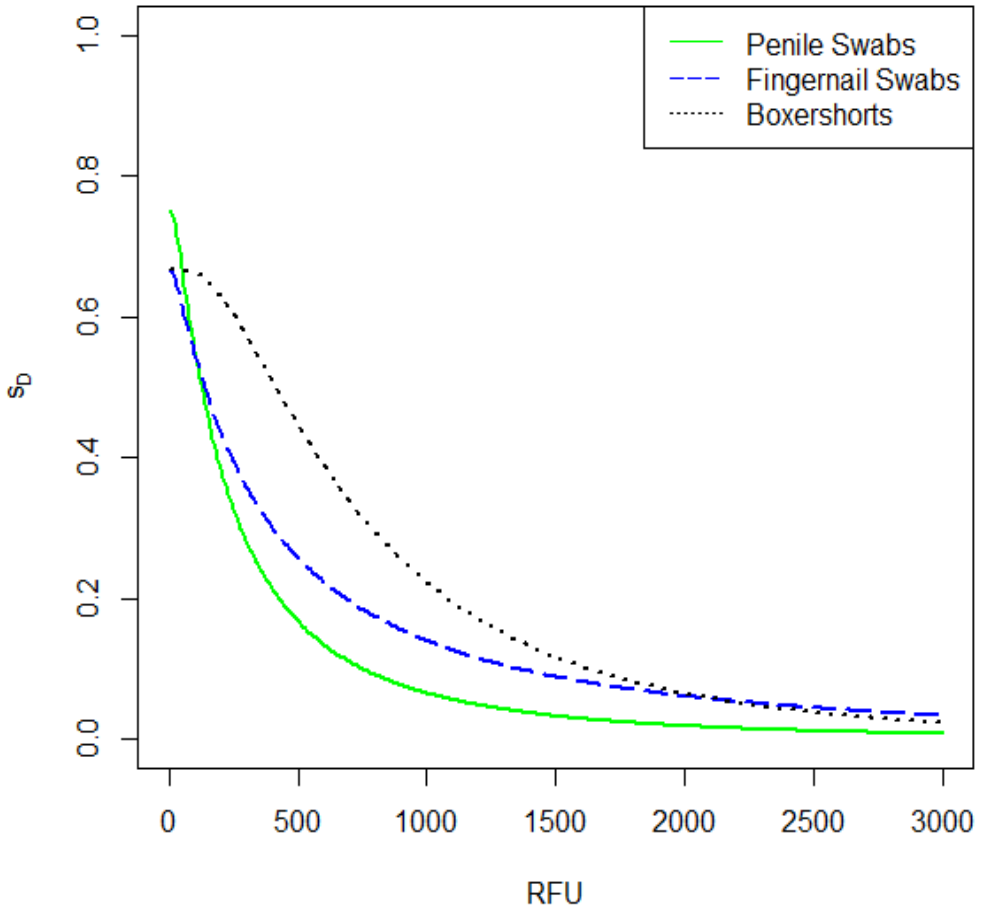
Boxer shorts direct transfer

- Not dependent upon time



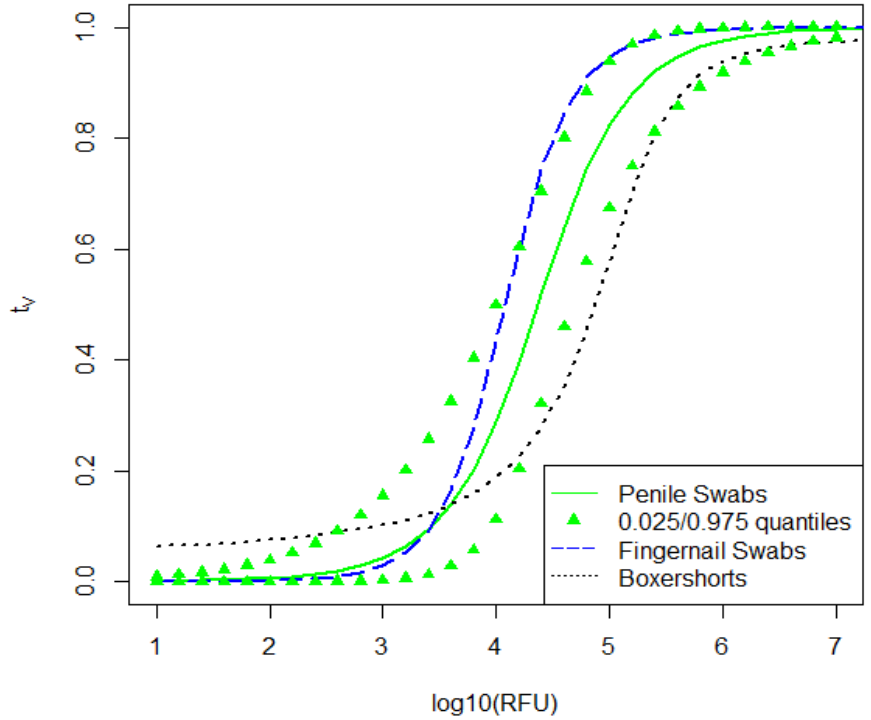
Results for indirect transfer (social activity)

- Also no time dependency with this model



Vaginal mucosa results – direct transfer

- Presence/absence of VM was scored using the (former) NFI method
- Note that the best indicator variable was log10(RFU) rather than Time since intercourse, hence we only use the former



Bayesian network - case example

Forensic examination of the victim and the suspect was performed:

- No semen or DNA "matching" the suspect was detected in the intimate samples collected from the victim.
- DNA "matching" the victim was detected on the penile swab, fingernail swab and boxershorts collected from the suspect.

Two sets of hypothetical findings (A and B)

- **A**: Samples collected 15 h after alleged offence; Positive test for vaginal mucosa; The $\log_{10}\overline{RFU}_{POI} = 5{,}4{,}5$ for penile swab, fingernail swab and boxershorts respectively.

- **B**: Samples collected 25 h after alleged offence; Negative test for vaginal mucosa; The $\log_{10}\overline{RFU}_{POI} = 4{,}3{,}5$ for penile swab, fingernail swab and boxershorts respectively.

Activity-related propositions

H_p: the suspect had vaginal intercourse with the victim

H_d: the suspect and the victim only had social interaction via cohabitation

BN case example (3)

		Penile swabs		Fingernail swabs		Boxershorts	
	Time	**Log10 LR** DNA+/Vag+	**Log10 LR** DNA+/Vag-	**Log10 LR** DNA+/Vag+	**Log10 LR** DNA+/Vag-	**Log10 LR** DNA+/Vag+	**Log10 LR** DNA+/Vag-
A	15 h	**8**	7	**3**	2	**11**	9
B	25 h	4	**3**	0.7	**0.8**	11	**9**

A (Time = 15 h): mRNA vag. mucosa POS, $\log_{10}\overline{RFU}_{POI} = 5{,}4{,}5$ for penile swabs, fingernail swab and boxershorts resp.

- **LR (log10) = 8, 3, 11**.

B (Time = 25 h): mRNA vag. mucosa NEG, $\log_{10}\overline{RFU}_{POI} = 4{,}3{,}5$ for penile swabs, fingernail swab and boxershorts resp.

- **LR (log10) = 3, 0.8, 9**.

Key findings

- There is much more information in the DNA result rather than the VM result (which adds very little), but improved VM methods will certainly result in improved LRs. Also, we currently have limited information about background/prevalent body fluid markers which will affect the outcome
- Boxer shorts provide a good source of evidence, especially when the offence is examined more than a day afterwards
- The BN framework provided here does not require a formal assessment at source level i.e. the absence of a positive VM test does not prevent assessment at activity level

Back to R v. Weller

- There has been some criticism of this case, since there was no attempt to analyse vaginal mucosa, which some argued was essential
- However, we have shown that the detection (or not) of VM has a small impact upon the LR compared to the DNA result
- In conclusion, the thinking was sound, and we now provide a method to calculate the activity level LR for such cases

Summary

- Observed higher persistence of DNA compared to mRNA
- Strong association between the $\overline{RFU}_{POI}$ values and positive / negative vaginal mucosa test
- The DNA quant ($\overline{RFU}_{POI}$) has a bigger impact on the resulting LR than the mRNA vaginal mucosa test
- Boxershorts can provide a good source of DNA evidence (not time dependent)

Thank you for your attention

Forensic Science International: Genetics 60 (2022) 102750

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Transfer, persistence and recovery of DNA and mRNA vaginal mucosa markers after intimate and social contact with Bayesian network analysis for activity level reporting

Helen Johannessen [a,*], Peter Gill [a,b], Gnanagowry Shanthan [b], Ane Elida Fonneløp [b]

[a] Department of Forensic Medicine, University of Oslo, Norway
[b] Department of Forensic Sciences, Oslo University Hospital, Norway

Limitations of qPCR and how to improve quantitation of DNA

Peter Gill

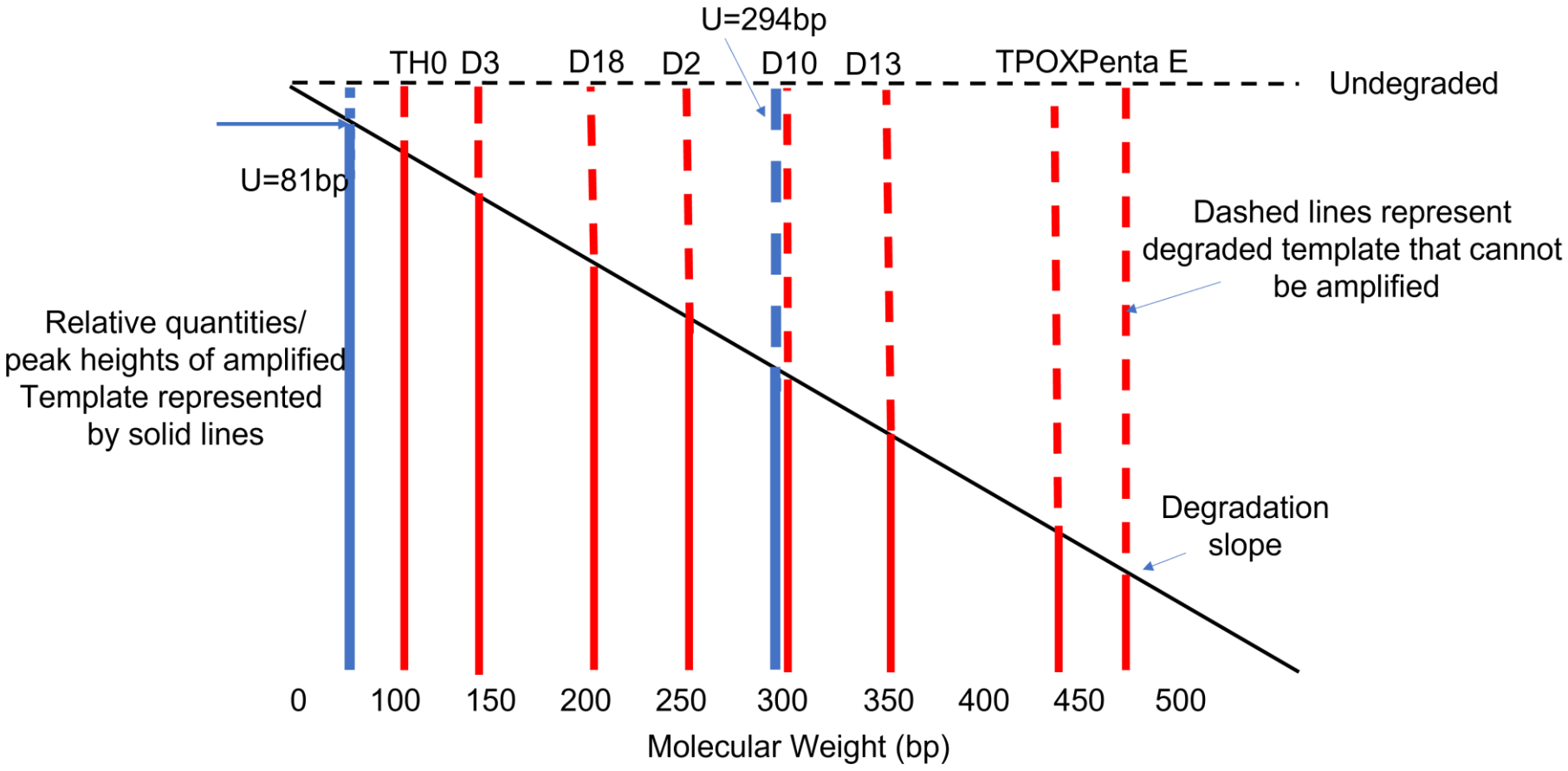
Motivation

- qPCR methods are in routine use
- How accurate are they?
- Example illustrates the use of the Promega Powerquant test, but all of qPCR methods follow the same idea
- Powerquant is advertised as a multicopy copy test. Target is not disclosed but it is not based upon STRs currently used in multiplexes.
- Number of copies may be variable per haploid genome (e.g. Plexor HY)
- Two types of targets:
  - Short target of 81bp
  - Long target of 214bp

Quantification method

- The short 81bp fragment is used to quantify and the long 214bp fragment is used to indicate whether degradation is present
- This works fine when the samples are pristine, but what happens if there is degradation?

qPCR with degraded material



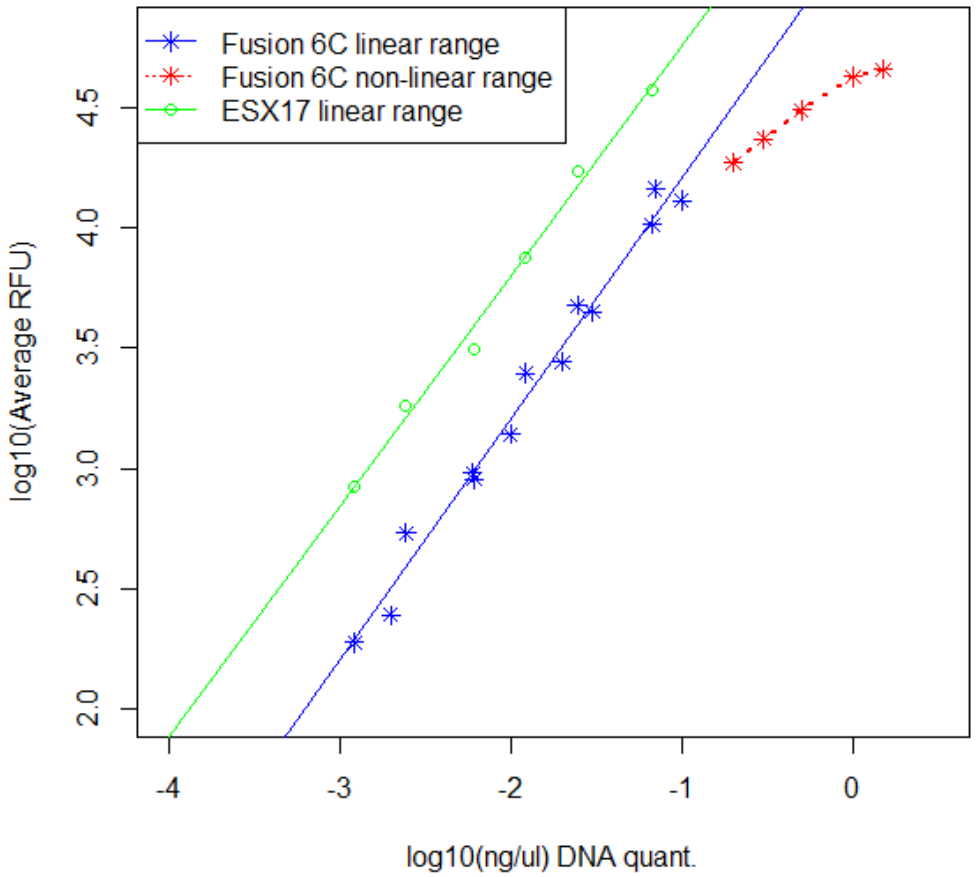
The amount of DNA represented by an STR multiplex is always over-estimated

An alternative method that uses the average RFU recovery of the multiplex

- Plot the average RFU recovery per locus for known quantities of control **(undegraded)** DNA Log log plots are linear and the regression coefficient=1
- Hence the relationship of quant vs. ave RFU is very easy to establish from the regression intercept coefficient (a)

$$Q = \frac{\overline{RFU}}{a}.$$

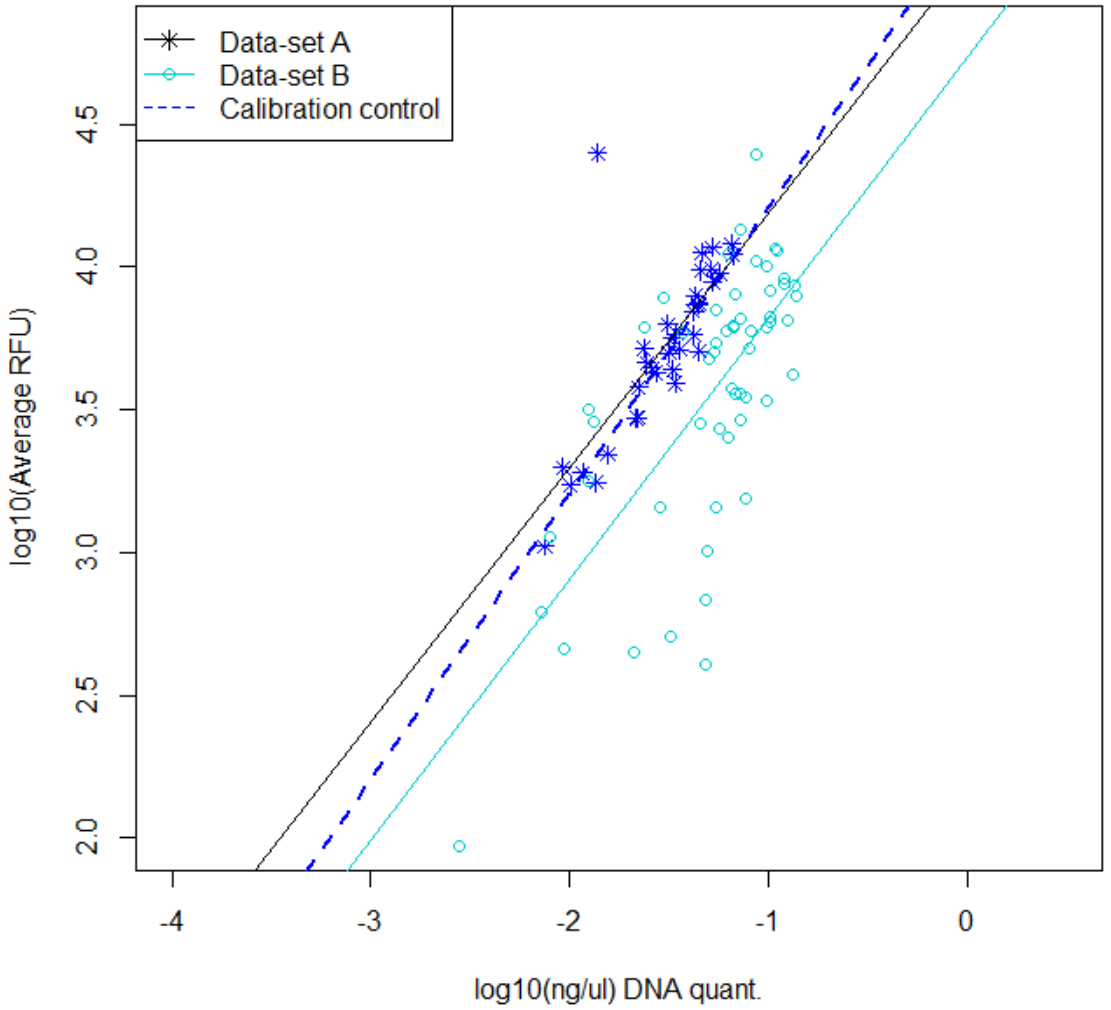
Calibration plots: Easy to generate



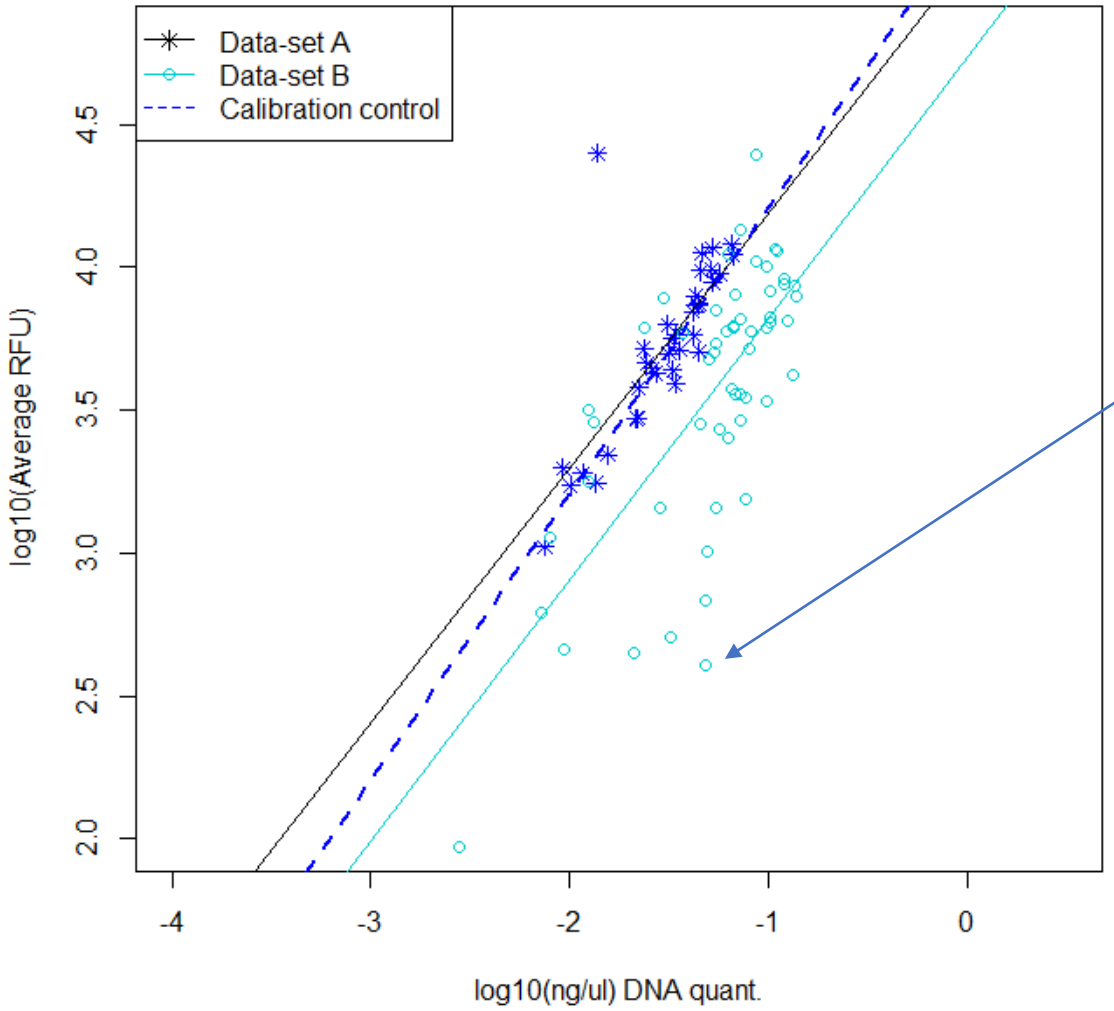
The quant value is from the Q_{81} Powerquant fragment

A comparison of two experiments where DNA is degraded

- So now we plot the data for degraded DNA – note the spread of data
- Set A: Fusion 6C – 158 samples of vaginal mucosa and epithelial cells
- Set B: Fusion 6C – 118 samples of epithelial cells from necks of simulated ‘victim’ assaults
- Plot log Powerplex Quant values vs log ave RFU values
- Interpret relative to the calibration control line



A closer look



According to Powerquant, the DNA quantity is 0.06ng/ul

But, the RFU estimate is much lower at 0.002ng/ul

The RFU method is based on the amount of amplifiable DNA present in the sample, rather than the total DNA >81 base pairs

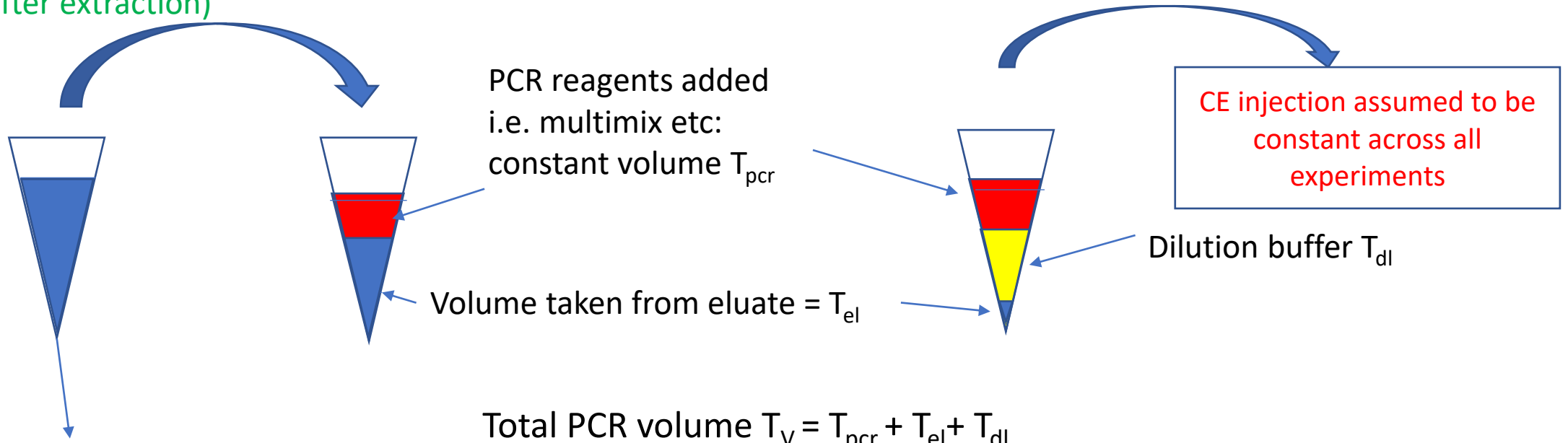
qPCR will always underestimate the DNA quant and this can be as much as two orders of magnitude

Calculation of dilution factor

(1) Elution Volume (E_V)
(the volume after extraction)

(2) PCR (no dilution of elution)

(3) PCR (with dilution of elution)



PCR reagents added
i.e. multimix etc:
constant volume T_{pcr}

CE injection assumed to be constant across all experiments

Dilution buffer T_{dl}

Volume taken from eluate = T_{el}

x ul taken for quantification
and the concentration is calculated as
C=ng/ul

Total PCR volume $T_V = T_{pcr} + T_{el} + T_{dl}$

Note that T_V should be constant for all experiments

Dilution factor = $T_{el} + T_{dl} / T_{el}$

RFU based measurement

- Quantitative measurement based upon the mean RFU per locus
- Calculated by summing the RFU values across the DNA profile and dividing by the number of loci (n)
- We only take account of the contribution of the POI, hence for mixtures it is necessary to calculate the mixture proportion (M_x) using probabilistic genotyping software

$$RFU_{POI} = M_x \times \frac{RFU_{tot}}{n} \times d_l$$

Where d_l is a dilution factor (if the sample is diluted before loading then the RFU_{POI} must be adjusted accordingly)

- This means that we generate virtual RFU values (*not the observed values*)

The quantity of amplifiable (degraded) DNA can only be estimated from RFU values

- To calculate the amount of amplifiable DNA, the calibration coefficient (*a*) is required, along with the elution volume (E_V)

Adjusted by the dilution factor to represent RFU/ul

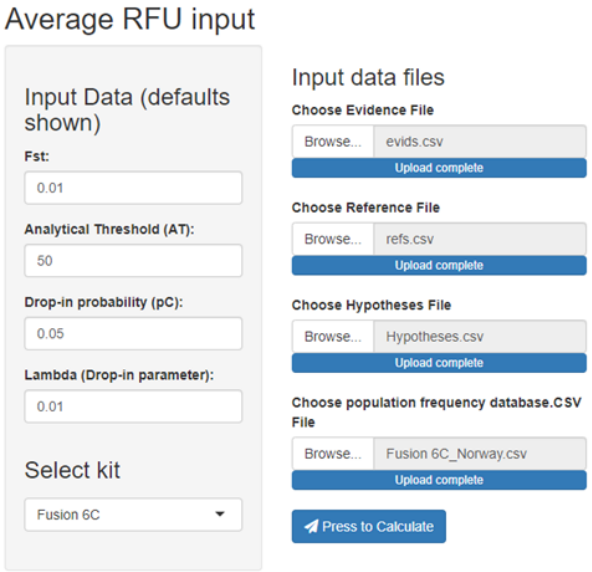
$$Q_{tot} = \frac{\overline{RFU}}{a} \times E_V$$

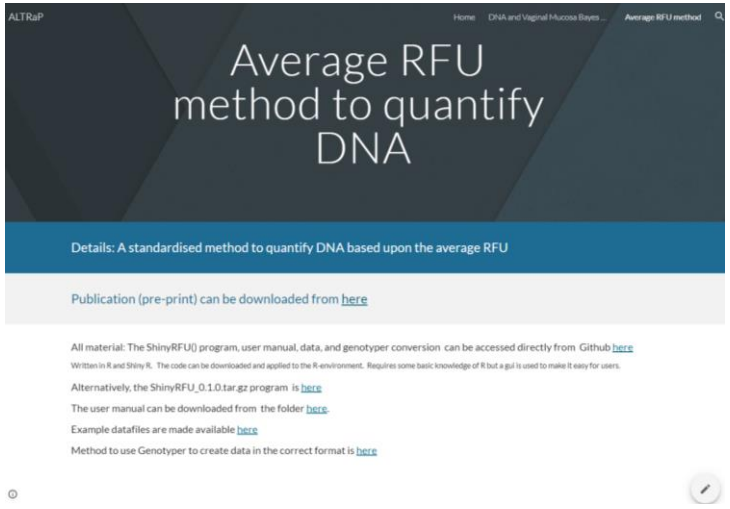
- This formula gives the <u>total</u> amount of DNA recovered, i.e. we compensate for different elution volumes and dilution factors
- Then we calculate the quantity of DNA attributed to a POI by multiplying Q_{tot} x M_{xPOI}
- *Where M_x is the mixture proportion from probabilistic genotyping*

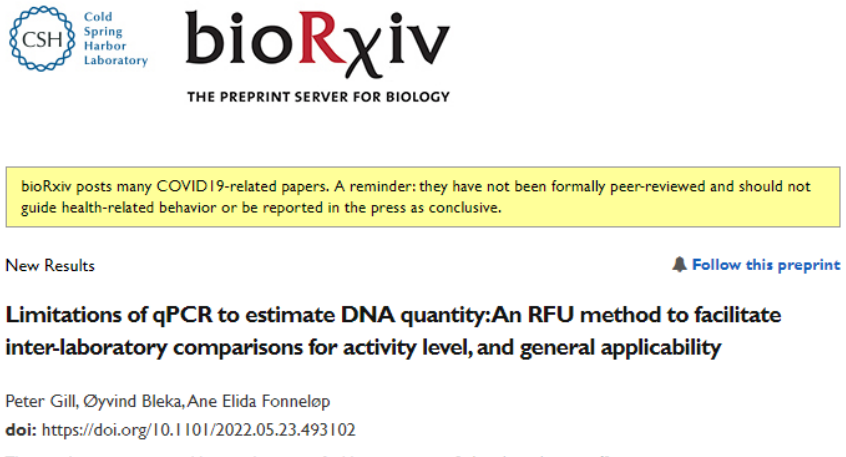
Automation of the calculations

- It is quite time consuming to carry out the necessary calculations, hence software is preferable.
- We have developed a ‘Shiny’ application called ShinyRFU()
- This program takes basic information and calculates average RFU values along with Mx values (based on EuroForMix), which are plugged into another spreadsheet that contains the dilution factor information

https://sites.google.com/view/altrap/average-rfu-method







Summary

- qPCR greatly overestimates DNA quant values for degraded DNA and can only be used as a rough indication of quantity where the purpose is to provide a prior indication of how much sample to load on CE
- If we want an accurate measurement of amplifiable DNA present, then this is obtained from the RFU measurements of the multiplex used
- Calibration is needed – easily carried out with c. 10 samples
- Method utilises probabilistic genotpying to estimate proportions of DNA recovery for specific contributors
- Can be used for:
  - Findings given activity level propositions
  - Rapid DNA
  - Direct PCR
- Programmed solutions to simplify the method

EDNAP/ENFSI Meetings Lisbon, Portugal Sep 27-30 2022

Forensic DNA Phenotyping - VISAGE and INFER

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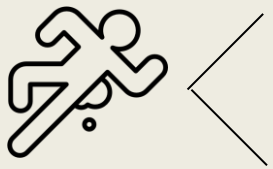
assoc. Prof. Institute of Legal Medicine, Medical University of Innsbruck, Austria

adj. Prof. Forensic Science Program, Penn State University, PA, USA

walther.parson@i-med.ac.at

Forensic DNA Phenotyping

Predictive analysis of externally visible traits (**appearance**), bio-geographic **ancestry** and **age** from the DNA of an unknown sample



No suspect

No DNA database match

We have:

- Extracted DNA
- DNA quants
- STR profile
- Amelogenin
- Single source/ mixture

Aim: provide investigative leads to reduce the pool of possible suspects

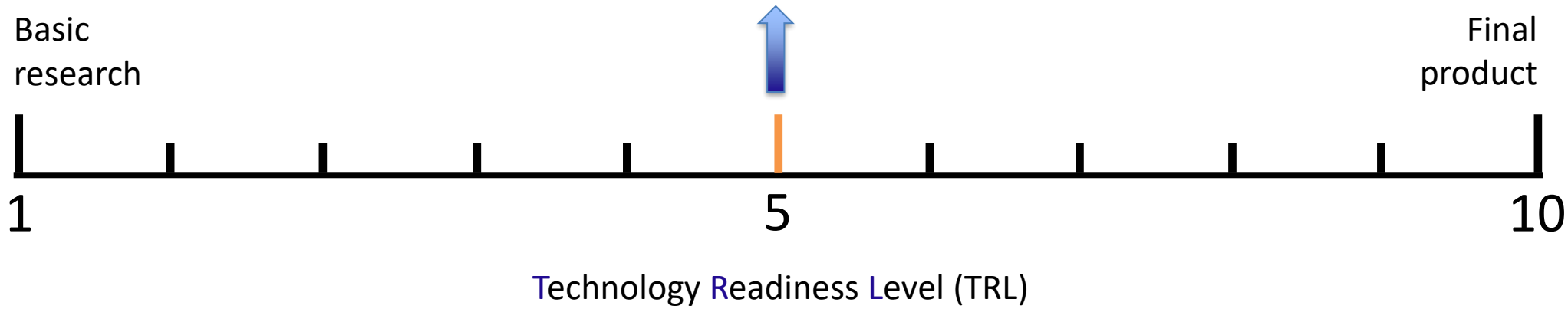
VISAGE
VISIBLE ATTRIBUTES THROUGH GENOMICS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

VISAGE (**VIS**ual **A**ttributes Through **GE**nomics)

We responded to a call of the **EU Horizon 2020** Work Program Secure Societies (SEC), Sub call: Fight against crime and terrorism, Forensics techniques on: a) trace qualification, and b) broadened use of DNA, TRL = 5.

Develop, validate and implement
genotyping and **statistical** prototype tools
for predicting **appearance**, **ancestry**, and **age**
from DNA traces
Study its **ethical**, **societal** & **regulatory** dimensions.



VISAGE
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VISAGE Working Packages (2017 – 2022)

WP1 MANAGEMENT

☑ WP2 MARKER DISCOVERY

☑ WP3 PROTOTYPE ANALYSIS TOOL DEVELOPMENT AND VALIDATION

WP4 STATISTICAL PREDICTION MODELLING AND SOFTWARE DEVELOPMENT

WP5 ETHICAL, SOCIETAL AND REGULATORY DIMENSION MAPPING

WP6 IMPLEMENTATION OF PROTOTYPE TOOLS IN RELEVANT ENVIRONMENT

WP7 EDUCATION AND TRAINING

VISAGE
VISIBLE ATTRIBUTES THROUGH GENOMICS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

Marker Discovery for Basic Prototype Tools

Appearance (M. Kayser & Team), **Ancestry** (C. Phillips & Team), **Age** (W. Branicki & Team)

Basic Tools

Appearance

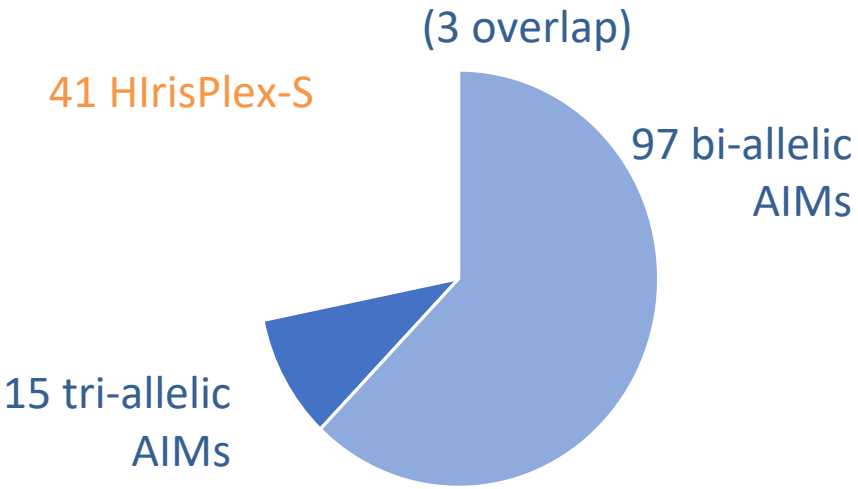
HIrisplex-S (41 SNPs): Chaitanya et al 2018 ***FSIG***
Single Base Extension, MPS

Ancestry

EFN + Kidd + TFS PID: 112 AIMs: Puente et al 2021 ***GENES***
Single Base Extension, MPS

Age

Blood (5 genes): Zbiec-Piekarska et al 2015 ***FSIG***
Pyrosequencing



153 SNP loci

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Marker Discovery for Predicting Appearance and Ancestry

Appearance (M. Kayser & Team), **Ancestry** (C. Phillips & Team)

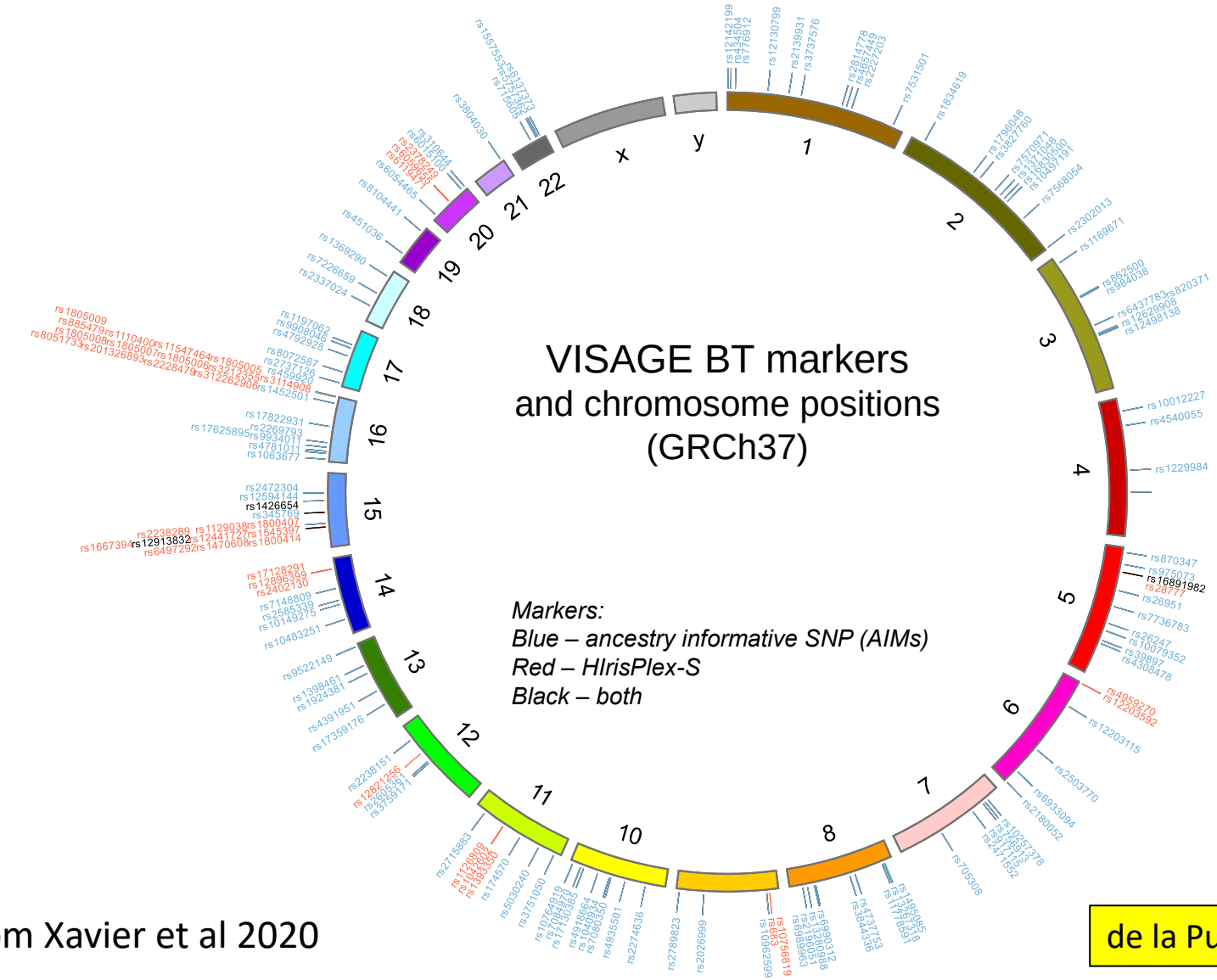


Fig. from Xavier et al 2020

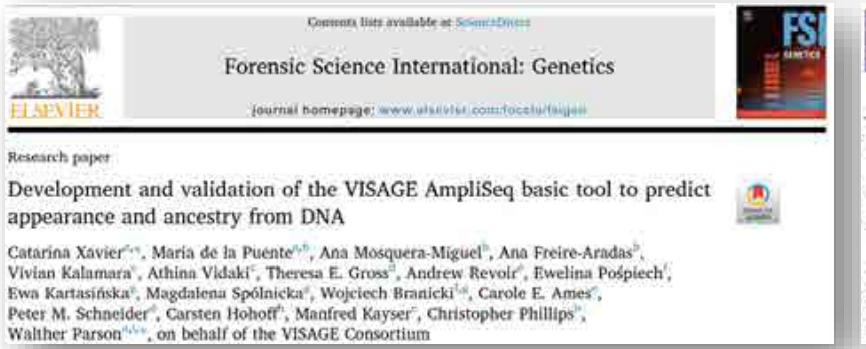
de la Puente et al (2022) ***GENES***

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

Development and Validation of Molecular Genetic Prototype Tools

W. Parson, C. Xavier, A. Heidegger, L. Palencia Madrid & Team

VISAGE Basic Tools to predict Appearance and Ancestry



Xavier et al (2020) ***FSIG***
AmpliSeq/Ion S5

Palencia-Madrid et al (2020) ***GENES***
PowerSeq/MiSeq FGx

Xavier et al (2022) ***GENES***
ForenSeq/MiSeq FGx

VISAGE

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

Marker selection for Basic AGE Prototype Tools

W. Parson, A. Heidegger, C. Xavier & Team

Appearance

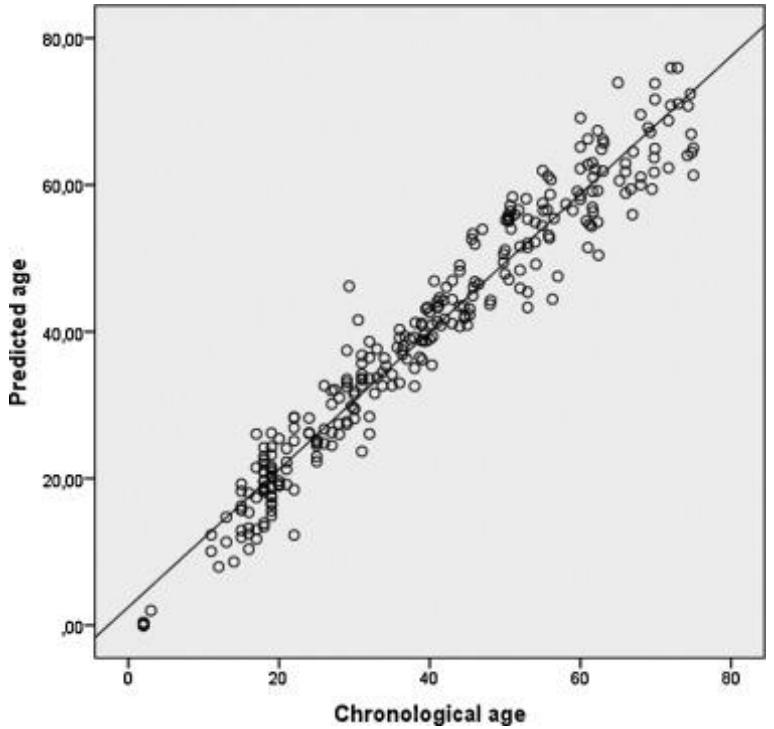
HIrisplex-S (41 SNPs): Chaitanya et al 2018 ***FSIG***
Single Base Extension, MPS

Ancestry

EFN + Kidd + TFS PID: 112 AIMs: Puente et al 2021 ***GENES***
Single Base Extension, MPS

Age

Blood (5 genes): Zbiec-Piekarska et al 2015 ***FSIG***
Pyrosequencing



VISAGE
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

Development of Prototype Tool for Age Prediction

VISAGE Basic Tools for Age Prediction in Blood

Forensic Science International: Genetics 48 (2020) 102322

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

Development and optimization of the VISAGE basic prototype tool for forensic age estimation

A. Heidegger[a], C. Xavier[a,*], H. Niederstätter[a], M. de la Puente[a,b], E. Pośpiech[c], A. Pisarek[c], M. Kayser[d], W. Branicki[c,e], W. Parson[a,f,*], on behalf of the VISAGE Consortium

Heidegger et al (2020) ***FSIG***

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

Marker Discovery for Enhanced Prototype Tools

Appearance (M. Kayser & Team), **Ancestry** (C. Phillips & Team), **Age** (W. Branicki & Team)

Basic Tools

Appearance

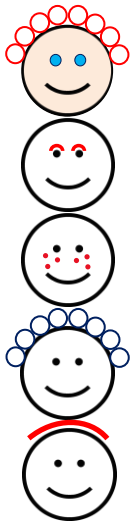
Hirisplex-S: Chaitanya et al 2018 ***FSIG***

Ancestry

112 AIMs: Puente et al 2022 ***GENES***

Age

Blood: Zbiec-Piekarska et al 2015 ***FSIG***



Enhanced Tools

Appearance

Chaitanya et al 2018 ***FSIG****:* Hirisplex-S

Peng et al 2019 ***JID****:* eyebrow color

Kukla-Bartoszek et al 2019 ***FSIG****:* freckles

Pośpiech et al 2018 ***FSIG****:* head hair shape

Chen et al ***in press****:* head hair loss in men

Ancestry

X-, Y-, aAIMs*:* subcont. ancestry; Manuscript ***submitted***

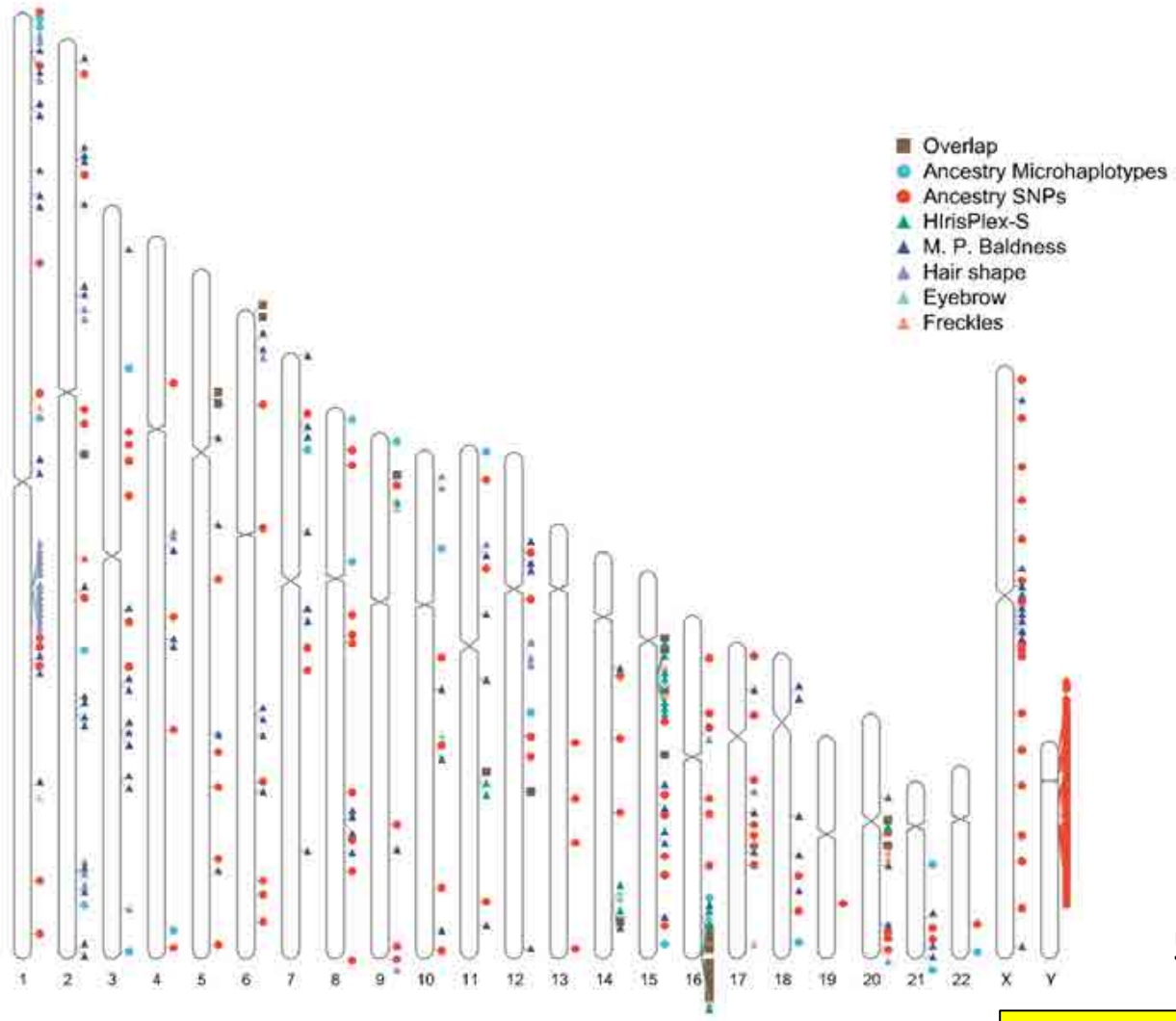
Age

Blood/saliva/bone: Wozniak et al 2021 ***AGING***

Semen: Pisarek et al 2021 ***AGING***

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

Development of VISAGE Enhanced Tool for App/Anc Prediction



523 loci

Fig. from Xavier et al accepted

Ruiz-Raminez et al in submission

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

VISAGE Enhanced Tool for Age Prediction in Blood/Buccal cells/Bone

www.aging-us.com AGING 2021, Vol. 13, Advance

Research Paper

Development of the VISAGE enhanced tool and statistical models for epigenetic age estimation in blood, buccal cells and bones

Anna Woźniak[1,*], Antonia Heidegger[2,*], Danuta Piniewska-Róg[3,*], Ewelina Pośpiech[4], Catarina Xavier[2], Aleksandra Pisarek[4], Ewa Kartasińska[1], Michał Boroń[1], Ana Freire-Aradas[5], Marta Wojtas[3], Maria de la Puente[2,5], Harald Niederstätter[2], Rafał Płoski[6], Magdalena Spólnicka[1], Manfred Kayser[7], Christopher Phillips[5], Walther Parson[2,8], Wojciech Branicki[1,4], VISAGE Consortium

Correspondence to: Wojciech Branicki, Walther Parson; **email:** wojciech.branicki@uj.edu.pl, walther.parson@i-med.ac.at

Keywords: DNA methylation, bisulfite amplicon MPS, epigenetic age prediction tool, age prediction in blood and buccal cells, age prediction in bones

Received: January 10, 2021 **Accepted:** February 16, 2021 **Published:**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

VISAGE Enhanced Tool for Age Prediction in Blood/Buccal cells/Bone

Tissue	KLF14[a] Chr 7	TRIM59[a] Chr 5	MIR29B2CHG[a] Chr 1	FHL2[a] Chr 2	ELOVL2[b] Chr 6	EDARADD[b] Chr 1	ASPA[c] Chr 17	PDE4C[c] Chr 19
Size	128bp	141bp	146bp	167bp	267bp	193bp	108bp	215bp
Blood	✓	✓	✓	✓	✓			✓
Buccal	✓		✓		✓	✓		✓
Bone	✓				✓ ✓		✓	✓ ✓

[a]Zbiec-Piekarska et al (2015) ***FSIG***
[b]Bekaert et al (2015) ***Electrophoresis***
[c]Wozniak, Heidegger et al (2021) ***Aging***

Wozniak, Heidegger et al (2021) ***Aging***
MiSeq FGx

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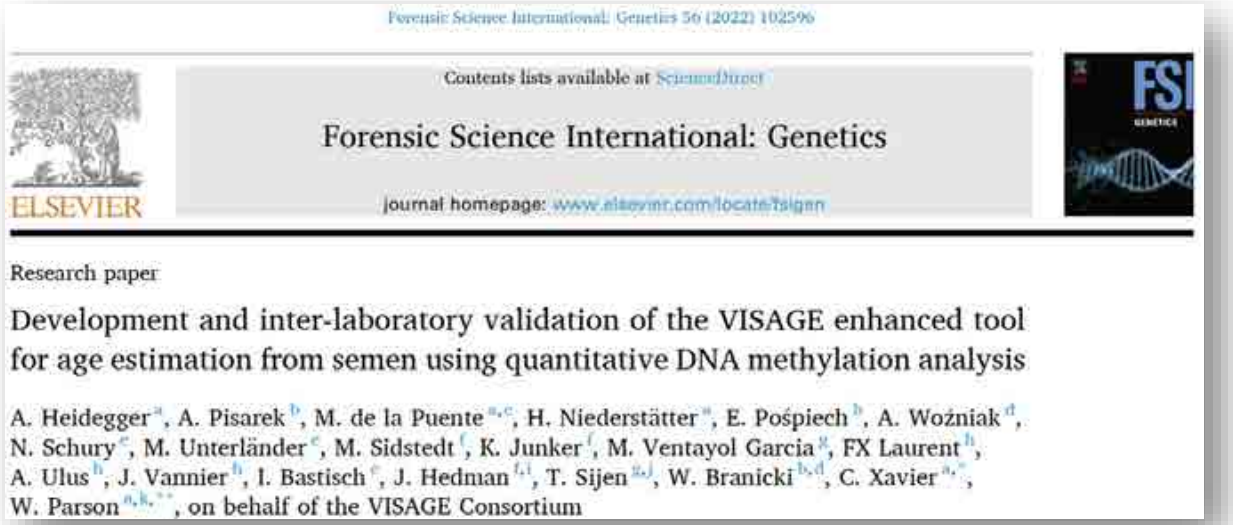
VISIBLE ATTRIBUTES THROUGH GENOMICS
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 740580.

VISAGE Enhanced Tool for Age Prediction in Semen



Forensic Science International: Genetics 56 (2022) 102596

Contents lists available at ScienceDirect

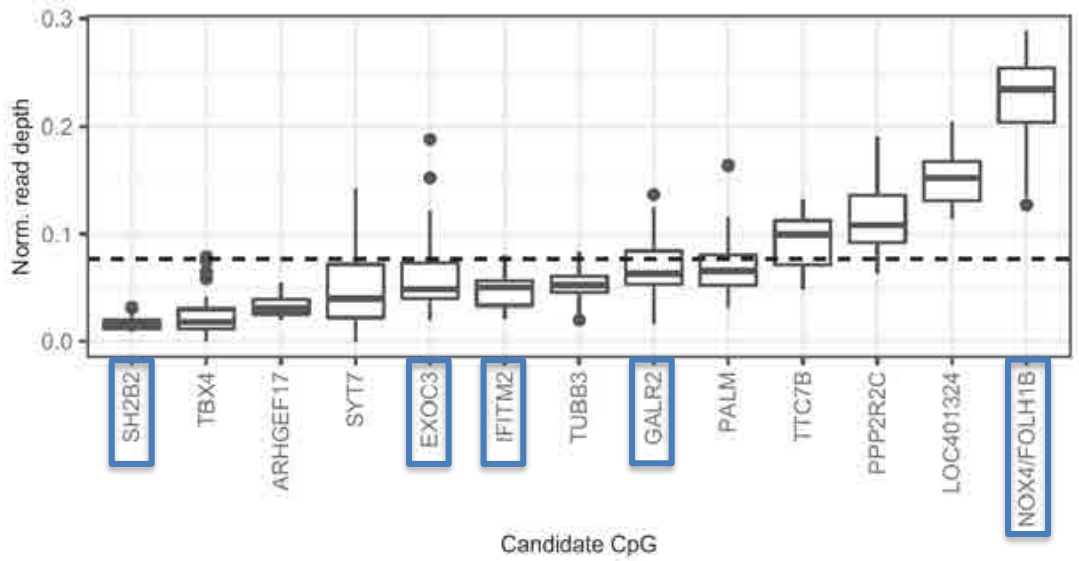
Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

Development and inter-laboratory validation of the VISAGE enhanced tool for age estimation from semen using quantitative DNA methylation analysis

A. Heidegger [a], A. Pisarek [b], M. de la Puente [a,c], H. Niederstätter [a], E. Pośpiech [b], A. Woźniak [d], N. Schury [e], M. Unterländer [e], M. Sidstedt [f], K. Junker [f], M. Ventayol Garcia [g], FX Laurent [h], A. Ulus [h], J. Vannier [h], I. Bastisch [c], J. Hedman [f,i], T. Sijen [g,j], W. Branicki [b,d], C. Xavier [a,*], W. Parson [a,k,**], on behalf of the VISAGE Consortium



Heidegger et al (2022) ***FSIG***
MiSeq FGx

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Objectives accomplished

The overall aim of VISAGE is/was to broaden the forensic use of DNA towards constructing composite sketches of unknown perpetrators from as many biological traces and sources and as fast as possible within current legal frameworks and ethical guidelines. Throughout its project time 2017-2021, the VISAGE Consortium has successfully addressed and fully accomplished its six objectives, as summarized below. The below mentioned references and reports can be found with their respective links to the open-access publications on the Scientific Publications and Reports parts of the VISAGE website.

Objective 1: Allocate previous and establish new DNA predictors for as detailed as possible information on appearance, age and ancestry.

Objective 1 was successfully addressed by work in workpackage 2 (WP2) led by Erasmus MC for appearance, USC for ancestry, and JU for age. In the early phase of the project, previously established DNA markers for appearance for 3 traits, ancestry for 5 continental regions (in part with newly established markers within VISAGE: De la Puente et al. 2021), and age from blood-derived DNA were ascertained, and delivered to MUI for developing the prototype VISAGE Basic labtools for appearance, ancestry and age in WP3. In parallel, new DNA markers were successfully discovered within the project via different approaches for i) additional six appearance traits as we described in several scientific publications (Xiong et al. 2019, Peng et al. 2019, Liu et al. 2019, Kukla-Bartoszeka et al. 2019, Chen et al. submitted) including one trait from earlier work of some VISAGE partners prior to VISAGE (Pospiech et al. 2018), ii) ancestry based on 7 continental regions (Phillips et al. in preparation) together with paternal ancestry from multiple regions, and iii) age from DNA of somatic tissues (Wozniak et al. 2021; Piniewska-Rog et al. 2021) as well as age from DNA of semen (Pisarek et al. 2021, Heidegger et al. 2021). These newly established DNA predictors for appearance, ancestry, and age, except those for two traits, and together with the previously established DNA predictors for the three appearance traits used in the prototype VISAGE Basic tool, were all delivered to MUI for developing the prototype VISAGE Enhanced labtools for appearance, ancestry and age in WP3. Staistical prediction modelling was done together with WP4 and the established prediction models were included in the statistical framework and prototype software developed in WP4. Objective 1 was fully accomplished within the project time.

Objective 2: Develop and forensically validate prototype tool(s) based on massively parallel sequencing (MPS) for simultaneously analysis of the identified DNA predictors of appearance, age and ancestry suitable for trace DNA.

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Scientific Publications

Review / Opinion articles

- Schneider PM, Prainsack B, Kayser M. The use of Forensic DNA Phenotyping in predicting appearance and biogeographic ancestry. Deutsches Arzteblatt International 2019;116:873-880.
- Vidaki A and Kayser M. Recent progress, methods and perspectives in forensic epigenetics. Forensic Science International: Genetics 2018;37:180-195.
- Parson W. Age estimation with DNA: from forensic DNA fingerprinting to forensic (epi)genomics: a mini-review. Gerontology. 2018;64(4):326-332.
- Vidaki A and Kayser M. From forensic epigenetics to forensic epigenomics: broadening DNA investigative intelligence. Genome Biol. 2017;18:238 .

Original research articles

- Gabrielle Samuel and Barbara Prainsack (2021). Shifting Ethical Boundaries in Forensic Use of DNA. Jahrbuch für Wissenschaft und Ethik 24/1: 155-172.
- Heidegger A, Pisarek A, de la Puente M, Niederstätter H, Pospiech E, Wozniak A, Schury N, Unterländer M, Sidstedt M, Junker K, Ventayol Garcia M, Lauren FX, Ulus A, Vannier J, Bastisch I, Hedman J, Sijen T, Branicki W, Xavier C, Parson W on behalf of the VISAGE Consortium. Development and inter-laboratory validation of the VISAGE enhanced tool for age estimation from semen using quantitative DNA methylation analysis. Forensic Science International: Genetics. 2021, 56: 102596
- de la Puente M, Ruiz-Ramírez J, Ambroa-Conde A, Xavier C, Pardo-Seco J, Álvarez-Dios J, Freire-Aradas A, Mosquera-Miguel A, Gross TE, Cheung EYY, Branicki W, Nothnagel M, Parson W, Schneider PM, Kayser M, Carracedo Á, Lareu MV, Phillips C, on behalf of the VISAGE Consortium. Development and evaluation of the ancestry informative marker panel of the VISAGE Basic Tool. Genes. 2021, 12(8):1284
- Pisarek A, Pośpiech E, Heidegger A, Xavier C, Papież A, Piniewska-Róg D, Kalamara V, Potabattula R, Bochenek M, Sikora-Polaczek M, Macur A, Woźniak A, Janeczko J, Phillips C, Haaf T, Polańska J, Parson W, Kayser M, Branicki W on behalf of the VISAGE Consortium. Epigenetic age prediction in semen - marker selection and model development. Aging. 2021, 13(5):19145-19164
- Piniewska-Róg D, Heidegger A, Pośpiech E, Xavier C, Pisarek A, Jarosz A, Wozniak A, Wojtas M, Phillips C, Kayser M, Parson W, Branicki W on behalf of the VISAGE Consortium. Impact of excessive alcohol abuse on age prediction using the VISAGE enhanced

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Reports

Report: Report on Three International Expert Symposia Disseminating the Results of the VISAGE Project (2021)

Report: Regulatory landscape of forensic DNA phenotyping in Europe (2018)

Deliverable_5.2: Societal, ethical, and regulatory dimensions of forensic DNA phenotyping.

Report: Recommendations to address the ethical and societal challenges of FDP.

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INFER - Introduction of forensic genomic tools for estimating Appearance, Ancestry and Age

ISF-IZ25-5793-2019-40

INFER Meeting Innsbruck Jan 2020

Evaluation, Validation and Implementation of Forensic DNA Phenotyping Tools

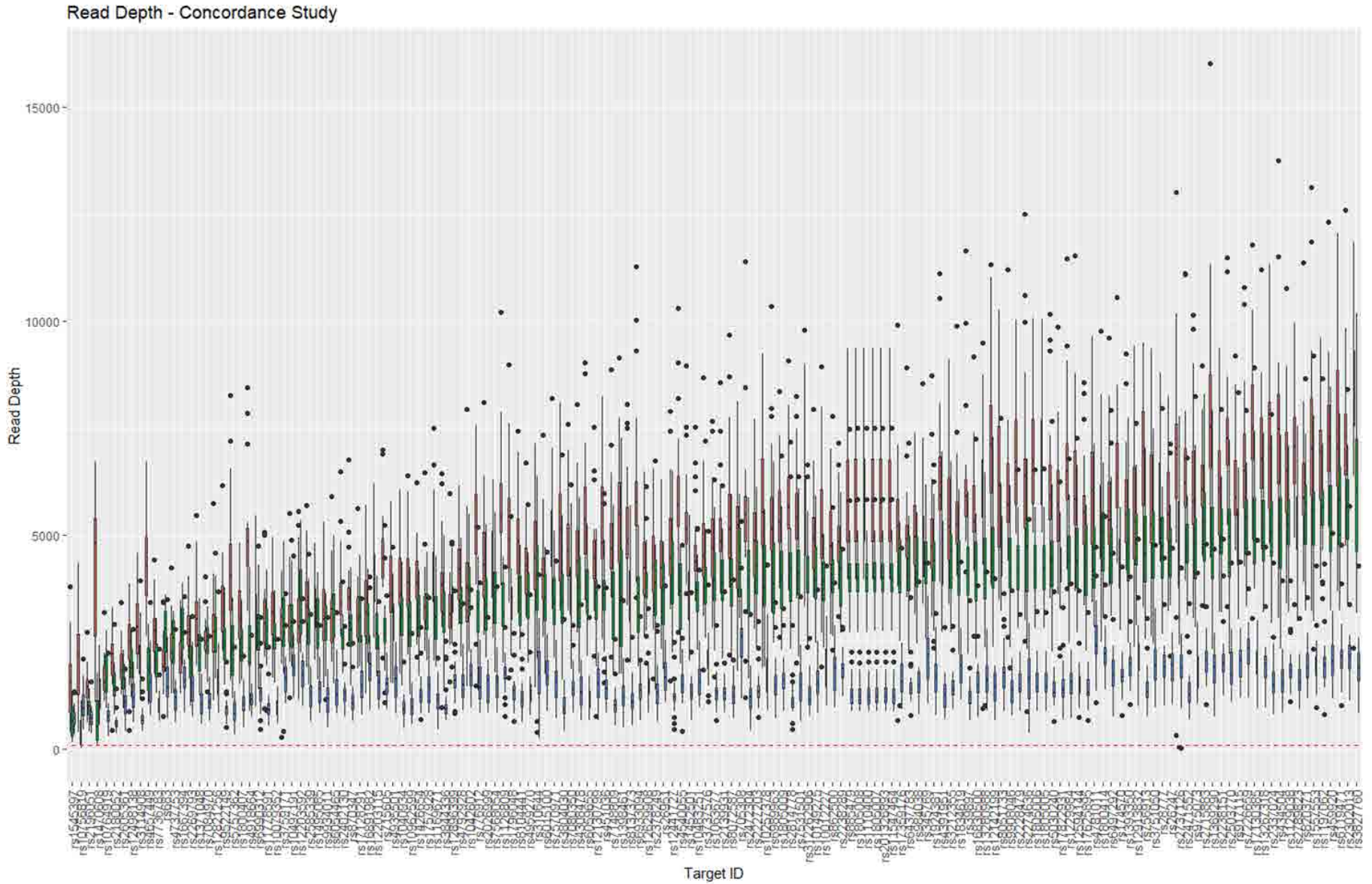
Larger sample-set including blood, buccal, saliva and semen samples

Non-European samples

Casework samples

This project has received funding from the European Union under grant agreement No IZ25-5793-2019-40

INFER - Predicting App/Anc with VISAGE BT



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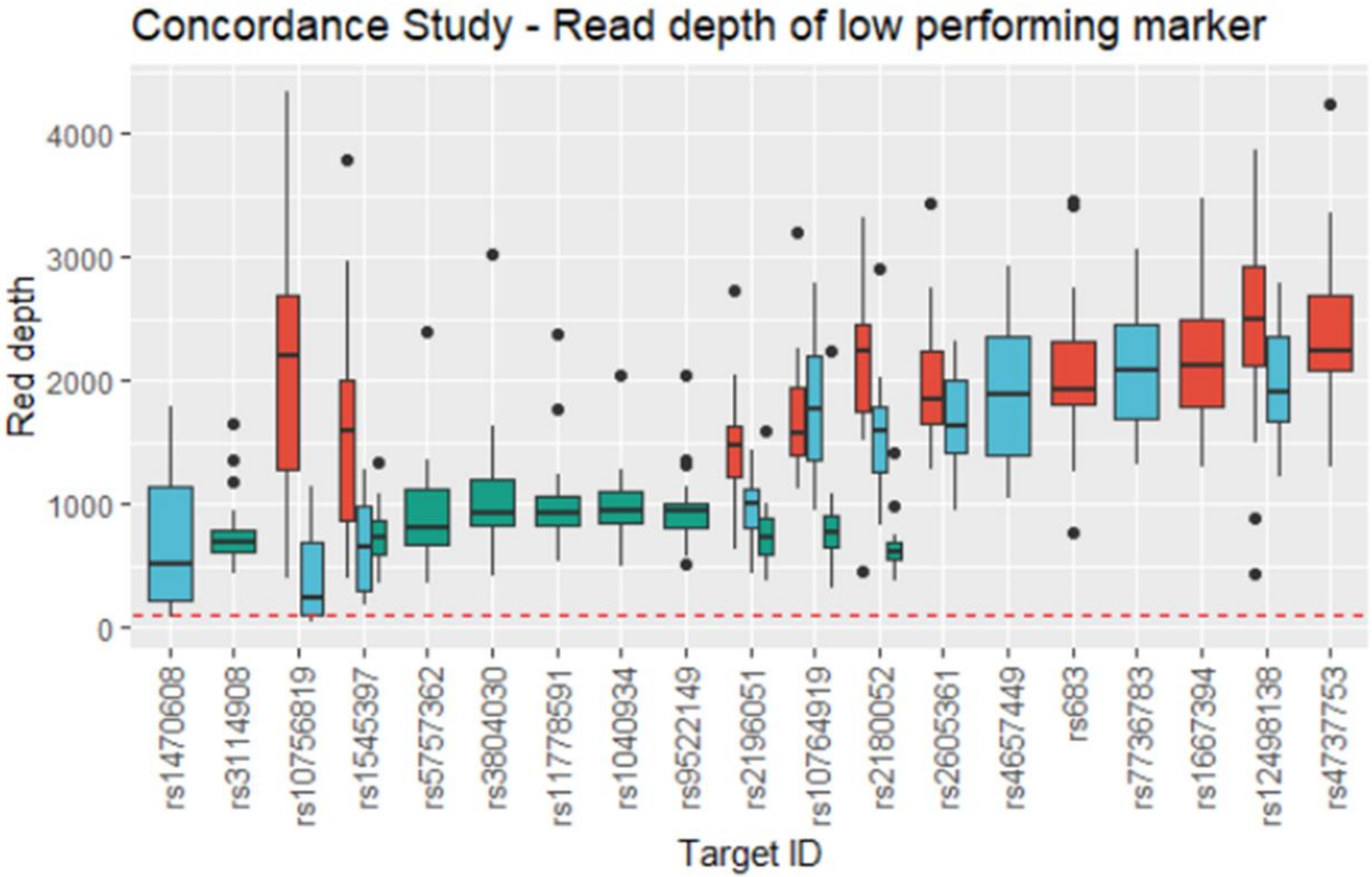
cc Lena Ewers, GMI

INFER - Predicting App/Anc with VISAGE BT



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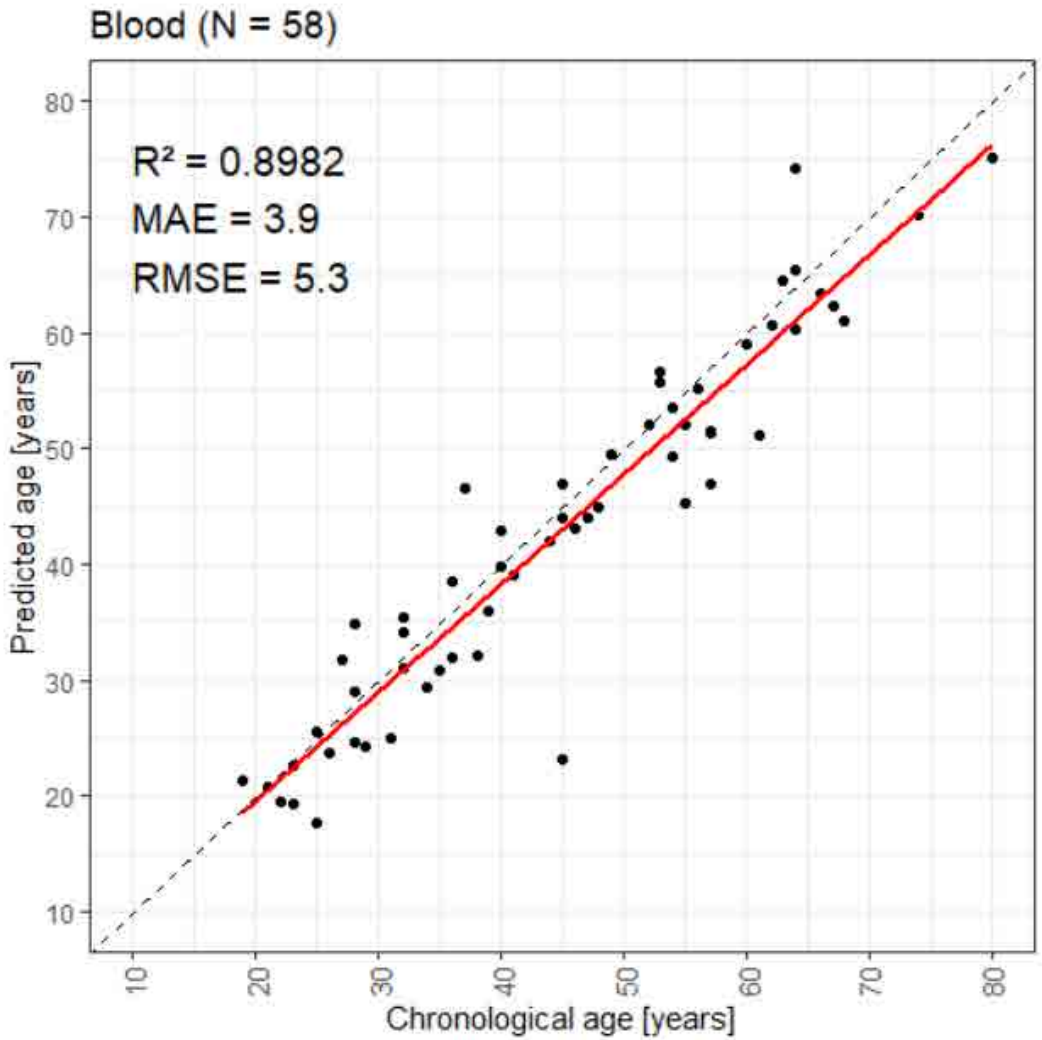
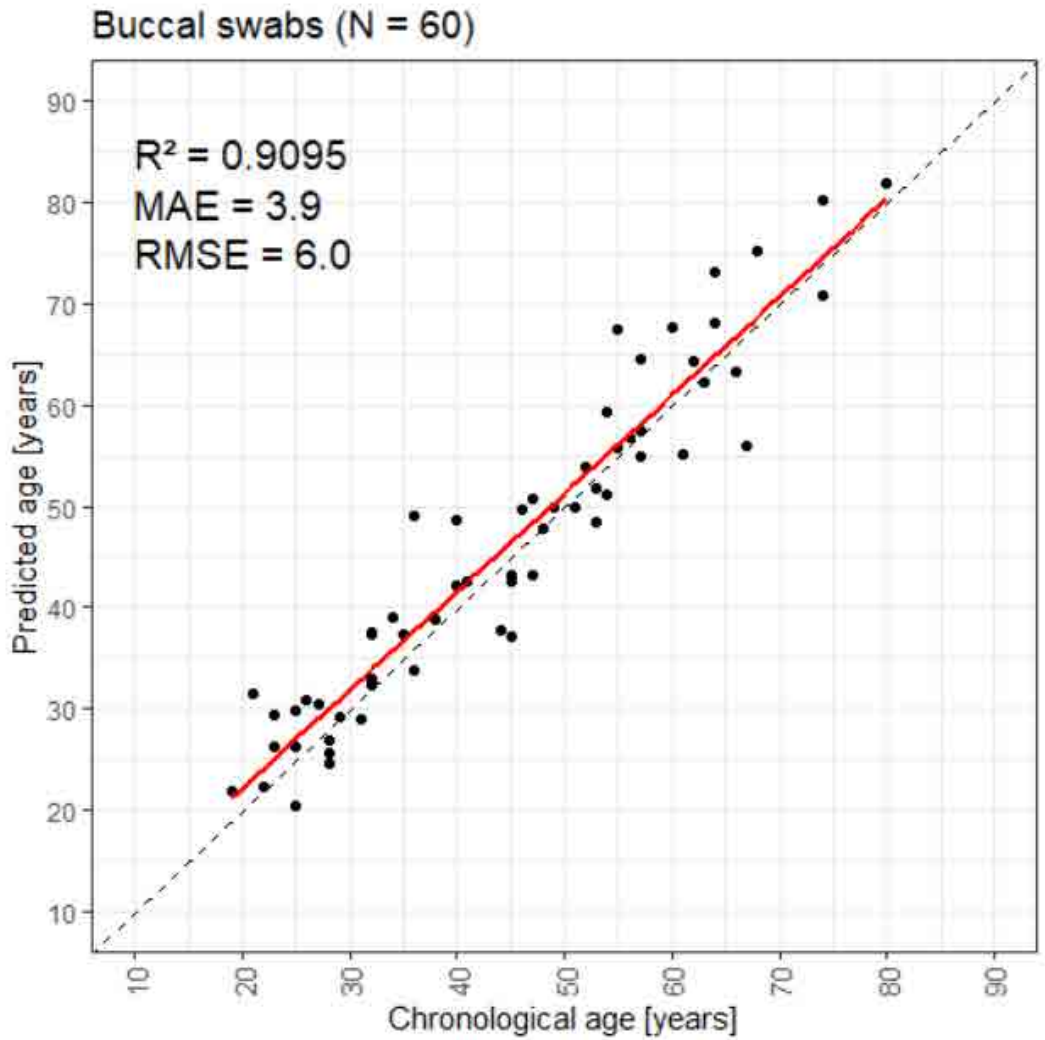
cc Lena Ewers, GMI

INFER - Predicting AGE with VISAGE ET Somatic





INFER; 3 labs; DNA input 20 ng; ~60 samples/flow cell

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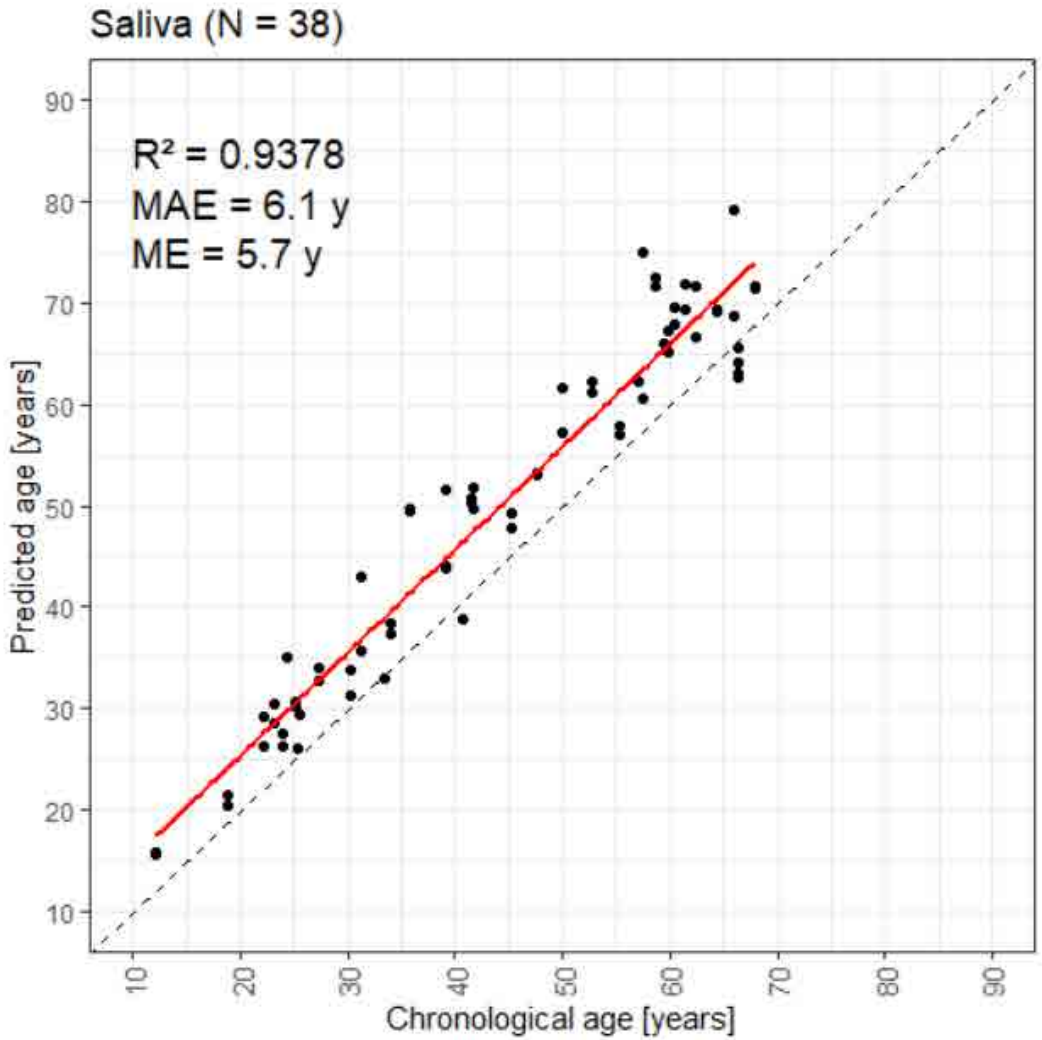
INFER - Predicting AGE with VISAGE ET Somatic



INFER; DNA input 50 ng; ~60 samples/flow cell

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