EUROPEAN DNA PROFILING GROUP (EDNAP) MEETING

Copenhagen, Denmark

28 April 2015

Host: Niels Morling
Chairman: Niels Morling.

A list of participants is attached.

Welcome
Professor Niels Morling welcomed members to Copenhagen.

Update on exercises

mRNA exercises Cordula Haas
The results of mRNA exercise 6 (skin and contact traces) has been published. After thorough investigations, it was decided not to perform the planned collaborative exercise concerning quantification of mRNA (presentation attached).

EDNAP ancestry informative marker exercise Chris Phillips
The manuscript with the results of the Ancestry Informative Marker (AIM) exercise has been reviewed. Christopher Philips, University of Santiago de Compostela (USC), is revising the manuscript.

A SNaPshot based method targeting18 common mtDNA mutations Arnoud Kal
Arnoud Kal presented the plans for the collaborative EDNAP exercise concerning typing of 18 mtDNA SNPs with the SNaPshot method (presentation attached). Titia Sitien and Arnoud Kal will send out reagents in the spring 2015. Participants are asked to submit the results before 15 September 2015.

Updates from other groups

ENFSI guideline for the formulation of evaluative reports in forensic science Niels Morling
The guidelines have been published.

Forensic Science Regulator, OK Gillian Tully
Gillian Tully gave an overview of the work of the Forensic Science Regulator (presentation attached).

EMPOP Walther Parson
Walther Parson gave an update on EMPOP-related publications, the database developments and the next EMPOP-related meetings. Four mtDNA articles have been published since the last meeting in Zürich. The EMPOP query engine is now capable of taking more complex phylogenetic events into consideration, i.e. insertions and deletions that simultaneously involve more than one nucleotide, e.g. 523del 524del (presentation attached).

Nomenclature of STR sequences Walther Parson

Walther Parson gave an update on the discussion of the nomenclature of STR sequences that are being produced in large numbers using massively parallel sequencing. The ISFG is not yet ready to formulate recommendations. The issue is being discussed in the forensic genetic community. A round table on STR nomenclature will take place at the upcoming ISFG conference in Krakow to discuss the different approaches.

High quality STR sequence database Walther Parson

Walther Parson and colleagues have updated STRbase (presentation attached). The system will be adapted for quality control of STR data and will most likely be used for the reviewing process of FSI Genetics. A new name for the database is needed to avoid confusion with NIST STRbase. "STRIDER", STR for Identity ENFSI Reference Database, has been suggested (presentation attached).

Interpol Richard Scheithaur

Richard Scheithaur gave a short summary of the DNA activities of Interpol.

EUROFORGEN-NoE – General update Peter Schneider

Peter Schneider gave an update concerning the project (attached).

EUROFORGEN-NoE - EuroForMix Peter Gill

Peter Gill introduced the EuroForMix software for the interpretation of results of investigations of DNA mixtures with 'continuous models'. The software is freely available R-package (two presentations attached).

Interpretation and communication of results Peter Gill

Peter Gill discussed the challenges of interpreting DNA results in crime case investigations. The communication of the results to the users was also discussed (presentation attached).

EDNAP web site update (www.isfg.org/EDNAP) Peter Schneider

Members are encouraged to visit the website. Suggestions are welcome.

Future activities Niels Morling

Please see the planned mtDNA exercise above.

Next meeting Niels Morling

The next EDNAP meeting will be held on 20 October 2015 in Santiago de Compostela.

Any other business Niels Morling

There was no other business.

Closing of the meeting

The meeting closed with sincere thanks to Niels Morling and colleagues at the laboratory in Copenhagen.

Attachments are found at the EDNAP website http://www.isfg.org/EDNAP/Meetings:

- List of participants
- Presentations
  - ENFSI guideline for the formulation of evaluative reports in forensic science
  - Peter Gill: EuroForMix (2 presentations)
  - Peter Gill: Interpretation and communication of results

- Cordula Haas: mRNA exercise
- Jodi Irwin: FBI update
- Walther Parson: EMPOP report
- Walther Parson: High quality STR database
- Peter Schneider: EUROFORGEN-NoE report
- Gillian Tully: Forensic Science Regulator, UK
- Maria Vouropoulou: Quantifiler Trio.

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mRNA quantification

Cordula Haas, Erin Hanson, Jack Ballantyne

EDNAP meeting, 28. April 2015, Copenhagen

mRNA profiling workflow

- RNA extraction
- DNase treatment (TURBO DNA-free kit)
- total mRNA quantification
- Reverse transcription (RT)
- body fluid specific PCR-multiplex
- Capillary electrophoresis

→ too little RNA into RT: no result
too much RNA into RT: cross contamination

Total mRNA quantification

- RiboGreen & Qubit (Fluorescence)
- RiboGreen & ELISA-Reader (Fluorescence)
- Bioanalyzer (Chip-Gelelectrophoresis)
- NanoDrop (Absorption A_{260})

Human specific mRNA quant assay - UCF

- developed by Jack Ballantynes group
- Housekeeping gene
- qPCR assay
- TaqMan MGB probe
- qPCR standard

- ➢ human specific
- ➢ abundant in body fluids
- ➢ sensitive

2 ul RNA into RT

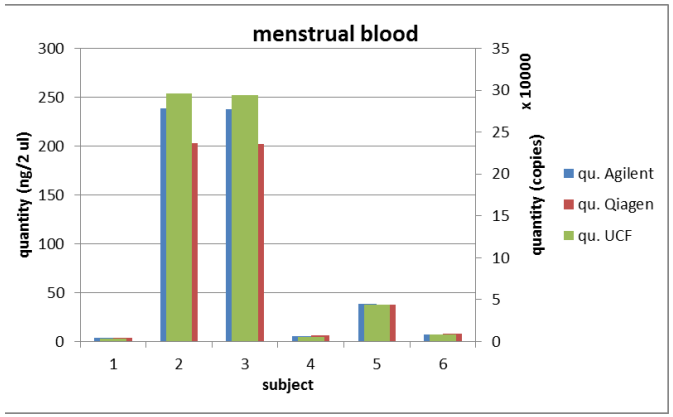
25 ng into RT

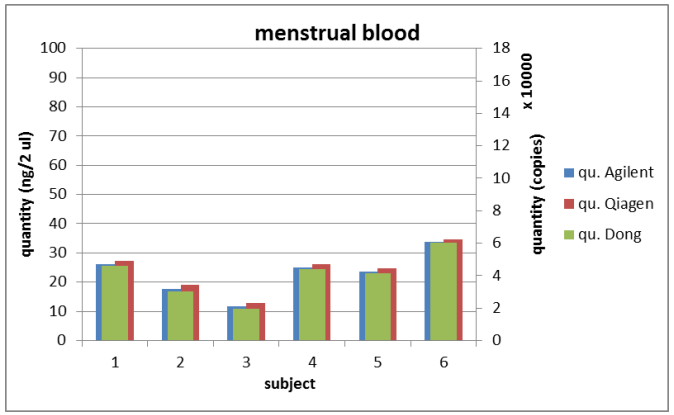
25 ng corresponds to

8.00 ul (1)	8.00 ul (4)
2.24* ul (2)	1.36 ul (5)
2.44* ul (3)	6.38 ul (6)

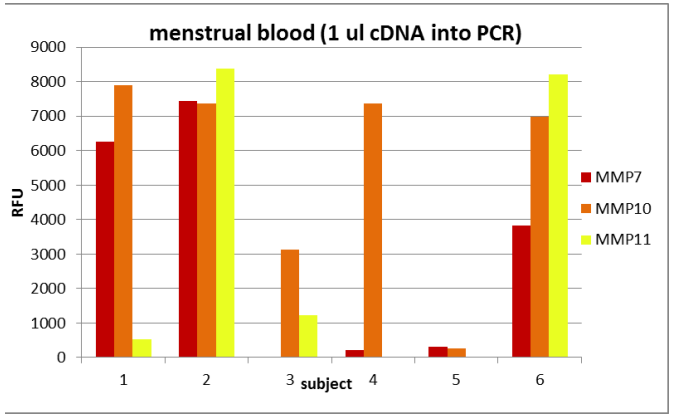
* 1:10 dilution

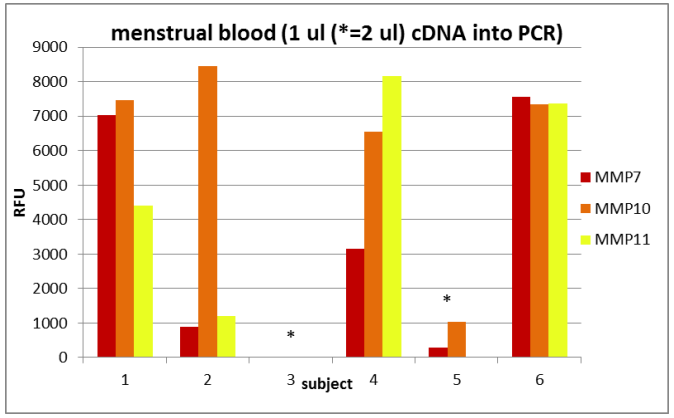
human-specific quantification





MB-specific marker expression





mRNA quantification – way forward?

- Correlation between RNA-concentration (copy numbers) and body fluid specific expression (peak height in RFU) only marginal
- Collaborative exercise?
- Test ‘no quant’ compared to ‘some sort of quant’ (however imperfect with respect to human specificity)?

‘no quant’ compared to ‘some sort of quant’

- 5 saliva and 5 vaginal donors
  saliva samples: 5 ul and 50 ul stains
  vaginal samples: ½ and 1/64 swabs
- Qiagen AllPrep RNA/DNA mini Kit
- RT a set input volume regardless of the quant (2 ul, 8 ul)
  RT a set using a defined total input (15 ng)
- assess the RTs with
  -a body fluid multiplex (including markers for all body fluids)
  -EDNAP vag and saliva triplexes

‘no quant’ compared to ‘some sort of quant’

Saliva

RNA mini Kit

		Qubit	RiboGreen	Quantus
		ng/ul	ng/ul	ng/ul
1	50 ul	2.3	2.9	1.7
1	5 ul	too low	0.7	0.4
2	50 ul	4.0	3.2	2.7
2	5 ul	too low	0.7	0.5
3	50 ul	3.4	4.6	3.4
3	5 ul	too low	0.7	0.6
4	50 ul	4.1	5.6	3.2
4	5 ul	too low	0.6	0.6
5	50 ul	too low	1.3	1.4
5	5 ul	too low	0.2	0.4

Vaginal secretion

AllPrep

		Qubit	RiboGreen	Quantus
		ng/ul	ng/ul	ng/ul
6	1/2	42.2	19.1	16.0
6	1/64	too low	0.6	0.2
7	1/2	too low	0.3	0.1
7	1/64	too low	0.5	0.2
8	1/2	too high	91.7	73.0
8	1/64	33.8	17.1	14.0
9	1/2	98.0	67.9	53.0
9	1/64	22.7	12.4	15.0
10	1/2	11.9	5.9	4.5
10	1/64	4.2	3.4	1.3

‘no quant’ compared to ‘some sort of quant’

Saliva

RNAminiKit

		Quantus	2ul into RT							8ul into RT						
			Saliva				Vag			Saliva				Vag		
		HS	HTN3	HTN1	STATH	MUC7	MYO	CYP	MUC4	HTN3	HTN1	STATH	MUC7	MYO	CYP	MUC4
		ng/ul	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu
1	50 ul	1.7	836	354	1014	1197	0	0	0	4565	344	2720	3826	0	0	0
1	5 ul	0.37	0	0	0	80	0	0	0	120	0	0	132	0	0	0
2	50 ul	2.7	568	147	399	526	0	0	0	2349	635	2275	1103	0	0	0
2	5 ul	0.53	0	0	0	0	0	0	0	167	0	54	95	0	0	0
3	50 ul	3.4	0	63	0	397	0	0	0	623	275	873	1073	0	0	1234
3	5 ul	0.56	0	0	0	94	0	0	0	0	0	0	246	0	0	0
4	50 ul	3.2	0	0	0	160	0	0	0	670	147	747	884	0	0	0
4	5 ul	0.55	0	0	0	0	0	0	0	0	0	0	114	0	0	0
5	50 ul	1.4	0	0	0	149	0	0	0	118	90	269	262	0	0	0
5	5 ul	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0

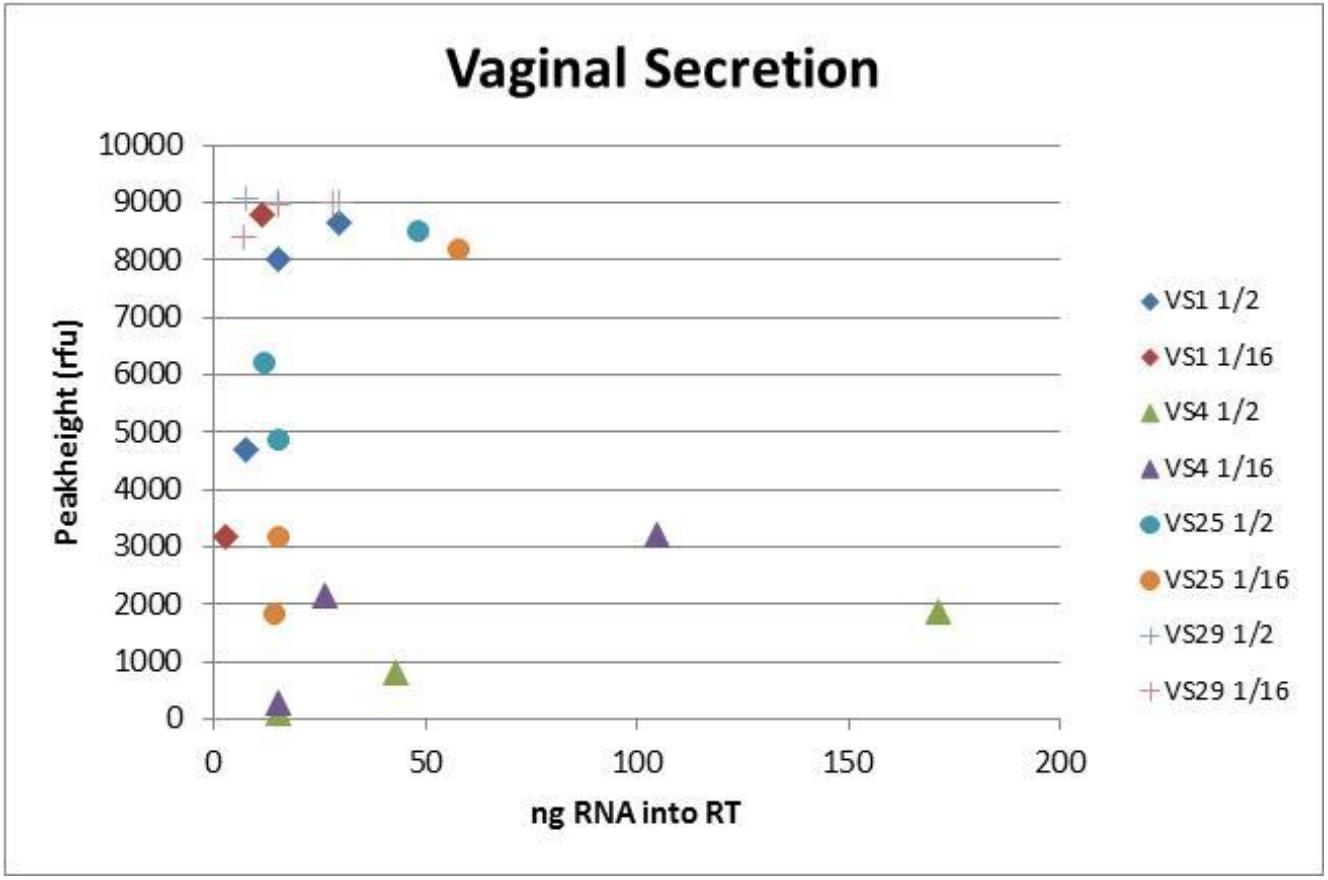
‘no quant’ compared to ‘some sort of quant’

Vaginal secretion

AllPrep

			2ul into RT							8ul into RT						
		Quantus	Saliva				Vag			Saliva				Vag		
		HS	HTN3	HTN1	STATH	MUC7	MYO	CYP	MUC4	HTN3	HTN1	STATH	MUC7	MYO	CYP	MUC4
		ng/ul	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu	rfu
6	1/2	16	0	0	0	0	150	8202	8032	0	0	0	0	104	8330	8123
6	1/64	0.17	0	0	0	0	0	0	1287	0	0	0	0	0	1364	4748
7	1/2	0.14	0	0	0	0	0	766	1167	0	0	0	0	338	0	2367
7	1/64	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	2597
8	1/2	73	0	0	0	0	1674	7950	7957	0	0	0	0	1491	8119	7976
8	1/64	14	0	0	0	0	939	8323	8216	0	0	0	0	833	8396	8128
9	1/2	53	0	0	0	0	623	8211	8263	0	0	0	0	480	8265	8288
9	1/64	15	0	0	0	0	198	8465	8324	0	0	0	0	546	8129	8218
10	1/2	4.5	0	0	0	0	451	952	8172	0	0	0	0	1349	1267	8129
10	1/64	1.3	0	0	0	0	375	0	7859	0	0	0	0	0	1455	8357

‘no quant’ compared to ‘some sort of quant’



2 ul, 8 ul, 15 ng RNA into RT, respectively

‘no quant’ compared to ‘some sort of quant’

UCF		DNA	RNA
Donor	**Swab size**	**Quant (ng/ul)**	**Quant (ng/ul)**
VS1	1/2	1004.0	3.7
	1/16	77.0	1.4
VS4	1/2	1292.0	21.4
	1/16	107.0	13.1
VS13	1/2	0.4	undet
	1/16	0.1	undet
VS25	1/2	120.0	6.0
	1/16	22.0	7.2
VS29	1/2	279.0	3.7
	1/16	58.0	3.5

20ul extract

14ul extract

ZH		DNA	RNA
Donor	**Swab size**	**Quant (ng/ul)**	**Quant (ng/ul)**
6	1/2	10.2	16.0
	1/64	0.8	0.2
7	1/2	6.0	0.1
	1/64	1.3	0.2
8	1/2	18.5	73.0
	1/64	3.4	14.0
9	1/2	14.6	53.0
	1/64	4.1	15.0
10	1/2	19.1	4.5
	1/64	4.4	1.3

80ul extract

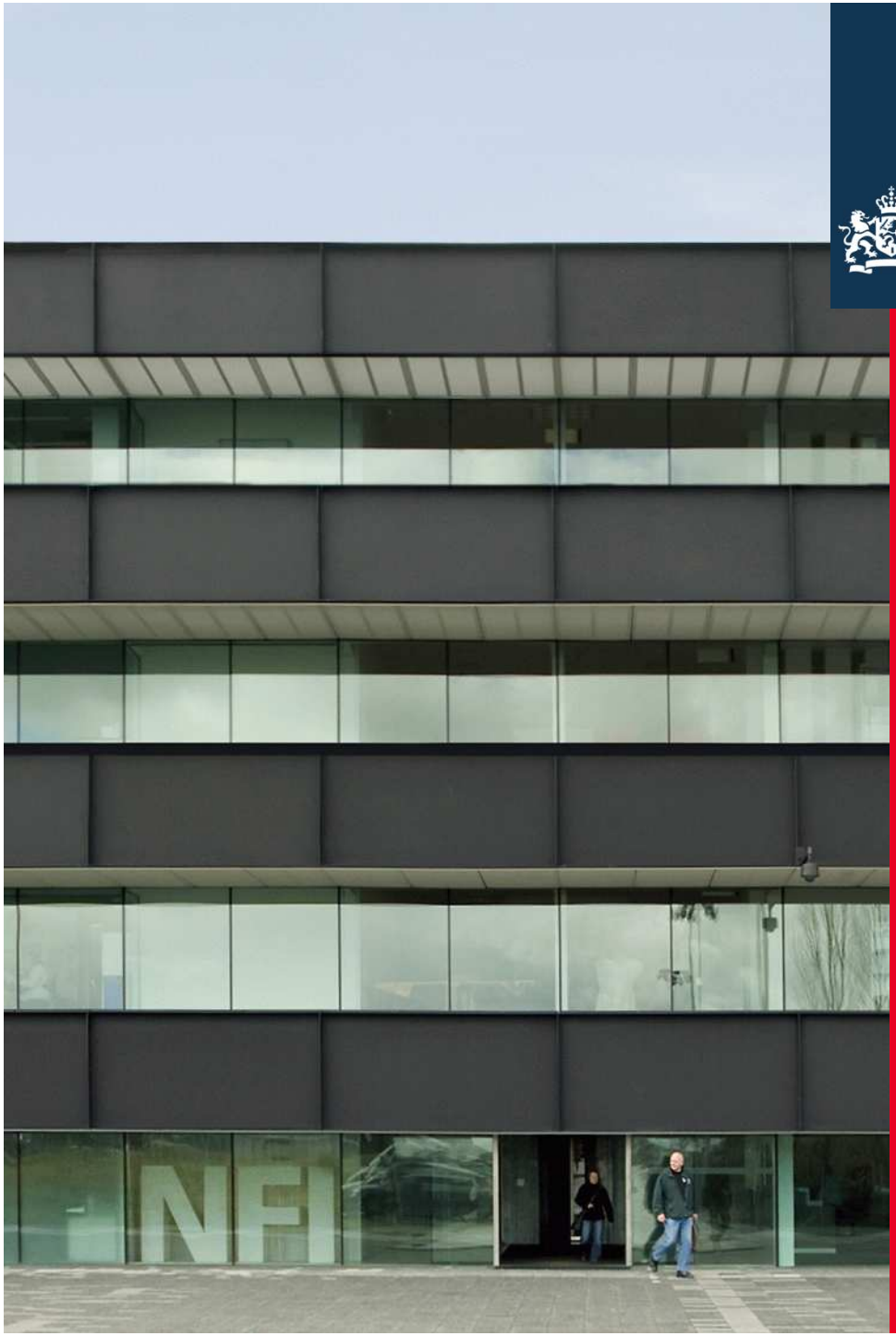
30ul extract

‘no quant’ compared to ‘some sort of quant’

- not much cross-reactivity
- no real advantage to RT a defined total input (15 ng) compared to a set input volume (2 ul, 8 ul)
- only marginal correlation between DNA and RNA quants
- no collaborative exercise

Thank you for your attention!

Jack Ballantyne, Erin Hanson,
Cordula Haas, Sabrina Ingold, Corinne Moser



Netherlands Forensic Institute
Ministry of Security and Justice

A SNaPshot targeting common mtDNA mutations

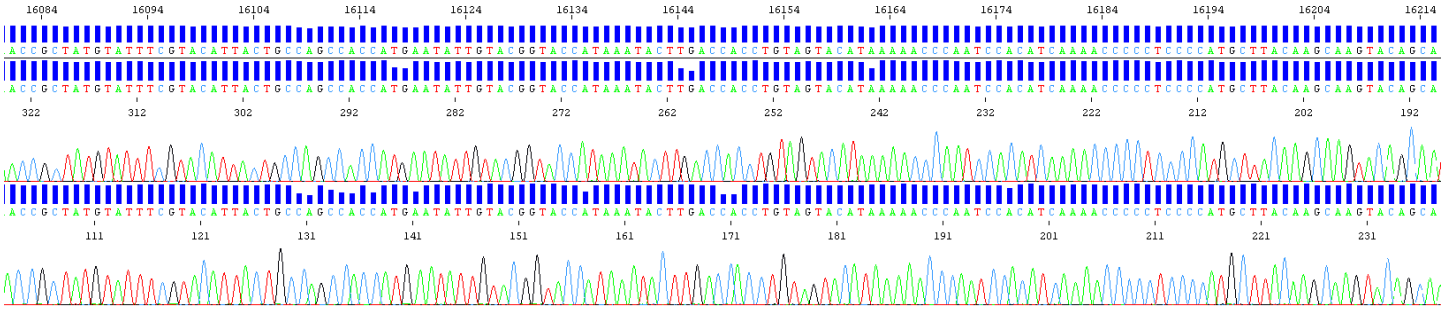
19 November 2014

Current method is time consuming

Mini-mtDNA method: 10 amplicons in 2 multiplexes

Sequencing reaction: 10x forward + 10x reverse = 20 sequencing reactions for 1 sample

- Time consuming
- Labour intensive
- Expensive
- Example: Case with 30 hairs → 600 sequencing reactions! (2011.09.15.067)



SNP analysis for mtDNA screening

Need for a quicker examination of mtDNA samples

- Selection of mtDNA samples for sequencing analysis
- Increasing sample throughput

Chemale et al. (2013) published a mtDNA screening tool

- SNaPshot assay targeting common SNPs in mtDNA HVS fragments
- Feasibility for degraded DNA?
- Focus on Brazilian population

Aim of project

Develop and optimise a SNaPshot assay targeting common mtDNA mutations in HVS fragments relevant to the Dutch Criminal casework, reflecting the individuals present in the National DNA database

Project carried out by:

Titia

Natalie

Gerda

Same PCR product for SNaPshot and mini-mtDNA

<u>Mini-mtDNA</u>

DNA (*5µl*)

↓

2x 5-plex PCR amplification (*50µl*)

↓

Purification of PCR product

↓

Sequencing PCR

↓

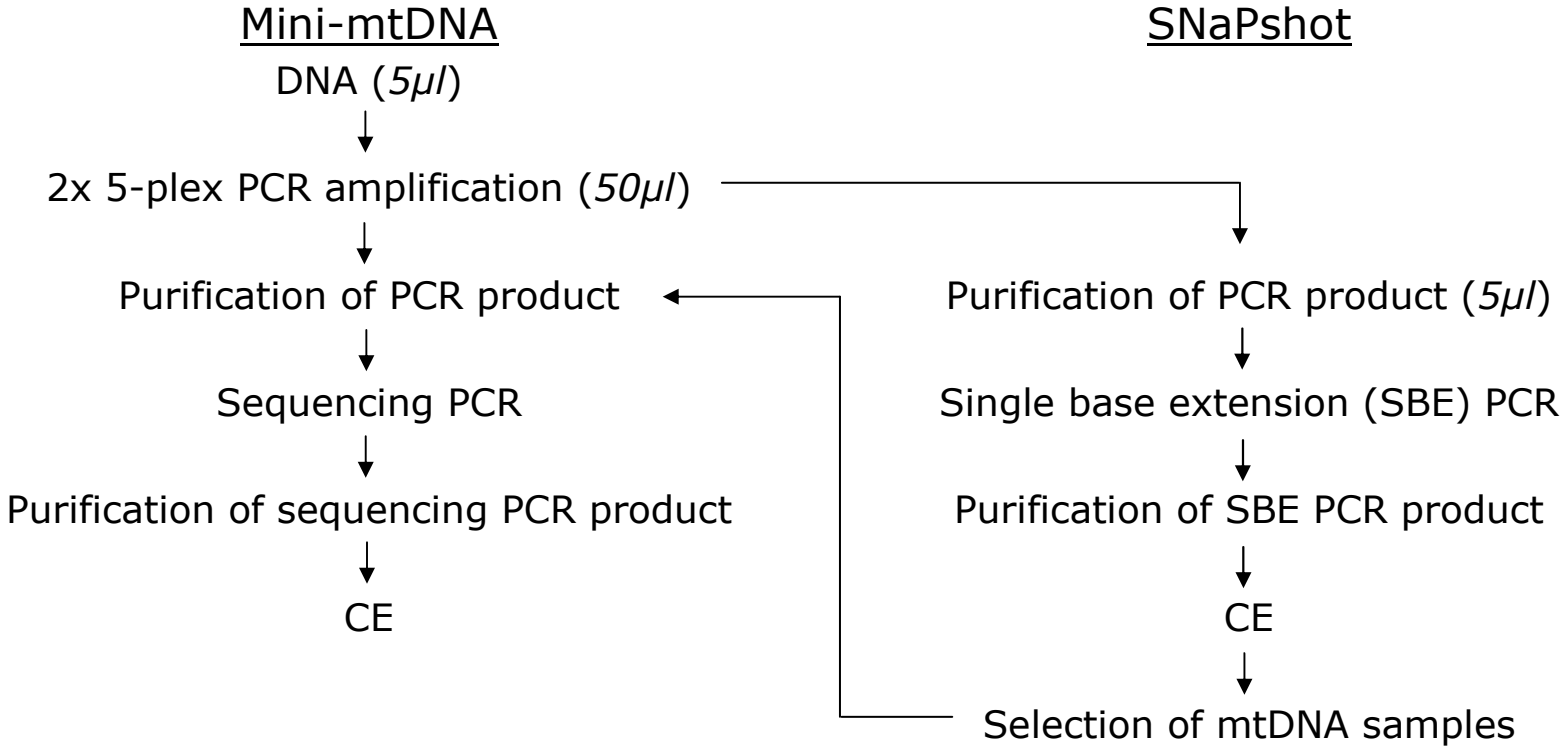
Purification of sequencing PCR product

↓

CE

Example: Case with 30 hairs → 600 sequencing reactions! (2011.09.15.067)

Same PCR product for SNaPshot and mini-mtDNA



Example: Case with 30 hairs → 600 sequencing reactions! (2011.09.15.067)

SNaPshot: Selection of 3 hair samples → 60 sequencing reactions

SNP selection

Selection criteria:

1. HVS fragments (mini-mtDNA)
2. Limited number of SNPs
3. High discrimination power
4. Haplogroup information
5. Non redundant SNPs
6. SNPs with high and low frequency in Dutch population

Final selection: 18 SNPs

Divided in two multiplex systems

-mp1: 9 SNPs (set1 mini-mtDNA)

-mp2: 9 SNPs (set2 mini-mtDNA)

SNP	Base change	Frequency	Haplogroup
73	A>G	0.5483	HV, H, V
146	T>C	0.0917	
	T>a	0.0001	
150	C>T	0.1023	
	C>g	0.0001	
152	T>C	0.2018	
182	C>T	0.0089	
185	G>A	0.0548	
	G>t	0.0031	
	G>c	0.0004	
195	T>C	0.1963	
	T>a	0.0002	
489	T>C	0.1091	M / J
497	C>T	0.0434	K
16126	T>C	0.1821	
16129	G>A	0.0662	
	G>c	0.0112	
16223	C>T	0.1285	
16270	C>T	0.0891	
16278	C>T	0.0657	
16294	C>T	0.1077	
	C>a	0.0003	
	C>g	0.0002	
16311	T>C	0.1692	
16362	T>C	0.0700	
16519	T>C	0.6441	

High power of discrimination for mtDNA

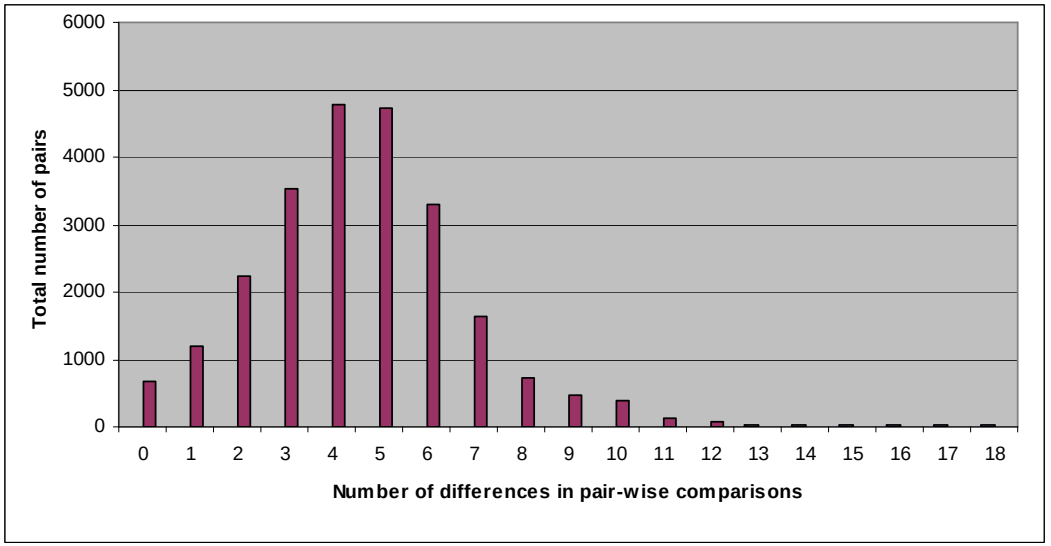
Power of the SNaPshot assay to discriminate mtDNA samples using the 18 SNPs selected

- Pair-wise comparisons between sequence data of 155 unrelated samples from NFI elimination dataset

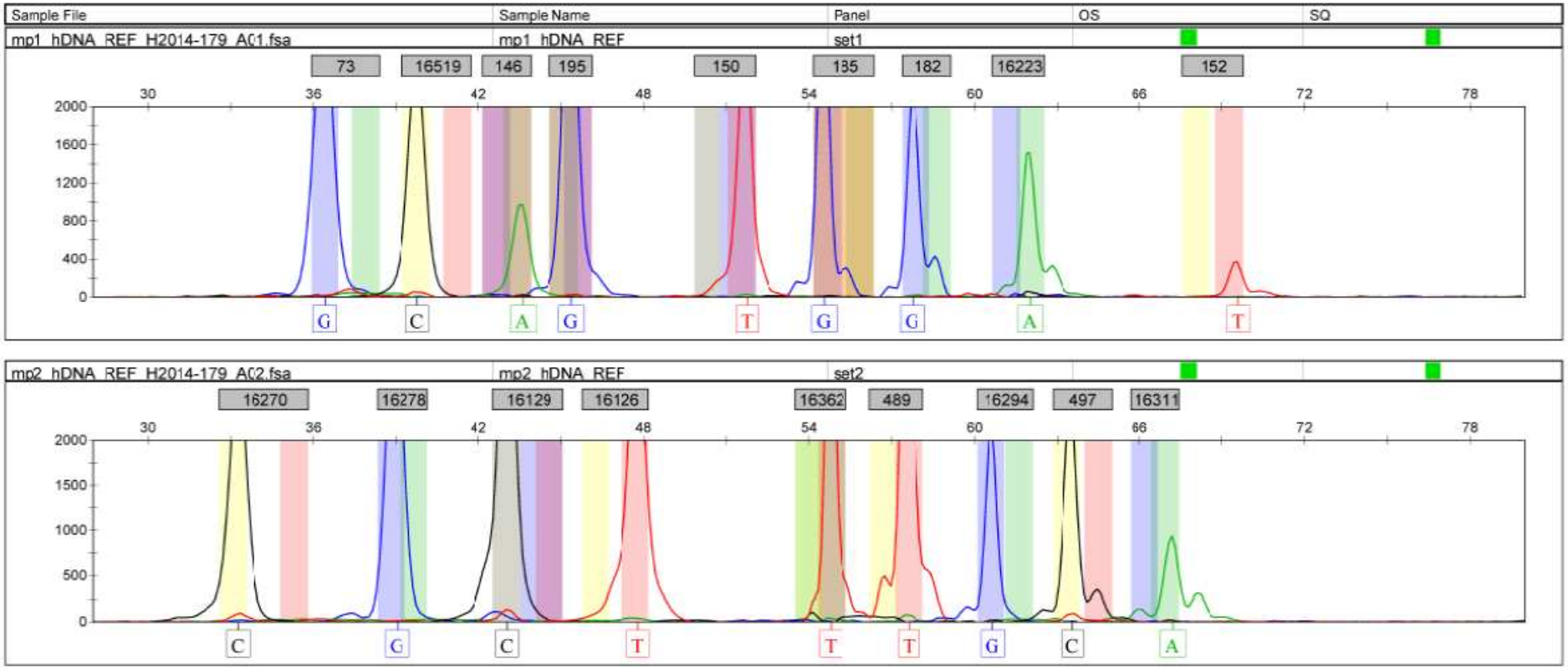
- Number of differences:
  0 – 15

Power of discrimination:

> 97.2%

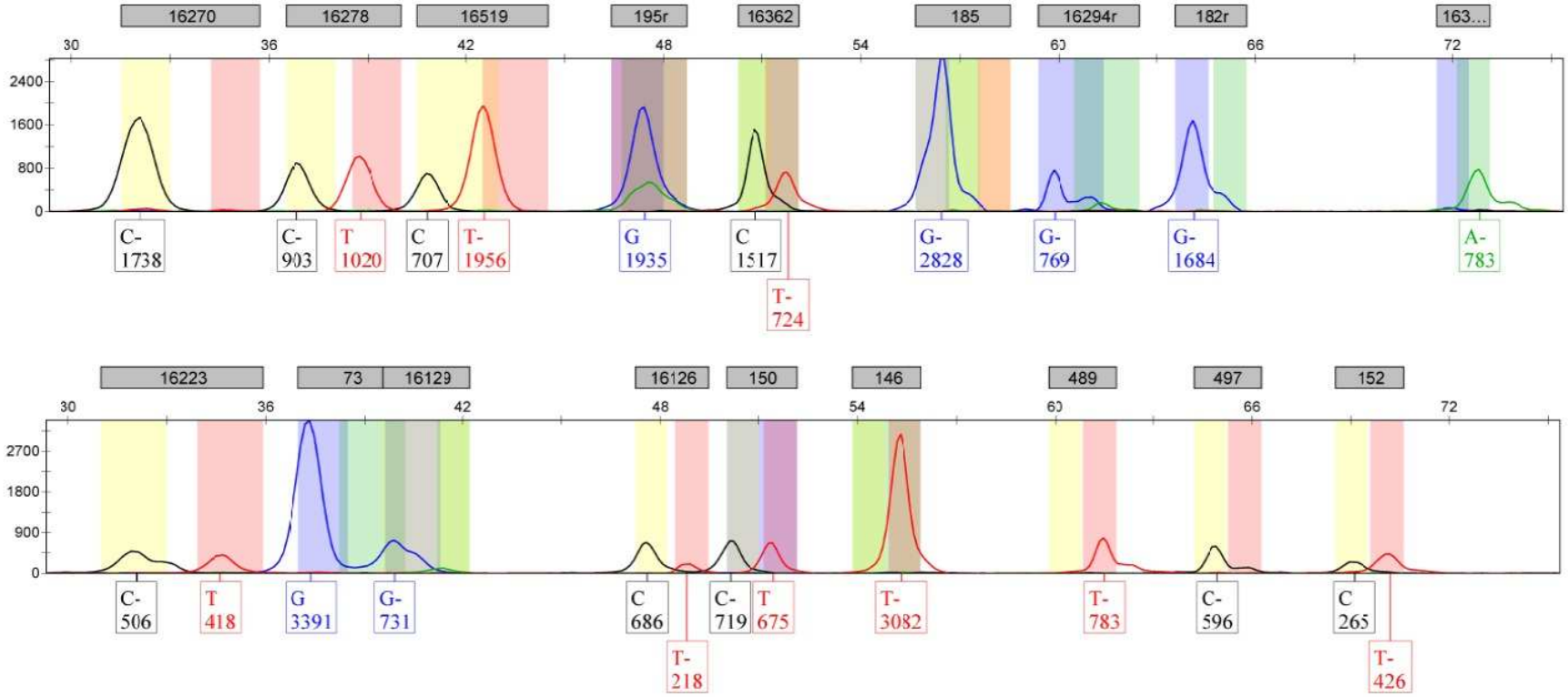


Optimised SNaPshot assay



Note: some extension primers have degenerate bases

SNaPshot of mixture



Optimised SNaPshot assay, summary

Reproducibility	✓
Inhibited samples	✓
Mixtures	✓
Concordance	✓
Manuscript	in preparation

Conclusion

MtDNA SNaPshot is a fast and efficient screening tool to discriminate mtDNA samples and facilitates the selection of samples for subsequent mtDNA sequencing

MtDNA SNaPshot can be incorporated into the existing workflow

MtDNA SNaPshot does not consume extra DNA extract

EDNAP Exercise proposal

Excercise on 10 samples (10 x SNaPshot, 2 x Sanger)

NFI provides:

- Protocols
- Primers
- Samples

Labs provide:

- All other chemistry

2015 Q1: start
2015 Q2: data collection
2015 Q3: data analysis, preparation of manuscript

Interested in joining the EDNAP excercise?

Contact:

Titia Sijen
T.sijen@nfi.minvenj.nl

Arnoud Kal
A.kal@nfi.minvenj.nl

Regulator's Update

Dr Gillian Tully

EDNAP

Copenhagen, 28 April 2015

Forensic Science Regulator

Overseeing Quality

Role of Regulator

Ensuring that provision of forensic science services across the CJS is subject to an appropriate regime of scientific quality standards

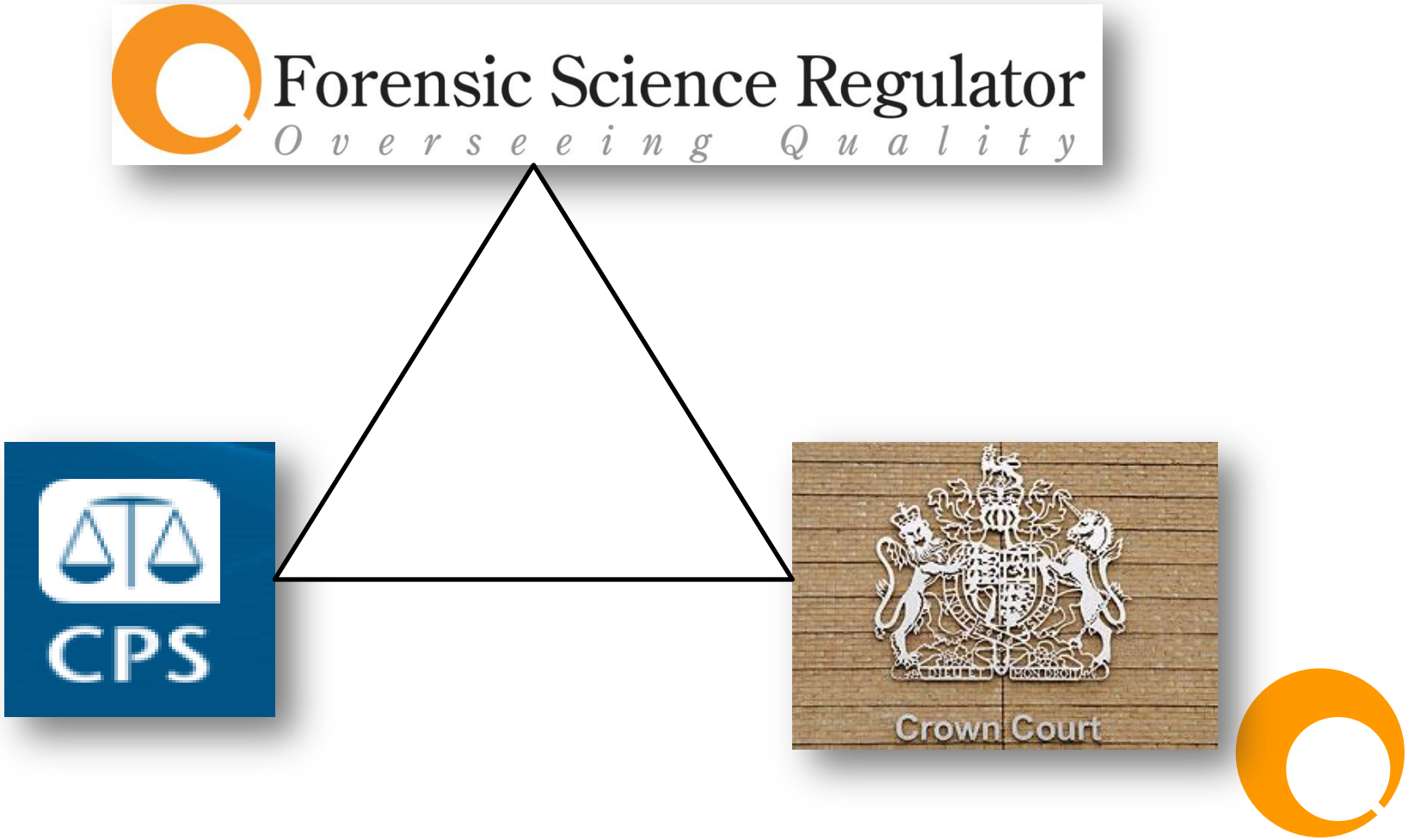
- Identifying requirement
- Leading development
- Providing guidance
- Investigating complaints

Covers all areas of forensic science

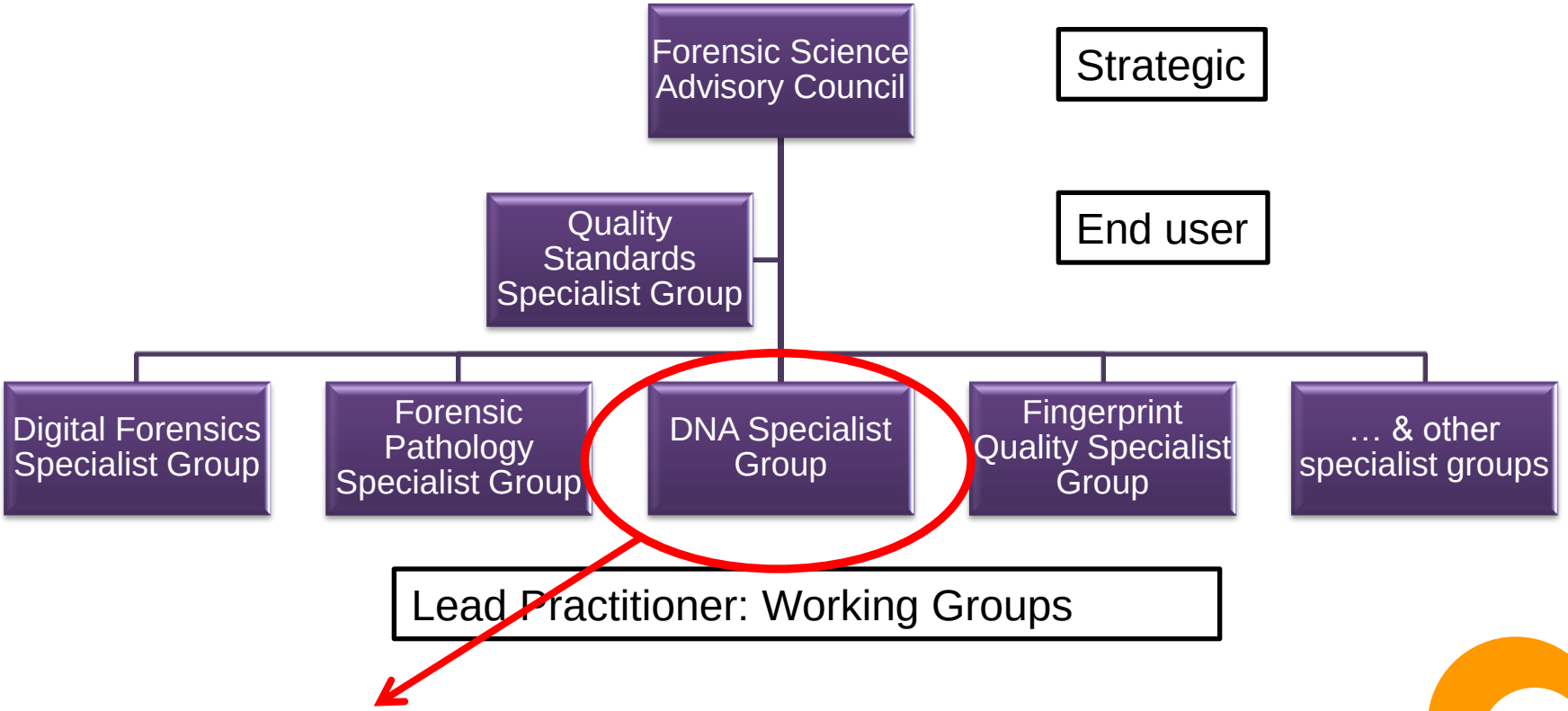
...but NOT regulating the market

Gill as Regulator

Expert Evidence Framework



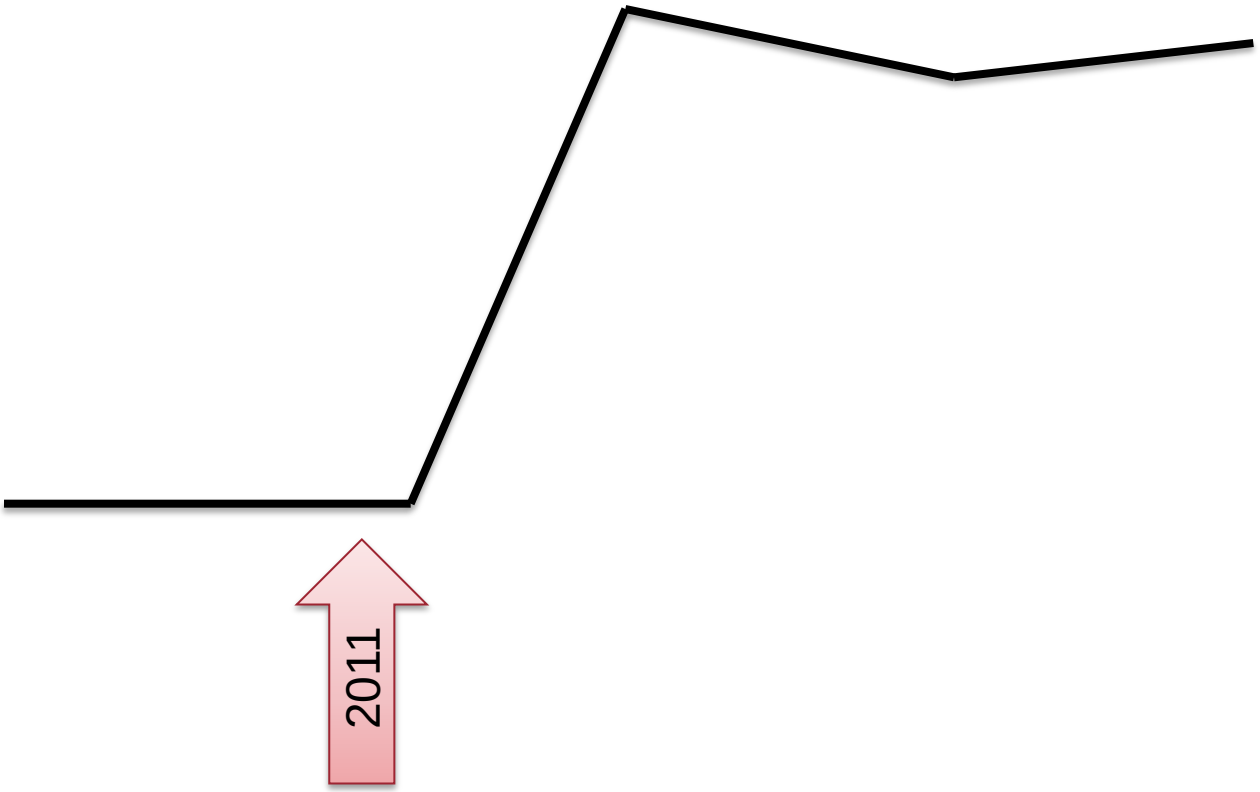
Regulation: Advice Structures



Denise Syndercombe-Court sits as ISFG representative

FSR Quality Framework

Errors, complaints and openness



Current Documents & Drafts

- The Management & Use of Staff Elimination Databases
  - FSR-P-302 Published 12 Sept 2014
- The control and Avoidance of Contamination in Laboratory Activities involving DNA Recovery & Analysis
  - FSR-G-208 Consultation completed
  - 161 specific points raised: being considered
  - Next back to DNA SG - summer

Current Documents & Drafts

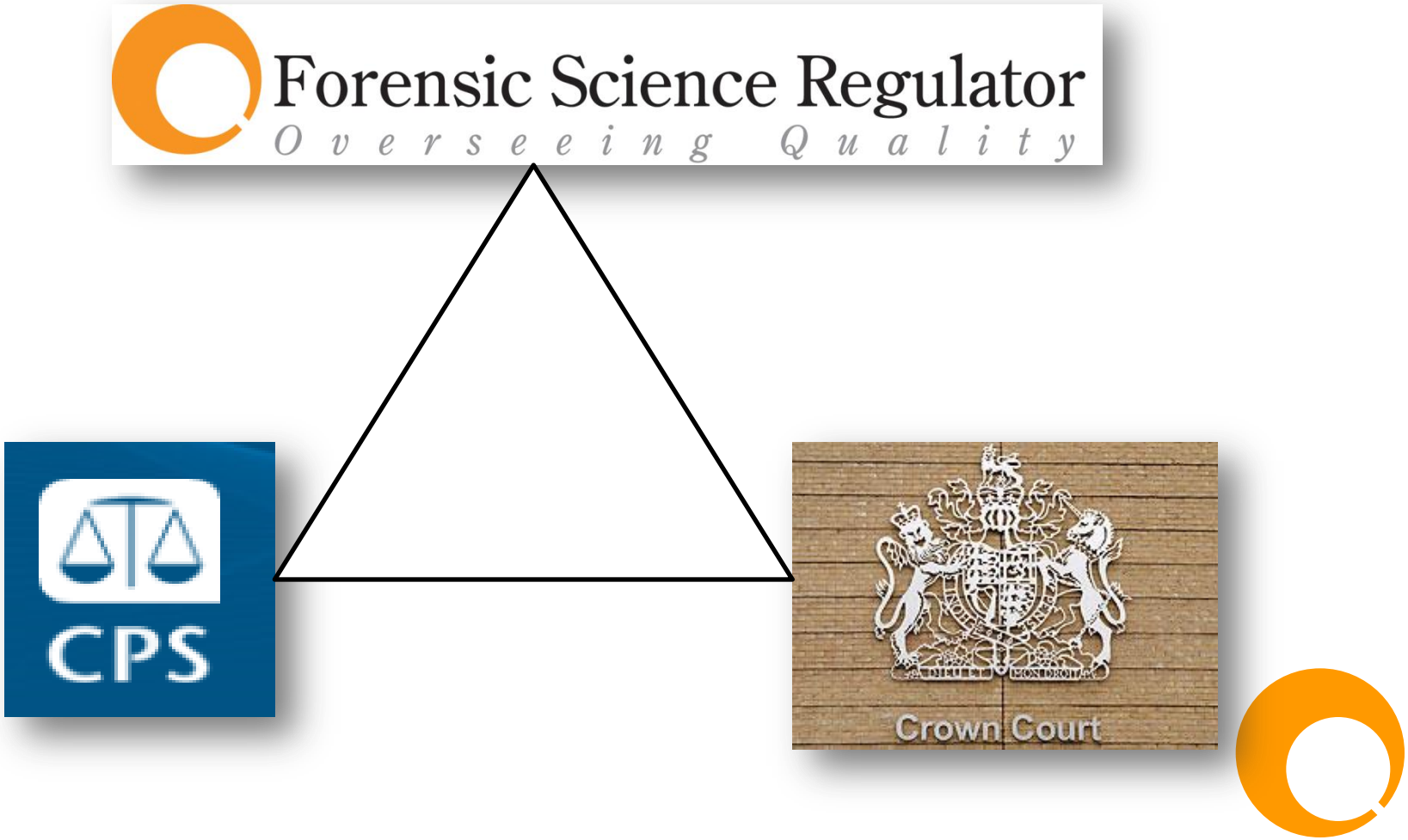
- The Control and Avoidance of Contamination in Crime Scene Examination involving DNA Evidence Recovery
  - FSR-G-206 Consultation closed
- Appendix: Bloodstain Pattern Analysis
  - FSR-C-102 Consultation closed
- Cognitive Bias Effects Relevant to Forensic Science Examinations
  - Consultation closed

Continuous improvement

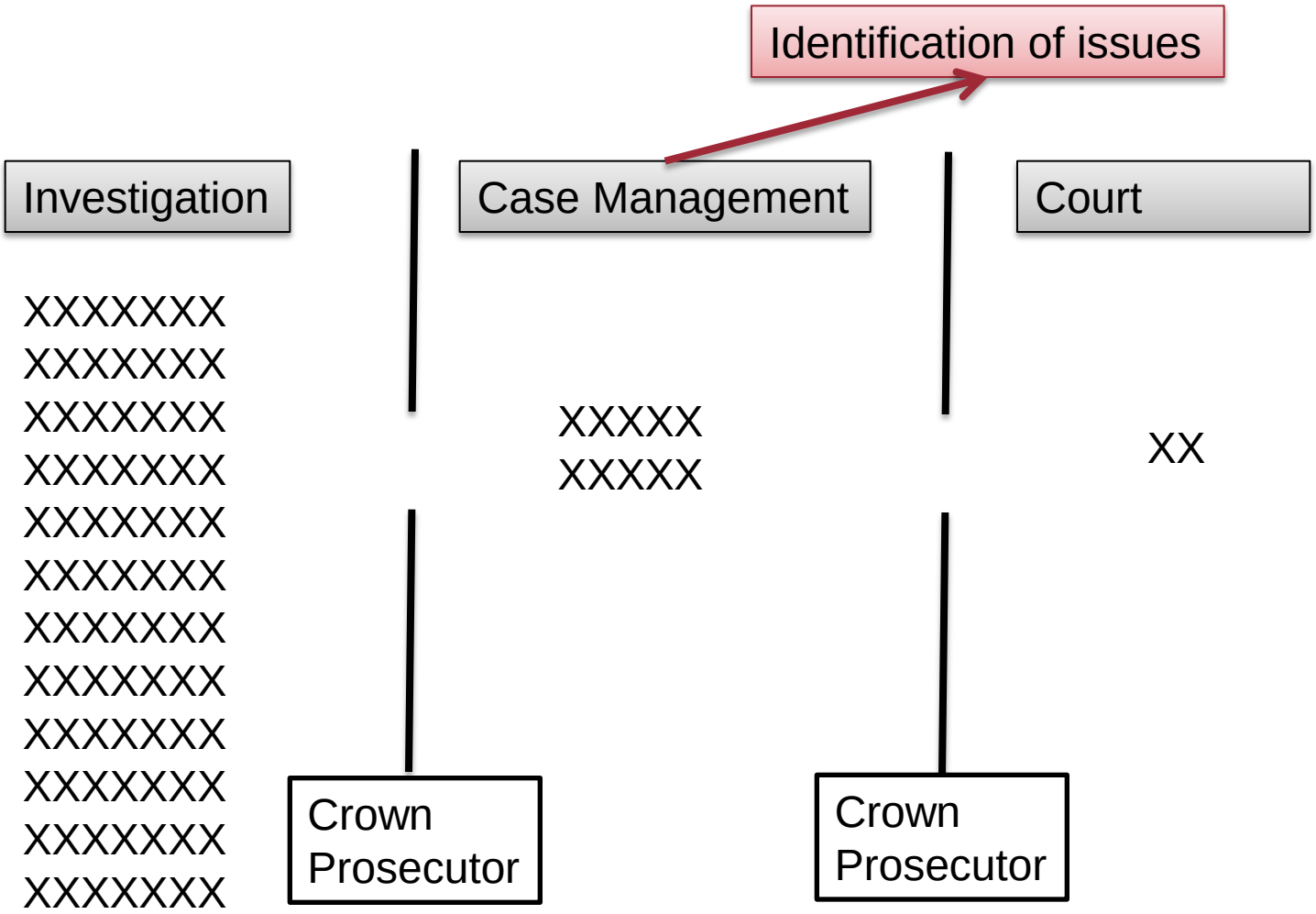
Other DNA-Related Activities

- Collaborative study on mixture analysis and interpretation
  - Organised on behalf of FSR by PFS & NIST
    - First since introduction of 17/20-plexes
    - Analytical variability
    - Interpretation variability
  - Jim Thompson presenting at Interpretation sub-group of ENFSI meeting
  - Designed to assess current status and stimulate improvement

Expert Evidence Framework



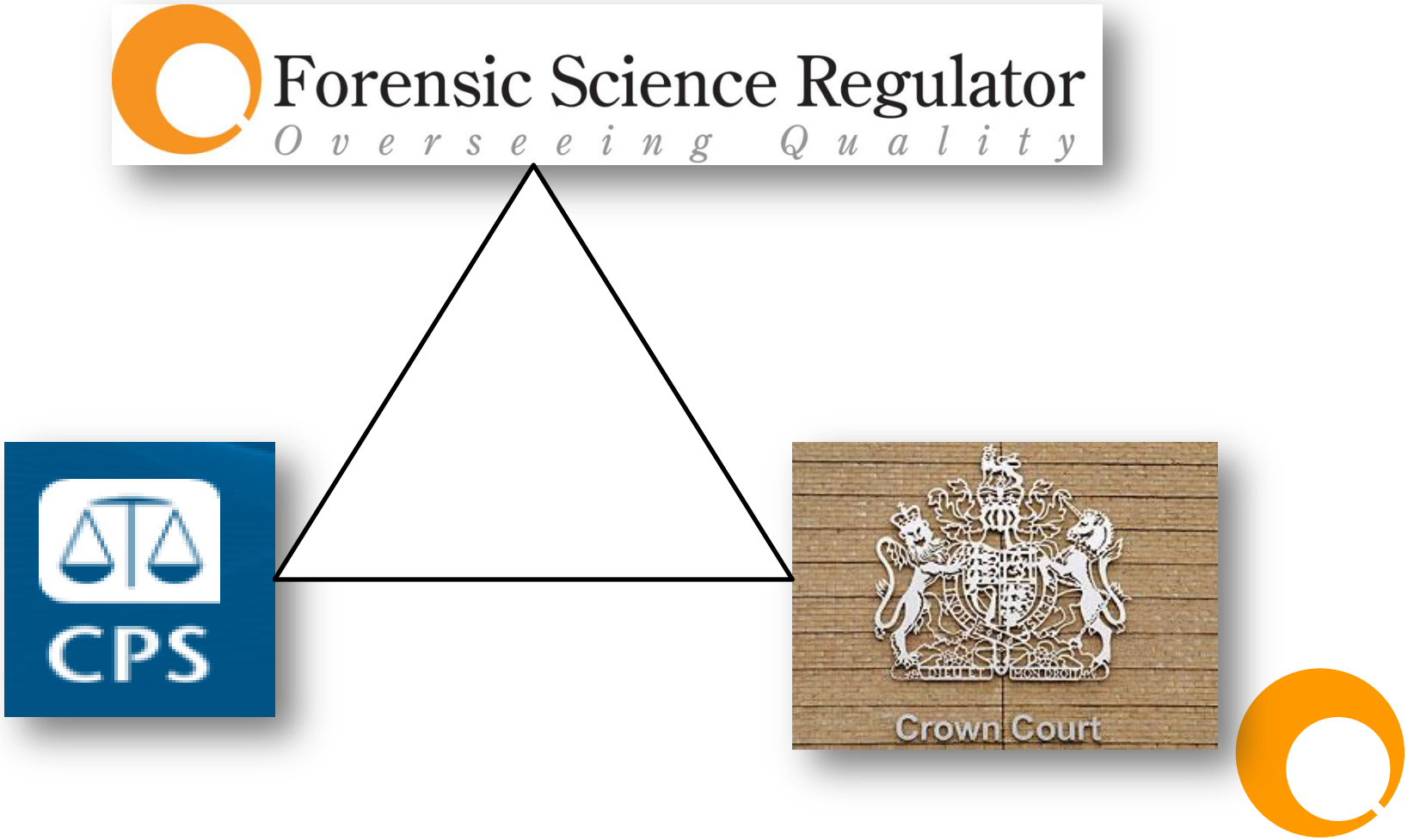
CPS Gatekeeping Role



1. To comply with the FSR's Codes of Conduct and Practice
2. To ensure Quality Standards and Assurance processes are applied which are nationally consistent and compliant with appropriate ISO standards, United Kingdom Accreditation Service (UKAS) accreditation, EU directives and clear development and validation processes...
3. To provide clear communication and interpretation of scientific processes, procedures, strengths, weaknesses and meaning.
4. To engage with Streamlined Forensic Reporting (SFR) process ...
5. To be fully aware of and compliant with CPIA Disclosure and Expert Witness obligations

http://www.cps.gov.uk/legal/s_to_u/scientific_evidence/core_foundation_principles_for_forensic_science_providers/

Expert Evidence Framework



Court

Criminal Practice Directions 2015

Part 33 – admissibility

- Extent & quality of data
- Validity of methods
- Safety of inference
- Uncertainty, accuracy, reliability
- Peer review
- Expert's field of expertise
- Completeness of information
- Following established practice

Judge is ultimate arbiter of admissibility
Working with senior judiciary

Thank you, good to be here!

..and thanks to The Forensic Science Regulation Unit:

June Guiness

Dr Jeff Adams

Simon Iveson

EDNAP Meeting, Copenhagen, Denmark, April 28, 2015

EMPOP Update

Dr. Walther Parson

assoc. Professor, Institute of Legal Medicine, Innsbruck

adj. Professor, Forensic Program, Penn State University, PA, USA

walther.parson@gmail.com

EMPOP update

1. New EMPOP related publications
    1. King et al (2014) Nature Communications
    2. Gomes et al. (2015) PLoS ONE
    3. Xavier et al. (2015) PLoS ONE
    4. Naue et al (2015) Mitochondrion
2. EMPOP database
3. EMPOP workshops

nature COMMUNICATIONS

ARTICLE

Received 5 Aug 2014 | Accepted 21 Oct 2014 | Published 2 Dec 2014

DOI: 10.1038/ncomms6631 OPEN

Identification of the remains of King Richard III

Turi E. King[1,2], Gloria Gonzalez Fortes[3,4,*], Patricia Balaresque[5,*], Mark G. Thomas[6], David Balding[6], Pierpaolo Maisano Delser[1], Rita Neumann[1], Walther Parson[7,8], Michael Knapp[9], Susan Walsh[10,11], Laure Tonasso[5], John Holt[12], Manfred Kayser[11], Jo Appleby[2], Peter Forster[13,14], David Ekserdjian[15], Michael Hofreiter[3,4] & Kevin Schürer[16]

527 years, oldest identification

full mitogenome sequence (J1c1)

2 living maternal relatives (19/21 gens)

discrepancy at 8994 - phylogenetic hotspot

Y-STR exclusion between 5 living relatives

nature COMMUNICATIONS

Evidence	LR	Posterior – sceptical prior	Posterior – 50/50 prior
All	**6.7 million**	**0.999994**	**0.9999999**
Genetic	79	0.67	0.987
Non-genetic	85,000	0.9995	0.999988
All exc. mtDNA	14,000	0.997	0.99993
All exc. Y	41 million	0.999999	1.0000000
mtDNA only	478	0.92	0.998
For illustrative purposes, below we give likelihood ratios calculated using the European mitochondrial DNA control region database			
mtDNA only	6847	0.994	0.9999
Genetic	1127	0.97	0.999
All	96 million	0.9999996	0.99999999

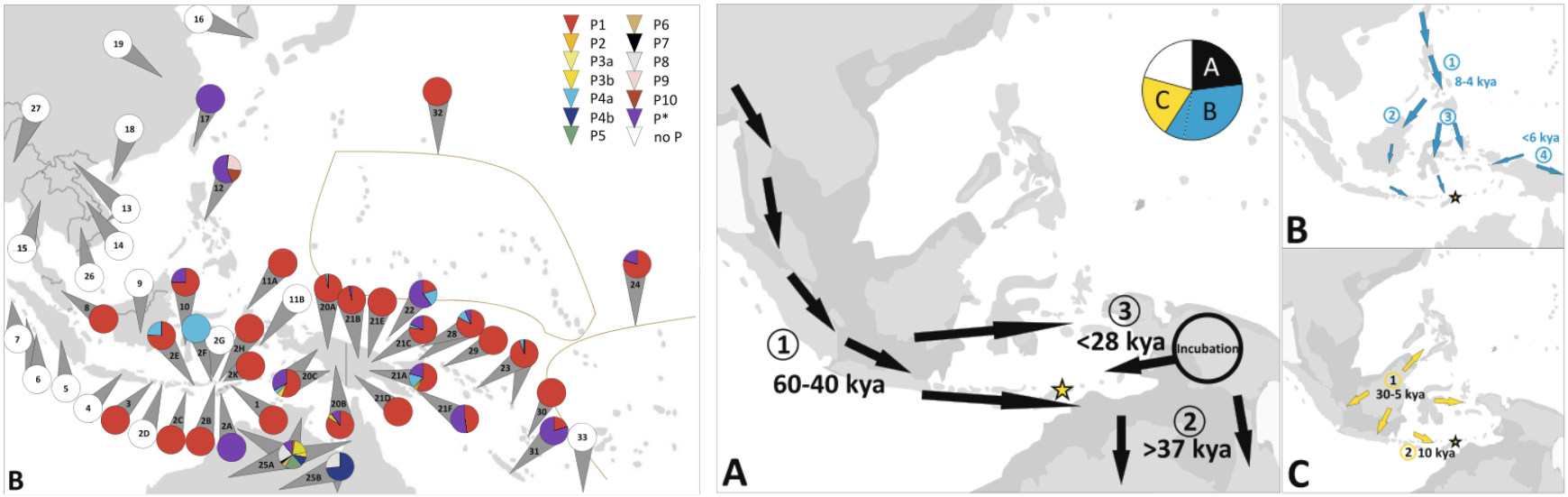
Gomes *et al. BMC Genomics* (2015) 16:70
DOI 10.1186/s12864-014-1201-x

BMC Genomics

RESEARCH ARTICLE Open Access

Human settlement history between Sunda and Sahul: a focus on East Timor (Timor-Leste) and the Pleistocenic mtDNA diversity

Sibylle M Gomes[1†], Martin Bodner[2†], Luis Souto[1,3], Bettina Zimmermann[2], Gabriela Huber[2], Christina Strobl[2], Alexander W Röck[2], Alessandro Achilli[4,5], Anna Olivieri[4], Antonio Torroni[4], Francisco Côrte-Real[6] and Walther Parson[2,7*]

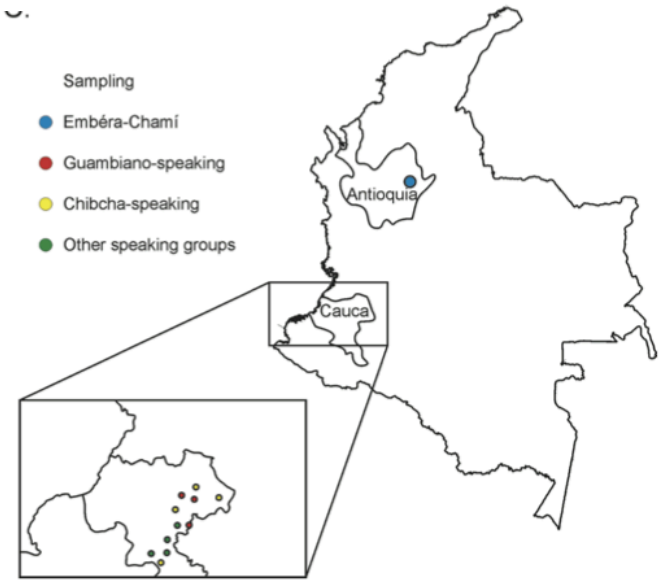


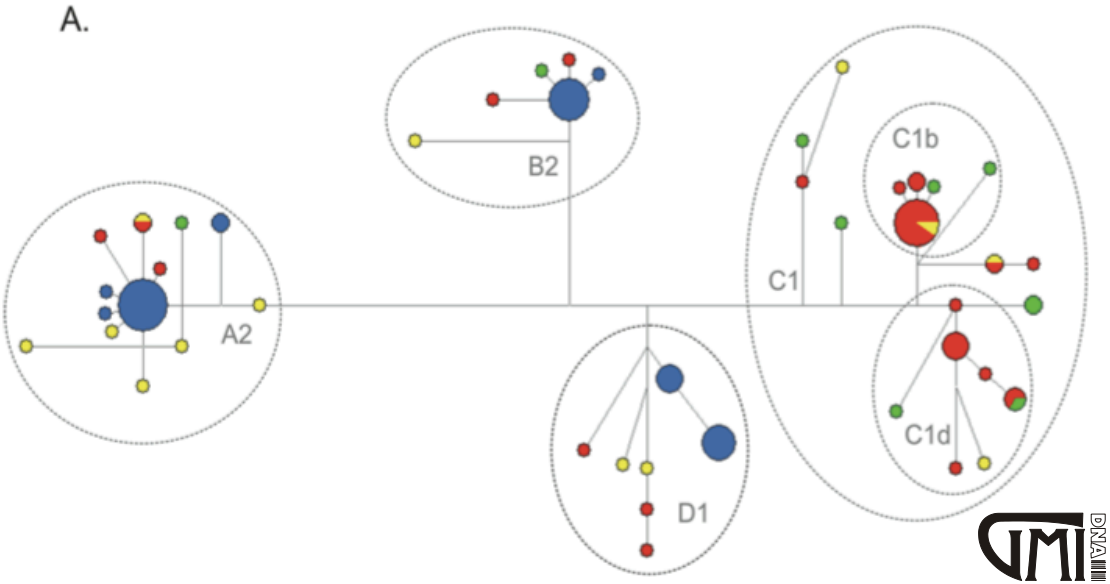
PLOS ONE

RESEARCH ARTICLE

Admixture and Genetic Diversity Distribution Patterns of Non-Recombining Lineages of Native American Ancestry in Colombian Populations

Catarina Xavier[1,2,3], Juan José Builes[4,5], Verónica Gomes[1,2], Jose Miguel Ospino[5], Juliana Aquino[6], Walther Parson[3,7], António Amorim[1,2,8], Leonor Gusmão[1,2,6], Ana Goios[1,2]*





Mitochondrion 20 (2015) 82–94

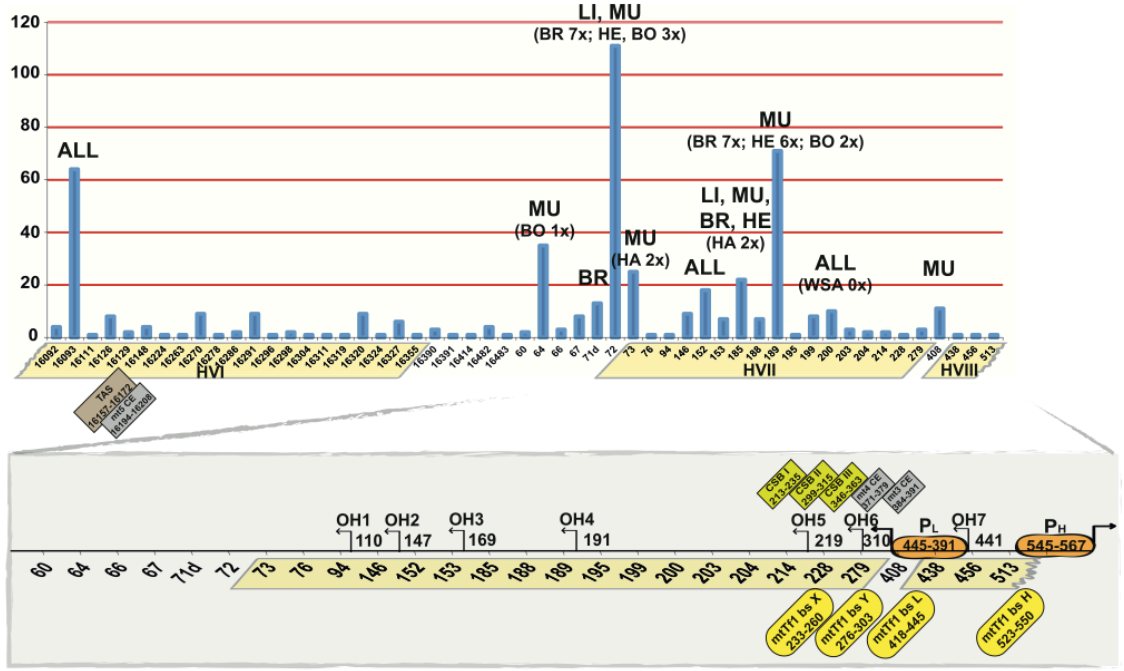
Contents lists available at ScienceDirect

Mitochondrion

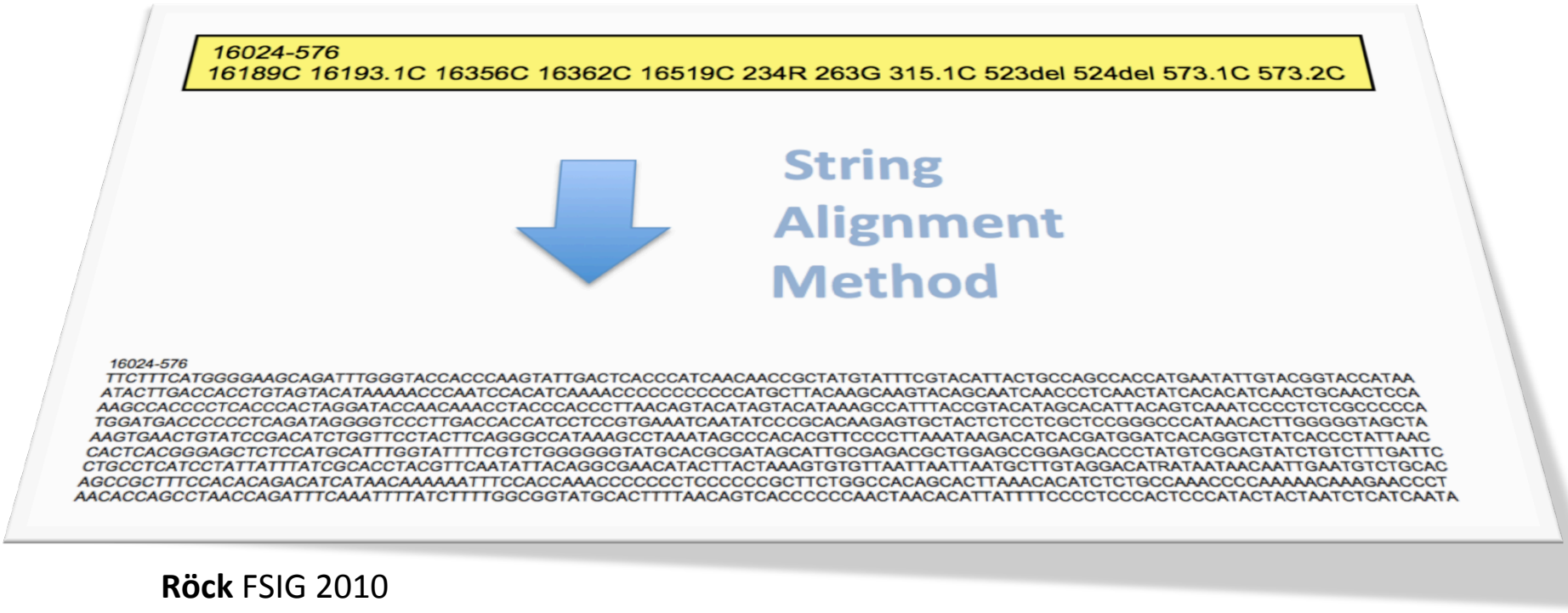
journal homepage: www.elsevier.com/locate/mito

Evidence for frequent and tissue-specific sequence heteroplasmy in human mitochondrial DNA

Jana Naue [a,b,*], Steffen Hörer [a], Timo Sänger [a], Christina Strobl [c], Petra Hatzer-Grubwieser [c], Walther Parson [c,d], Sabine Lutz-Bonengel [a]



2. EMPOP database - Alignment free searches



Röck FSIG 2010

SAM - alignment-free search software

guarantees that matches are found regardless of alignment and notation of haplotypes

Database matches and neighbors

	# differences	Example 1	Example 2
matches	0	0	0
neighbors	1	0	274
	2	0	3,847
	3	1	14,519

# of neighbors important for understanding matches

Problem of determining neighbors in an alignment free search

16304C 16519C 263G 315.1C 456T 523del 524del

16304C 263G 315.1C 456T 523del 524del - neighbor at 1

16304C 16519C 263G 315.1C 456T - neighbor at **1**

Database matches and neighbors

16304C 16519C 263G 315.1C 456T 523del 524del

16304C 263G 315.1C 456T 523del 524del - neighbor at 1

16304C 16519C 263G 315.1C 456T - neighbor at **1**

523/4del are two individual differences, but **one** phylogenetic event (because we never observed just one nucleotide being inserted/ deleted)

CCAGC ACACACACAC CGCTGC - rCRS

CCAGC ACACACAC CGCTGC

CCAGC ACACAC CGCTGC

CCAGC ACACACACACAC CGCTGC

Database matches and neighbors

Problem of telling the search engine what is a phylogenetic event if sequences are unaligned (have no positional numbers)

Solution:

"Event-based SAM"

1) SAM - set # neighbors high enough to include all indels
2) Determine **events** - based on observed length variants

(may also be relevant to MPS STRs)

Frequent events in the CR

AC-repeat between

514 and 525

“Chibcha-deletion”

6 bp deletion between 105/106 and 110/111

…

Rare events in the CR

16033+CTCTGTTCTTTCAT (14)

398+ACCAGATTTCAAAT (14)

291+ACATCATAACAAAAAA (16)

563+AACAAAGAAC...AAA (204)

…

Increasing the number of neighbours included in a query has a significant impact on the performance of the search engine (time)

Optimize programming code

Currently data mining for full mitogenomes for determining events in the coding region

EMPOP mtDNA database, v3/R11

Logged in Walther Parson | logout

Home News Introduction Contribute Your Account Terms of Use

QUERY POPULATIONS TOOLS

Query Result Details Neighbors

Sample ID (none specified)
Ranges 16024-576
Profile 16304C 263G 315.1C 456T 523- 524-

198 of 198 haplotypes shown

Origin					Metapopulation	Cost	Count	Mutations	Ignored Mutations	Haplogroup		Publications
Continent	Region	Country	Province	City						Rank 1	Rank 2	
Europe	Southern Europe	Portugal	Central Portugal		Eurasian	0.42	2	C16519T (0.25) AC524- (0.17)	C309.1-	H5	H5	Rocha 2012
America	Northern America	United States of America	Texas		Eurasian	0.42	2	C16519T (0.25) AC524- (0.17)	C309.1- C309.2-	H5	H5	AFDIL 2012

Metapopulation	Cost	Count	Mutations	Ignored Mutations	Haplogroup	
					Rank 1	Rank 2
Eurasian	0.42	2	C16519T (0.25) AC524- (0.17)	C309.1-	H5	H5
Eurasian	0.42	2	C16519T (0.25) AC524- (0.17)	C309.1- C309.2-	H5	H5

3. EMPOP Meetings

Title: EMPOP advanced practical course

Date: August 31, 2015

Time: 9:00 – 18:30

Workshop presenters: Catarina Xavier, Martin Bodner, Walther Parson

3. YHRD/EMPOP Meeting

Save the date

Haploid Markers Meeting

May 19-21 2016, Berlin

20th anniversary

EDNAP Meeting, Copenhagen, Denmark, April 28, 2015

STRbASE ENFSI DNA WG STR Population Database, v2

ENFSI DNA WG STRbase

Update and development

Dr. Walther Parson

assoc. Professor, Institute of Legal Medicine, Innsbruck

adj. Professor, Forensic Program, Penn State University, PA, USA

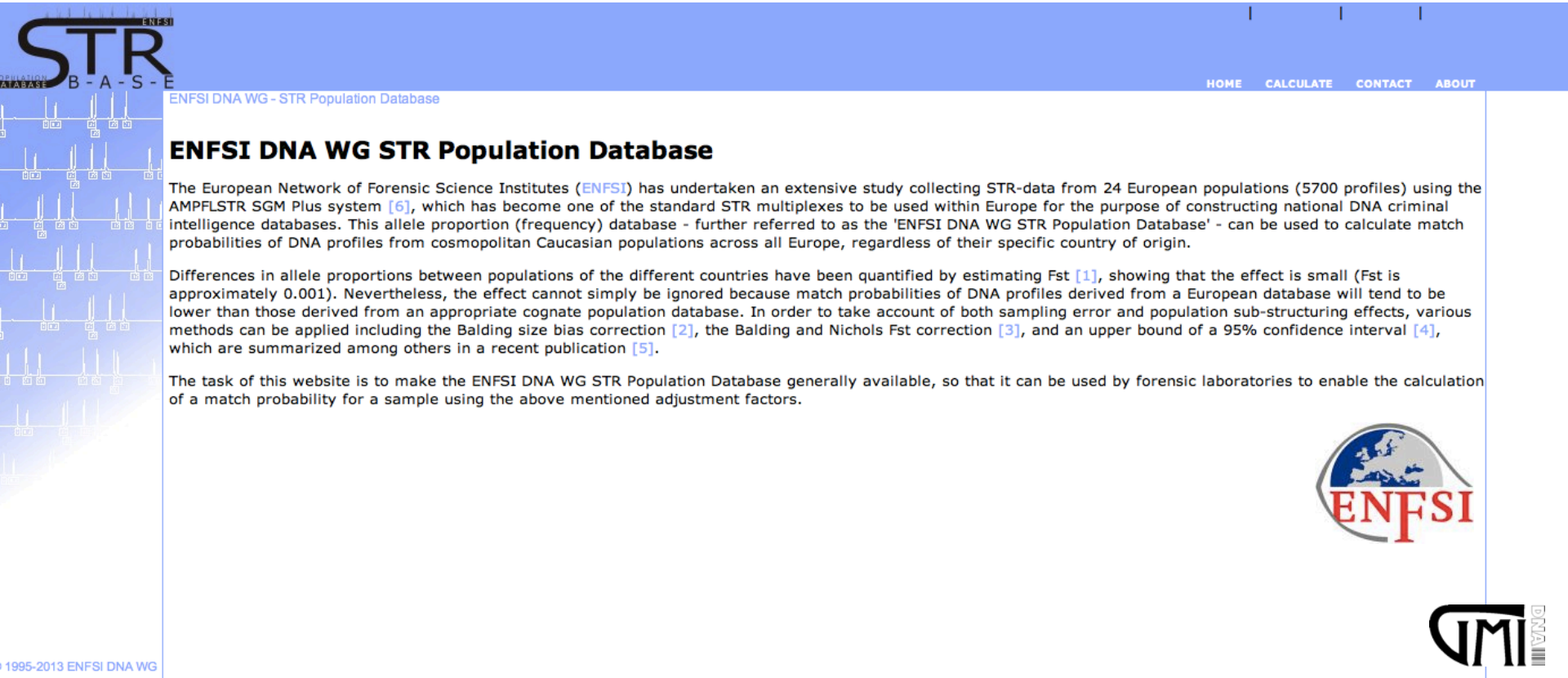
walther.parson@gmail.com

History

2001 Collaborative ENFSI DNA WG population study SGMplus
24 populations, approx. 5700 samples

2003 Gill et al FSI 131 (2003) 184-196

2004 STRbase V1 (GMI funded)



Formulae

A comparison of adjustment methods to test the robustness of an STR DNA database comprised of 24 European populations

Peter Gill[a,*], Lindsey Foreman[b], John S. Buckleton[c], Christopher M. Triggs[d], Heather Allen[a]

Actual matching probability

$P_m = 2p_ip_j$	Heterozygotes
$P_m = p_i^2$	Homocygotes
$P_m = 2p_i - p_i^2$	Single alleles

Balding & Nichols (1994)

$P_m = \frac{2(\Theta + (1-\Theta)p_i)(\Theta + (1-\Theta)p_j)}{(1+\Theta)(1+2\Theta)}$	Balding-Nichols heterozygotes
$P_m = \frac{(2\Theta + (1-\Theta)p_i)(3\Theta + (1-\Theta)p_i)}{(1+\Theta)(1+2\Theta)}$	Balding-Nichols homocygotes
$P_m = \frac{(2\Theta + (1-\Theta)p_i)(3\Theta + (1-\Theta)p_i)}{(1+\Theta)(1+2\Theta)}$	Balding-Nichols single alleles

Balding size bias correction (1995)

$P_m = \frac{2(x_i+2)(x_j+2)}{(n+4)^2}$	heterozygotes
$P_m = \frac{(x_i+2)^2}{(n+4)^2}$	homocygotes

Confidence Intervals (NRC-Report 1996)

$Var(ln(2p_ip_j)) \approx \frac{p_i + p_j - 4p_ip_j}{2Np_ip_j}$	Confidence interval heterozygotes
$Var(ln(2p_i^2)) \approx \frac{2(1-p_i)}{Np_i}$	Confidence interval homocygotes
$Var(ln(2p_i - p_i^2)) \approx \frac{2(1-p_i)^3}{Np_i(2-p_i)^2}$	Confidence interval single alleles

$\Gamma = \sqrt{V_1 + V_2 + ... + V_k}$

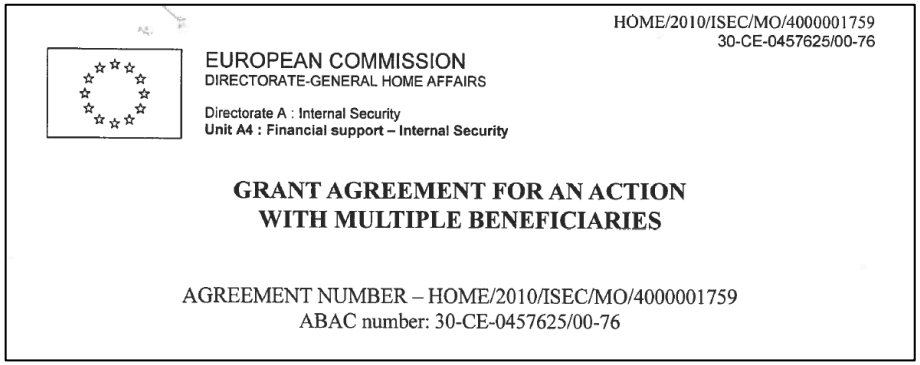
$Upperbound = log^{-1}(log_{10}(P_m)) + 1.96\Gamma$

Query genotype added to database

STRbASE

Successful Monopoly 2010 Application

„Upgrading the ENFSI STRbase“



HOME/2010/ISEC/MO/4000001759
30-CE-0457625/00-76

EUROPEAN COMMISSION
DIRECTORATE-GENERAL HOME AFFAIRS

Directorate A : Internal Security
Unit A4 : Financial support – Internal Security

GRANT AGREEMENT FOR AN ACTION WITH MULTIPLE BENEFICIARIES

AGREEMENT NUMBER – HOME/2010/ISEC/MO/4000001759
ABAC number: 30-CE-0457625/00-76

Financial aspect:

Personnel cost	0 Euro
IT-Hardware	19,000 Euro
depreciation rate	7,499 Euro
ENFSI DNA WG	3,000 Euro
	10,499 Euro

Operational aspect:

Application 2010
Project start 2012
Project end 2014

STRbASE

Successful Monopoly 2010 Application

„Upgrading the ENFSI STRbase“

Project Activity Timeline Form (ENFSI Monopoly Programme Bid 2010)

Project Title : Upgrading the ENFSI STR BASE

Project Leader : Ingo Bastisch

Project Activity	MONTH (YEAR 1)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Jän.12	Feb.12	Mär.12	Apr.12	Mai.12	Jun.12	Jul.12	Aug.12	Sep.12	Okt.12	Nov.12	Dez.12
STR loci												
Collation of STR population data, review process												
Population genetic analysis												
Dissemination of data												
New database software												
Review of current web architecture and software												
Server infrastructure/setup												
Analysis & specification of requirements												
Database design (ERM)												
Development												
Quality Control, software testing, documentation												
Deployment of new software												

Project Activity	MONTH (YEAR 2)											
	13	14	15	16	17	18	19	20	21	22	23	24
	Jän.13	Feb.13	Mär.13	Apr.13	Mai.13	Jun.13	Jul.13	Aug.13	Sep.13	Okt.13	Nov.13	Dez.13
STR loci												
Collation of STR population data, review process												
Population genetic analysis												
Dissemination of data												
New database software												
Review of current web architecture and software												
Server infrastructure/setup												
Analysis & specification of requirements												
Database design (ERM)												
Development												
Quality Control, software testing, documentation												
Deployment of new software												

Project Activity	MONTH (YEAR 3)											
	25	26	27	28	29	30	31	32	33	34	35	36
	Jän.14	Feb.14	Mär.14	Apr.14	Mai.14	Jun.14	Jul.14	Aug.14	Sep.14	Okt.14	Nov.14	Dez.14
STR loci												
Collation of STR population data, review process												
Population genetic analysis												
Dissemination of data												
New database software												
Review of current web architecture and software												
Server infrastructure/setup												
Analysis & specification of requirements												
Database design (ERM)												
Development												
Quality Control, software testing, documentation												
Deployment of new software												

STRbASE

Extension of STR-Markers

Forensic Science International: Genetics 6 (2012) 819–826

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

European Network of Forensic Science Institutes (ENFSI): Evaluation of new commercial STR multiplexes that include the European Standard Set (ESS) of markers

L.A. Welch [a,*], P. Gill [b,i,**], C. Phillips [c], R. Ansell [d], N. Morling [e], W. Parson [f], J.U. Palo [g], I. Bastisch [h]

[a] Centre for Forensic Science, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow G42 9TA, United Kingdom
[b] Department of Forensic Genetics, Norwegian Institute of Public Health, Oslo, Norway
[c] Forensic Genetics Unit, University of Santiago de Compostela, Spain
[d] Swedish National Laboratory of Forensic Science, Sweden
[e] Department of Forensic Medicine, University of Copenhagen, Denmark
[f] Institute of Legal Medicine, Innsbruck Medical University, Muellerstrasse 44, 6020 Innsbruck, Austria
[g] Laboratory of Forensic Biology, University of Helsinki, Finland
[h] Bundeskriminalamt, Germany
[i] University of Oslo, Oslo, Norway

High quality STR genotypes

STRbASE

ENFSI DNA WG
STR Population Database, v2

Dec 17 2014

QUERY ... ABOUT FREQUENCIES FORMULAE CONTACT TERMS OF USE

SGMplus
Identifiler
Powerplex 16
Powerplex 18
Powerplex 21
Fusion
ESSplex
ESSplex SE
NGM
NGM-SE
ESI-16
ESX-16
ESI-17
ESX-17
✓ Globalfiler

Query

The second ... STR loci defined in the specifications of the ENFSI DNA WG. Additional loci included in commercia... high quality population data are available. Those loci are dimmed in the input form.
More...

Kit

FST 0.01 output with comma as decimal separator

D3S1358 VWA D16S539 CSF1PO TPOX

Y indel D8S1179 D21S11 D18S51 DYS391

D2S441 D19S433 TH01 FGA

D22S1045 D5S818 D13S317 D7S820 SE33

- check/uncheck all
- AUSTRIA
- BELGIUM
- BOSNIA AND HERZEGOWINA
- CZECH REPUBLIC
- DENMARK
- FINLAND
- FRANCE
- GERMANY
- GREECE
- HUNGARY
- IRELAND
- MONTENEGRO
- NORWAY
- POLAND
- SLOVAKIA

STRbASE

ENFSI DNA WG
STR Population Database, v2

QUERY | BATCH QUERY | ABOUT | FREQUENCIES | FORMULAE | CONTACT | TERMS OF USE

The CSV file requires *commas (,)* as delimiters and *double quotes (")* as field enclosure characters.
Download a sample CSV file.

FST 0.01 output with comma as decimal separator ☐

File format ◉ CSV ○ GeneMapper

CSV file Datei auswählen Keine Datei ausgewählt

☑ check/uncheck all
- ☐ AUSTRIA
- ☐ BELGIUM
- ☐ BOSNIA AND HERZEGOWINA
- ☐ CZECH REPUBLIC
- ☐ DENMARK
- ☐ FINLAND
- ☐ FRANCE
- ☐ GERMANY

Query | sample1 | sample2 | sample3 | sample4 | sample5 | sample6 | sample7 | sample8 | sample9 | sample10 | sample11 | sample12

Origin	Actual Matching Probability	Balding Size Bias Correction	Balding & Nichols FST	NRC II Upper Bound Confidence Intervals
AUSTRIA	3.7701e-11	4.9920e-11	1.1486e-10	2.0862e-10
BELGIUM	3.7032e-11	5.1496e-11	1.2325e-10	2.3834e-10
CZECH REPUBLIC	5.1497e-11	6.9368e-11	1.4980e-10	3.0055e-10
FRANCE	1.2532e-8	1.5323e-8	2.6431e-8	5.3260e-8
GERMANY	5.4901e-11	6.0192e-11	1.5974e-10	1.4510e-10
HUNGARY	4.0100e-11	5.4661e-11	1.3480e-10	2.4414e-10
SLOVAKIA	1.0727e-10	1.3608e-10	3.0290e-10	5.1774e-10
SLOVENIA	5.9386e-9	7.4287e-9	1.3523e-8	2.7422e-8
AUSTRIA, BELGIUM, CZECH REPUBLIC, FRANCE, GERMANY, HUNGARY, SLOVAKIA, SLOVENIA	4.7203e-11	4.8699e-11	1.4265e-10	8.3008e-11
Europe	4.5991e-11	4.6677e-11	1.3899e-10	6.7821e-11
Entire Database	4.5991e-11	4.6677e-11	1.3899e-10	6.7821e-11

Frequencies

These tables include allele frequencies and number of samples (n) from the most recent database release sorted by marker and country.
This data can be downloaded as XML file.

VWA

Allele	AUSTRIA	BELGIUM	BOSNIA AND HERZEGOWINA	CZECH REPUBLIC	DENMARK	FINLAND	FRANCE	GERMANY	GREECE	HUNGARY	IRELAND	MONTENEGRO	NORWAY	PO
	222	206	171	200	200	230	208	662	208	224	304	200	202	
11								7.5529e-4						
12									4.8077e-3					
13			1.1696e-2					2.2659e-3	2.4038e-3	2.2321e-3			2.4753e-3	
14	1.0586e-1	1.0680e-1	1.1111e-1	1.0000e-1	7.0000e-2	1.3043e-1	8.6539e-2	9.7432e-2	9.3750e-2	1.1161e-1	1.1349e-1	1.4500e-1	8.6634e-2	7.76
15	9.2342e-2	1.2136e-1	1.2573e-1	9.7500e-2	9.7500e-2	5.2174e-2	1.2740e-1	1.0347e-1	7.9327e-2	1.1384e-1	1.0197e-1	9.0000e-2	9.9010e-2	8.49
16	1.7568e-1	1.9903e-1	2.0468e-1	1.7500e-1	2.6000e-1	1.7609e-1	2.4038e-1	2.2130e-1	1.6827e-1	2.0536e-1	2.1875e-1	1.7500e-1	2.2277e-1	2.23
17	2.8604e-1	2.7185e-1	2.3977e-1	3.1250e-1	2.3000e-1	2.7174e-1	2.3317e-1	2.5453e-1	3.1731e-1	3.0134e-1	2.7138e-1	2.8750e-1	2.8960e-1	2.76
18	2.5901e-1	2.0146e-1	2.1053e-1	2.2750e-1	2.4000e-1	2.0435e-1	2.1154e-1	2.2054e-1	2.4279e-1	1.7634e-1	1.9243e-1	2.1250e-1	1.9802e-1	2.47
19	7.2072e-2	8.0097e-2	9.0643e-2	7.2500e-2	8.2500e-2	1.3696e-1	8.6539e-2	8.6103e-2	7.4519e-2	7.1429e-2	9.3750e-2	7.2500e-2	8.6634e-2	8.00
20	9.0090e-3	1.9418e-2	5.8480e-3	1.5000e-2	1.7500e-2	2.1739e-2	1.4423e-2	1.2840e-2	1.4423e-2	1.5625e-2	8.2237e-3	1.7500e-2	1.4852e-2	9.70
21					2.5000e-3	6.5217e-3		7.5529e-4	2.4038e-3	2.2321e-3				

TH01

Allele	AUSTRIA	BELGIUM	BOSNIA AND HERZEGOWINA	CZECH REPUBLIC	DENMARK	FINLAND	FRANCE	GERMANY	GREECE	HUNGARY	IRELAND	MONTENEGRO	NORWAY	POLA
	222	206	171	200	200	230	208	662	208	224	304	200	202	
5	2.2522e-3	2.4272e-3						1.5106e-3		2.2321e-3			2.4753e-3	

Future developments

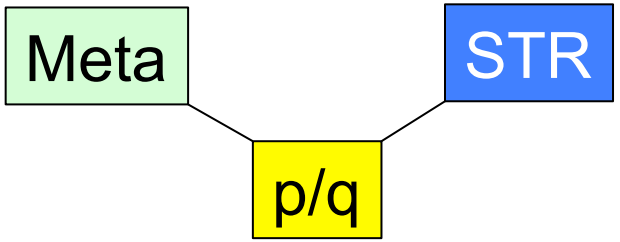
Further extension of markers and populations

EMPOP example

Quality control of datasets by database curators

Provision of (shuffled) genotypes via the database

Sample ID	TH01	TH01	VWA	VWA	FGA	FGA
Anonym0001	6	9.3	16	17	20	24
Anonym0002	7	7	13	14	18	18
Anonym0003	8	9	12	16	22	24
Anonym0004	9.3	9.3	11	13	19	20
Anonym0005	7	9.3	12	18	23	24



Current discussions in EDNAP, ENFSI and with FSIG

Adaptation of formulae

Currently based on ENFSI study and paper 2003

More/alternative approaches to be included

Collaboration with research groups

Layout may be subject to changes to easy readability

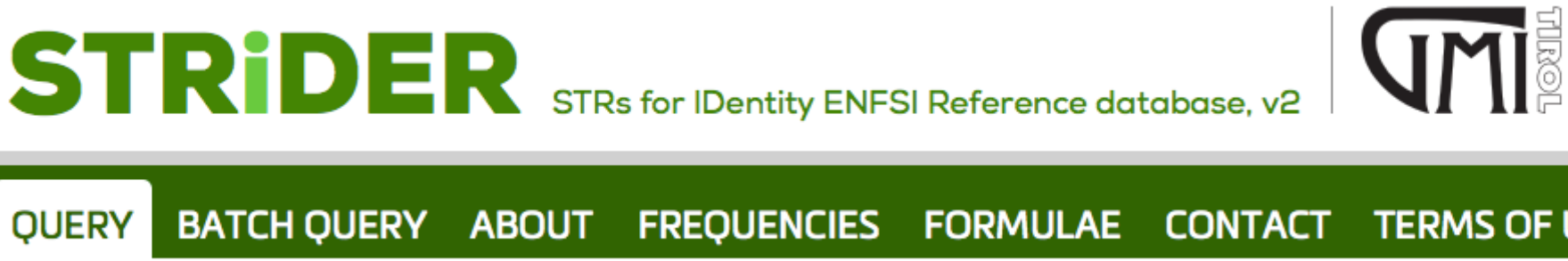
Future developments

Funding application Monopoly 2014

- International application (AT, CZ, DE, ES, FR, NL, NO, PL, SE)
- New query engine (string-based) to meet NGS formats
- Extension of STR maskers and populations
- Update and provision of online quality control tools
- User-friendly access from other platforms (mobile devices)
- Link to other software packages (LRmix, …)

Proposal currently on waiting list (Apr 2015)

New name to avoid confusion with NIST STRbase



ack. Chris Phillips, USC

1. To achieve a steady, effective pace
2. To attain a maximum level of competence

EUROFORGEN-NoE Update: EDNAP Meeting Copenhagen 2015

Peter M. Schneider

Institute of Legal Medicine

University of Cologne (Germany)

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Recent activities

- **Expansion of the EUROFORGEN Consortium**
  - 3 new projects with 4 partners
- **Study on DNA profiling success rates**
  - Based on actual casework results
- **The Virtual Institute**
  - and how to get there
- **Dissemination and Training news**
  - and other sources of support

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These 3 projects and 4 partners have been accepted:

- **EP4:** Dr. C. Haas, Zürich
  "Association of a Body Fluid with a DNA Profile by Targeted RNA and DNA Deep Sequencing"
- **EP5:** Dr. M. Vennemann, Münster; Dr. L. Dennany, Glasgow
  "Development of innovative electrochemical biosensor technologies for the detection of tissue specific DNA methylation“
- **EP6:** Prof. M. Kayser, Rotterdam
  "Forensic DNA phenotyping of hair structure for investigative purposes"

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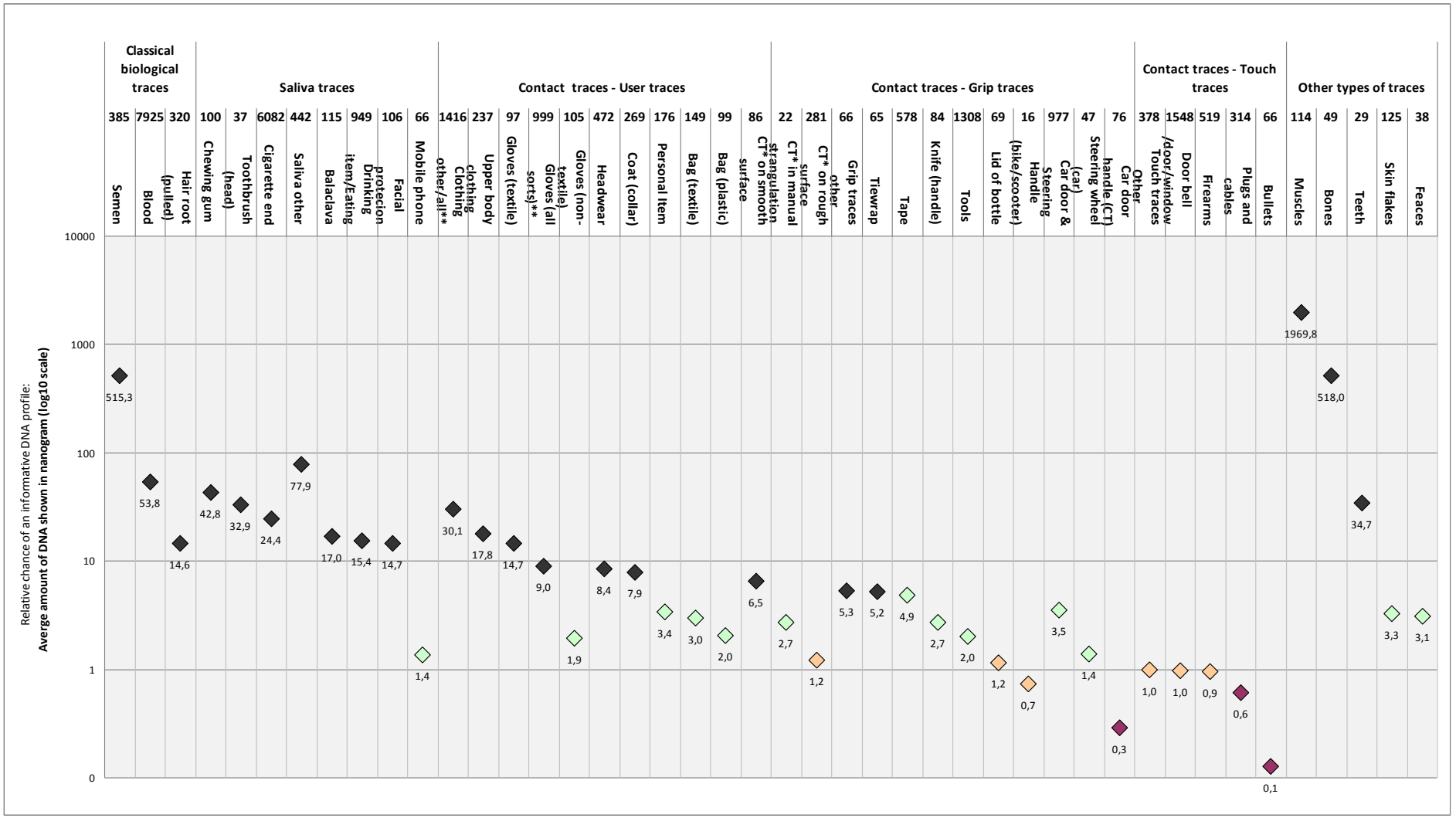
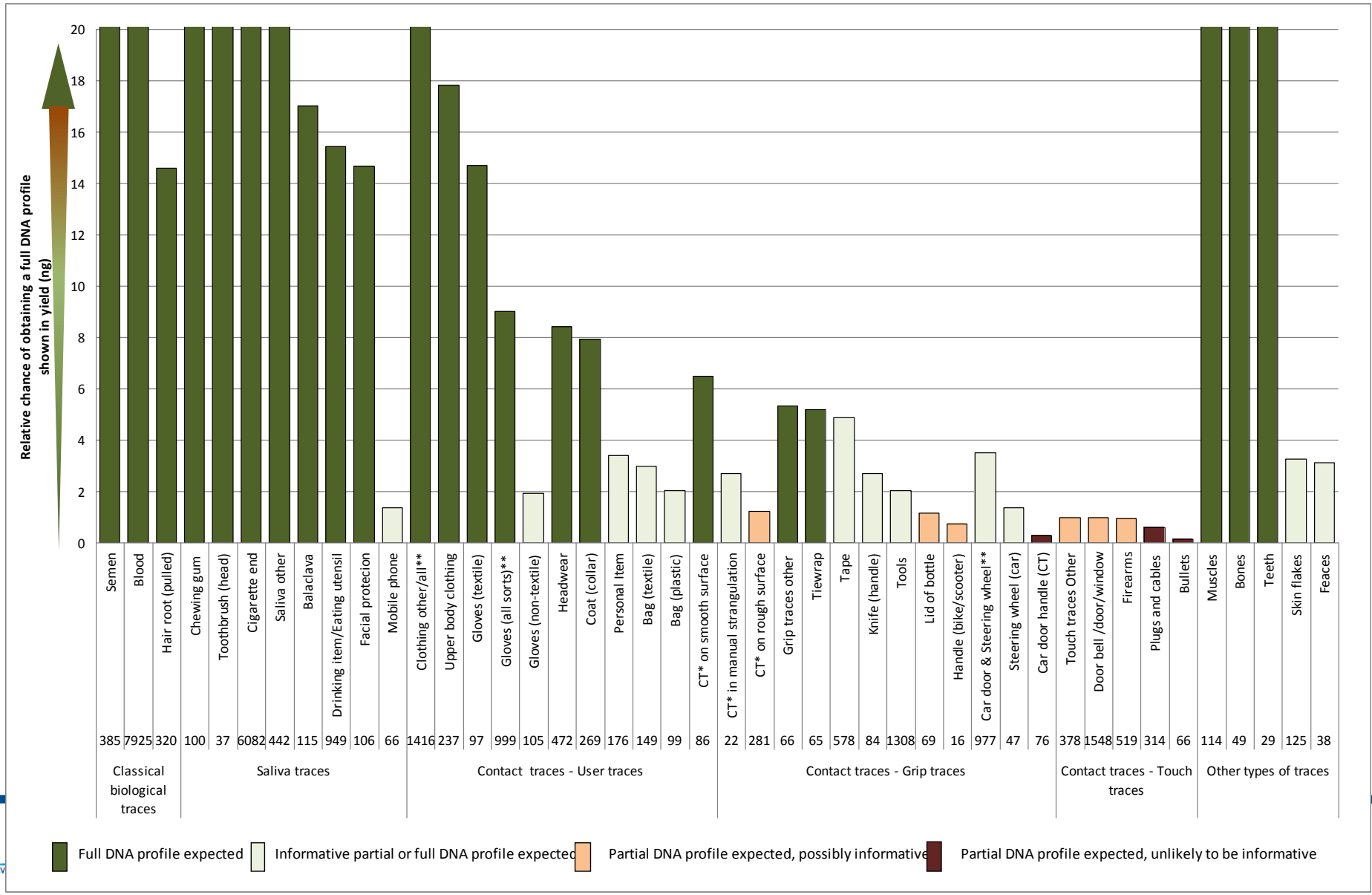
WP3-EP1: Study on DNA profiling success rates (NFI)

- To determine the relative chance of obtaining a DNA profile per sample category using data of six EUROFORGEN laboratories.
- Data were compiled by the NFI – report to be published
- A total of 27,401 casework samples analysed after December 2012 in six forensic laboratories is used.
- 44 categories of typical crime scene samples, each sample category containing data from 16 to 7,925 samples.
- Blood stains, cigarette ends and the collar of a coat are more useful sample types than the handle of a knife, a plastic bag or ‘plugs and cables’.
- 32% of analysed samples are contact traces, and about 20% produce informative partial/full profiles

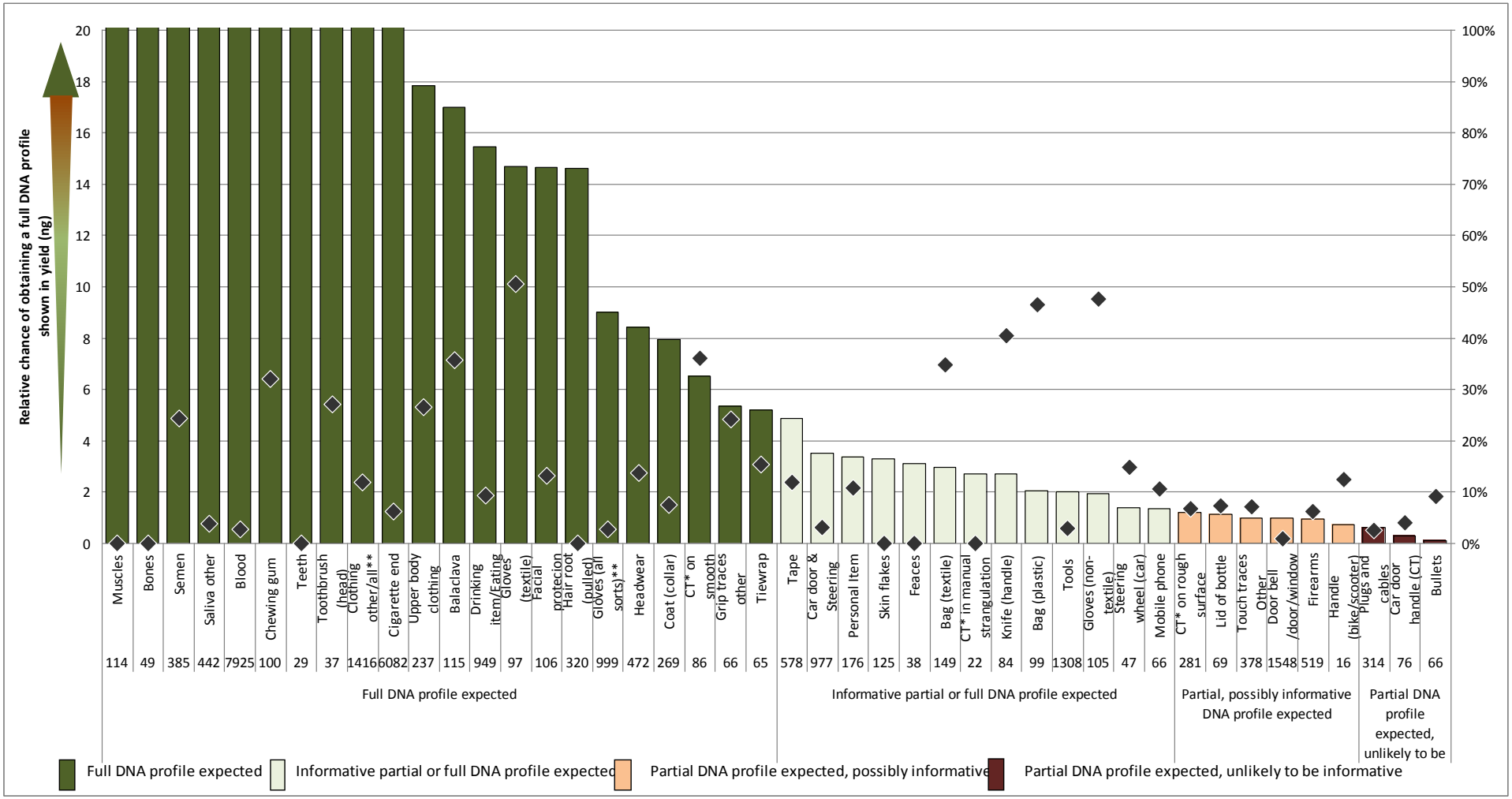
Average total DNA yield in ng of six laboratories – log10 scale



DNA profiling success rates of typical crime scene samples



DNA profiling success rates of typical crime scene samples including mixture information



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DNA yields based on sample types (I)

Expected results	Average yield (ng)	Sample category	Details regarding sample category	% Samples in dataset	% mixtures
Full profile	1969,8	Muscles	Usually obtained from unidentified bodies	0,4%	0%
	518,0	Bones	Usually obtained from unidentified bodies	0,2%	0%
	515,3	Semen	Intimate swabs and stains	1,4%	24%
	77,9	Saliva other	Spit, toothpicks, apple cores, drinking straws, etc.	1,6%	4%
	53,8	Blood	All stain sizes	28,9%	3%
	42,8	Chewing gum		0,4%	32%
	34,7	Teeth		0,1%	0%
	32,9	Toothbrush (head)		0,1%	27%
	30,1	Clothing other/all	Data originating from laboratories not using subcategories	5,2%	12%
	24,4	Cigarette end	Ends of cigarette, sigar end smoked joints	22,2%	6%
	17,8	Upper body clothing	Blouse, t-shirts, sweaters , etc.	0,9%	27%
	17,0	Balaclava	Can contain both saliva and epithelial cells	0,4%	36%
	15,4	Drinking item/Eating utensil	Plastic bottles, glasses, coffee cups, cans, spoons, etc.	3,5%	9%
	14,7	Gloves (all sorts)	Data originating from laboratories not using subcategories	0,4%	51%
	14,7	Facial protecion	Mouthcaps, safety glasses, facial masks, etc.	0,4%	13%
	14,6	Hair root (pulled)		1,2%	0%
	9,0	Gloves (textile)	Non disposible multiple wear gloves, etc.	3,6%	3%
	8,4	Headwear	Caps, hats, helmets, etc.	1,7%	14%
	7,9	Coat (collar)		1,0%	7%
	6,5	Contact trace on smooth surface		0,3%	36%
	5,3	Grip traces other	Grabbed clothing , flashlight, etc.	0,2%	24%
	5,2	Tiewrap		0,2%	15%

DNA yields based on sample types (II)

Expected results	Average yield (ng)	Sample category	Details regarding sample category	% Samples in dataset	% mixtures
Informative partial/full profile	4,9	Tape		2,1%	12%
	3,5	Car door & Steering wheel	Data originating from laboratories not using subcategories	3,6%	3%
	3,4	Personal Item	Watches, (sun)glasses, jewelery, etc.	0,6%	11%
	3,3	Skin flakes	Epithelial cells, for instance dandruff	0,5%	0%
	3,1	Feaces		0,1%	0%
	3,0	Bag (textile)	Bags designed for long(er)term use	0,5%	35%
	2,7	Contact trace in manual strangulation	DNA collected from the perpetrator of the skin of the victim	0,1%	0%
	2,7	Knife (handle)		0,3%	40%
	2,0	Bag (plastic)	Largely disposable bags, single use	0,4%	46%
	2,0	Tools	Screwdrivers, crowbars, hammers, etc.	4,8%	3%
	1,9	Gloves (non-textile)	Disposable gloves for example worn in drugslabs	0,4%	48%
	1,4	Steering wheel (car)		0,2%	15%
	1,4	Mobile phone		0,2%	11%
Partial, possibly informative profile	1,2	Contact trace on rough surface	Stones, bricks, etc.	1,0%	7%
	1,2	Lid of bottle	Lids from drinking bottles or items like jerrycans	0,3%	7%
	1,0	Touch traces other		1,4%	7%
	1,0	Door bell /door/window		5,6%	1%
	0,9	Firearms		1,9%	6%
	0,7	Handle (bike/scooter)		0,1%	13%
Partial profile, unlikely to be informative	0,6	Plugs and cables	Battery chargers, electric tools, etc.	1,1%	3%
	0,3	Car door handle (CT)		0,3%	4%
	0,1	Bullets		0,2%	9%

The Virtual Institute of Research for Forensic Genetics

EUROFORGEN
Network of Excellence

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  - Recommended open software
  - Train-the-trainers section

Virtual Institute for Forensic Genetic Research in Europe

Our website will provide a framework for exchange of expertise and data, not only between consortium members but with any other individuals or institutions working in forensic genetics in Europe. It will bring together the knowledge and resources centered on forensic genetics tools and education at a European level, and allow researchers, forensic practitioners, stakeholders and legal experts to interact with the network. Currently, the following resources are available:

- **EUROFORGEN Course Material>: Up-to-date lectures and presentations on major topics of forensic genetics derived from the "Train the Trainers" workshop series.**
- **EUROFORGEN publications>:** Original publications from EUROFORGEN Consortium members available for downloading.
- **Recommended Open Software>:** a list with open software tools is displayed together with a brief description on their applications.
- **Train-the-Trainers Section>:** it contains the "TTT Blog", a discussion forum to post comments and questions related to training issues, to get directly into contact with the EUROFORGEN trainer team.

Please use the blog for your feedback, and your suggestions for improvement. The contents will be regularly updated and expanded.

Your EUROFORGEN-NoE team.

Quicklinks

You can apply for → **short term fellowships**.

The European landscape in forensic genetics: → **Geographical display and contact data**

→ The winners are found! The EUROFORGEN competitive call review led to 3 proposals with highest score.

Newsletter (4/2014)

Dowload here

Consortium

- **Dedicated "for members only" area of website**
  - Can only accessed after individual registration, and obtaining a user name and password
  - All colleagues working in institutions that have submitted their contact data by submitting a questionnaire in the initial inquiry will be admitted
  - Please do not hesitate to inquire if you are not sure about the participation of your lab!

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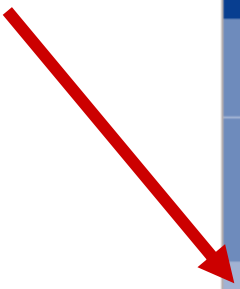
The Virtual Institute of Research for Forensic Genetics

EUROFORGEN Network of Excellence



EUROFORGEN Network of Excellence

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- The Project
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  - European landscape in forensic genetics
  - Directory of Forensic Genetic Research Laboratories in Europe
  - European Virtual Institute of Research in Forensic Genetics
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European Virtual Institute of Research in Forensic Genetics - access query

You are interested in becoming a member of the European Virtual Institute of Research in Forensic Genetics?

If you are a scientist working at a forensic genetics laboratory, or a professional working in an institution of the justice system, you are invited to join the Virtual Institute. Please see our Newsletter 3/2014 for further details.

Please enter your personal contact data, and the data of your institution below. We will verify your request and come back to you in the following days.

One requirement to get access to the EUROFORGEN-NoE Virtual Institute of Research in Forensic Genetics is the participation of your institution by submitting the EUROFORGEN-NoE questionnaire.

Your EUROFORGEN-NoE team.

- **Privileged access to new content:**
  - **Course Material:** Up-to-date lectures and presentations on major topics of forensic genetics derived from the "Train the Trainers" workshop series.
  - **Publications:** Original publications (PDF) from Consortium members available for downloading.
  - **Open Software:** a list with open source / accessible software tools is displayed together with a brief description on their applications.
  - **Train-the-Trainers Section:** a discussion forum to post comments and questions related to training issues, to get directly into contact with the EUROFORGEN trainer team.

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WP4: Ethical and legal aspects, and the societal dimension of forensic genetics - Publications

- **Ethical, Social and Policy Aspects of Forensic Genetics: A Systematic Review**
  - on history of forensic genetics, situates current research in this field within the broader research and innovation agenda of the EU
- **Public perspectives on established and emerging forensic genetics technologies in Europe: A preliminary report**
  - insights into some discussions around public perspectives on forensic genetic technologies, as well as an introduction to the diverse range of organized public actors interested in forensic genetics
- **A comparative audit of legislative frameworks within the European Union for the collection, retention and use of forensic DNA profiles**
  - a number of countries have started to amend and revise existing laws – in most cases to facilitate the use of DNA data and the database storage of DNA profiles from suspects and convicted offenders
  - Will be published soon!

Consortium publications

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WP5: Education, Training and Career Development

- **Three 'Train the Trainers' workshops in Copenhagen**

20-22 participants from all European countries

Subject: Statistical methods in forensic genetics

1st Workshop 7-10 October 2013

2nd Workshop 20-23 May 2014

3rd Workshop 20-23 April 2015

Organized by: Niels Morling

Teachers: Thore Egeland (team leader)

Guro Dørum, Oskar Hansson, Daniel Kling

- **Pre-Congress Workshops at the next ISFG Congress in Kraków 2015 will be supported by EUROFORGEN**

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The Short Term Fellowship Program

- **First Call 2013**
  - 14 fellowships awarded to 13 colleagues from 9 countries
  - Details on website

- **Second Call 2014-2015**
  - 20 new fellowships open
    - Laboratory visits for 3-5 days
    - Active participation in workshops related to EFG aims
    - Other research/training activities related to scope of WPs 2-5
  - Application details on the website
  - Travel support up to EUR 500

… and finally announcing:

- International dissemination conference “DNA in Forensics 2016”
  - **Topics**
    - Integrated presentation of the network's activities
    - Covering results from all work packages
    - Dissemination of the results addressing the relevant stakeholders, end users (police and security agencies, policymakers and to the wider public)
    - A session on ethical, legal and social issues in forensic genetics

Date and place?

  - **Organization**
    - To be organized with entire consortium
    - Open to the public
    - Accepting contributions from the scientific community
    - Details of the conference will be widely announced in order to ensure the attendance of key agencies and journalists across Europe

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find us on Facebook

Please do not forget to join our Facebook group!
… already 194 members!

Thank you very much for your attention!

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within the 7th Framework Programme

EuroForMix

A user-friendly software for evaluating STR/SNP profiles using peak height information

Øyvind Bleka, MSc
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UNIVERSITY OF OSLO

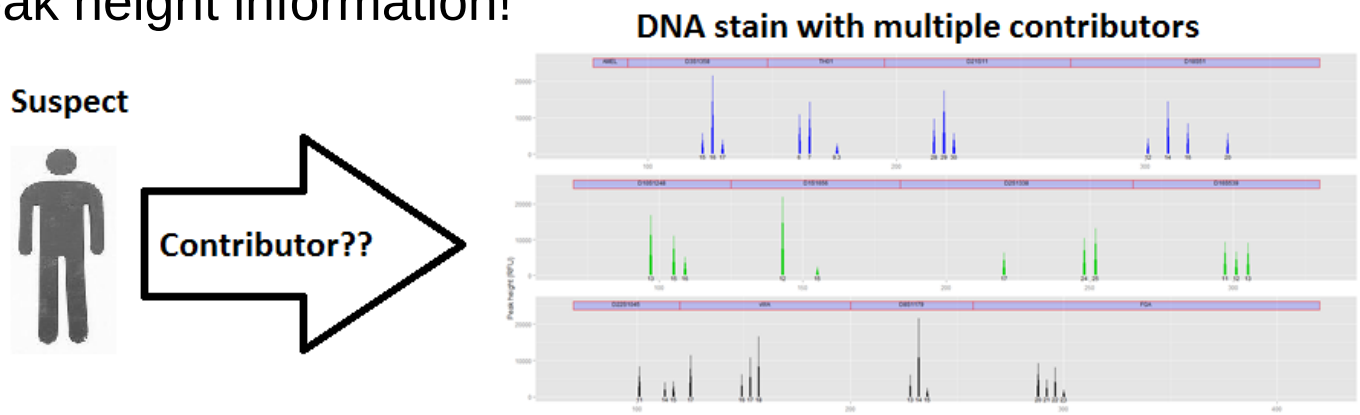
Norwegian Institute of Public Health

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

About EuroForMix

- A Graphical User Interface which implements and extends the continuous model from Cowell et.al (2015).
- Parameters for mixture proportion, peak height distribution, stutter proportion and degradation are automatically taken into account.
- No need for calibration, but prior information can be specified.

- Weight of evidence (WoE) of an obtained crime sample now uses peak height information!



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Features

- The continuous model in EuroForMix supports:
  - Multiple contributors in hypothesis
    - Can condition on any number of reference profiles
    - Can specify any number of unknowns (practical limit is 4)
  - Replicated samples
    - No need for making a consensus sample
  - Stutters
  - Allele drop-out
  - Allele drop-in with a peak height model
  - Coancestry effect (Fst-correction)
  - Degradation of peak heights over fragment length

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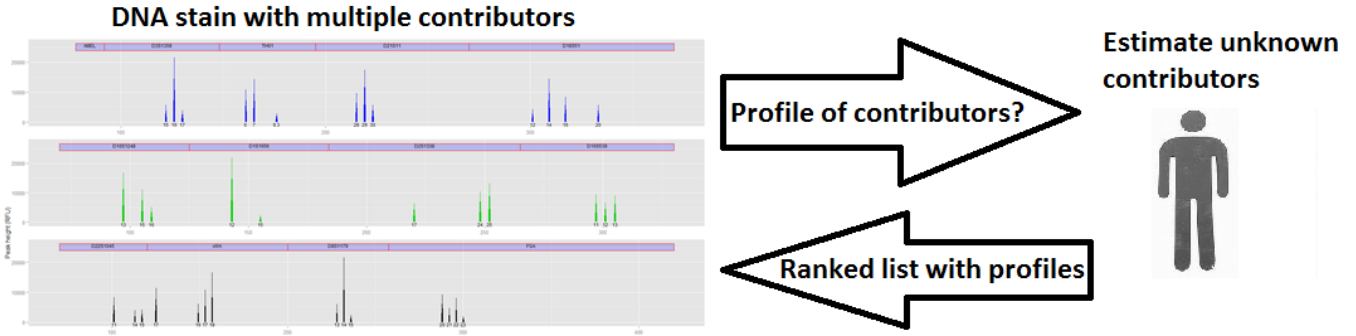
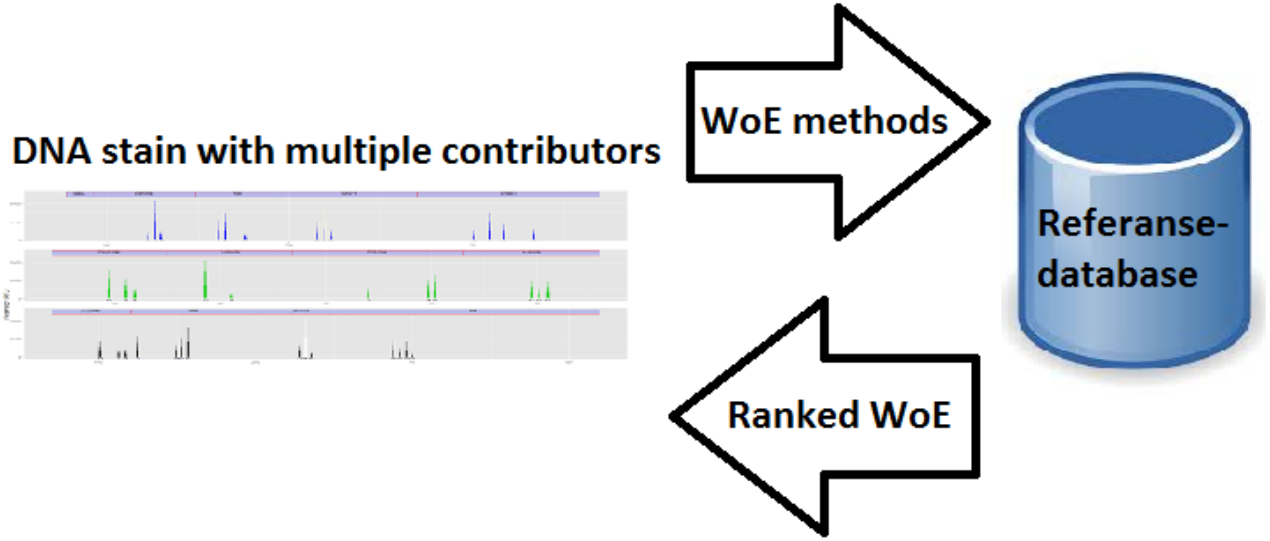
Other applications

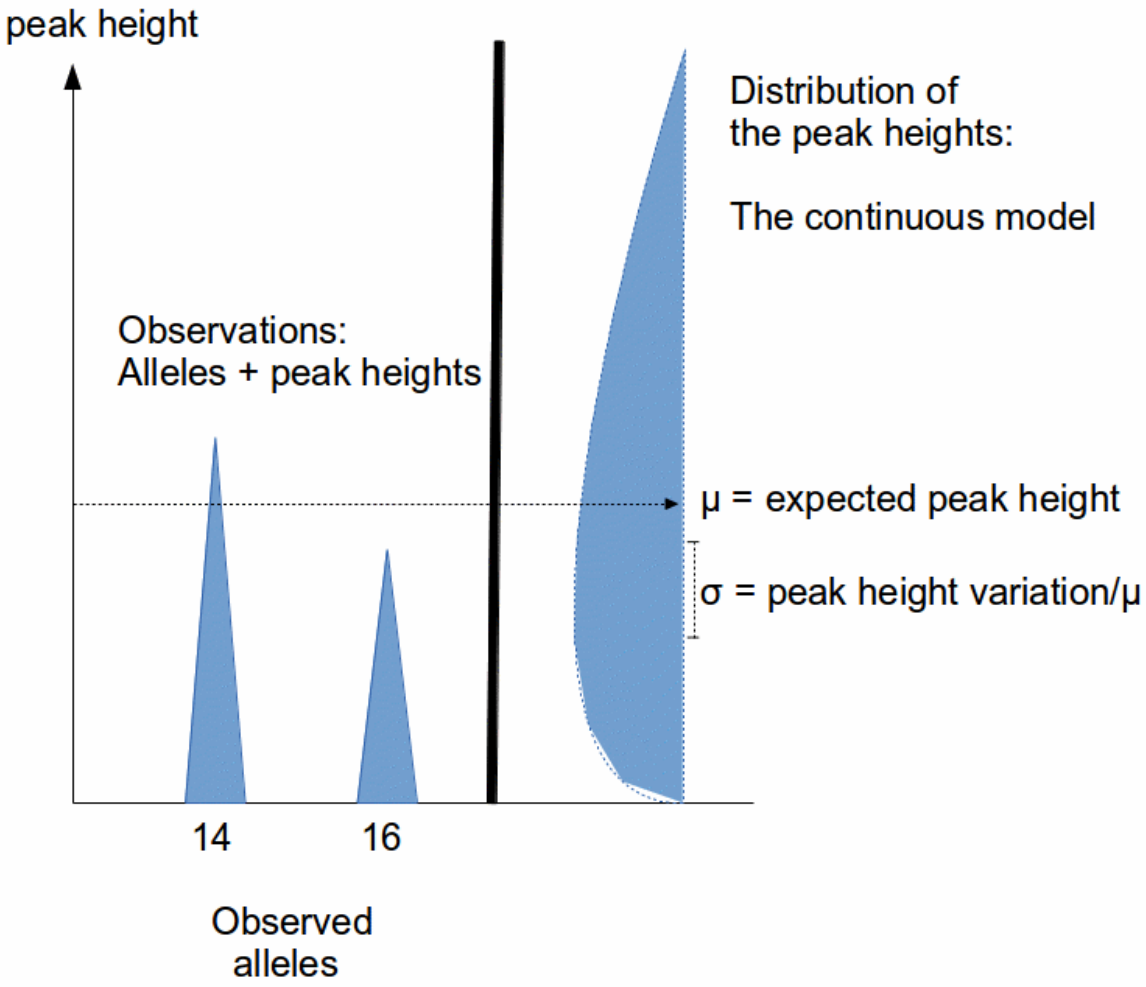
Deconvolution:



Database search:



The continuous model



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Inference approaches

To obtain $\boldsymbol{P}(\boldsymbol{E}|\boldsymbol{H})$, the probability of observed sample $\boldsymbol{E}$ given hypothesis $\boldsymbol{H}$, an inference approach must be applied

- EuroForMix supports two approaches:

Approach 1) Maximum likelihood estimation

$\boldsymbol{P}(\boldsymbol{E}|\boldsymbol{H})$ estimated with $\max_{\theta} p(E|H,\theta)$

Approach 2) Bayesian (integrates out model parameters)

$\boldsymbol{P}(E|H)$ estimated with $\int_{\theta} p(E|H,\theta)p(\theta)d\theta$

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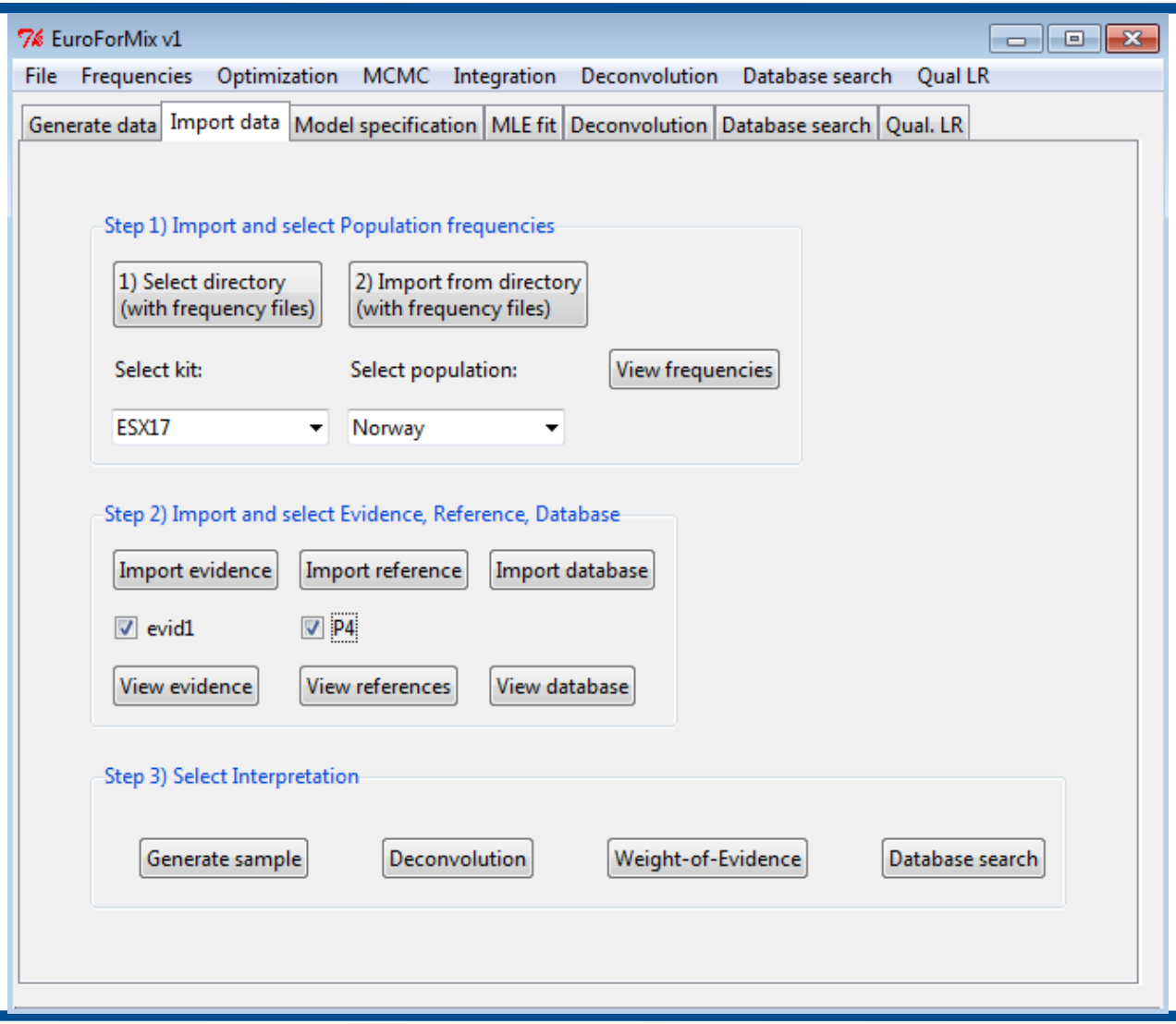
The GUI: Import



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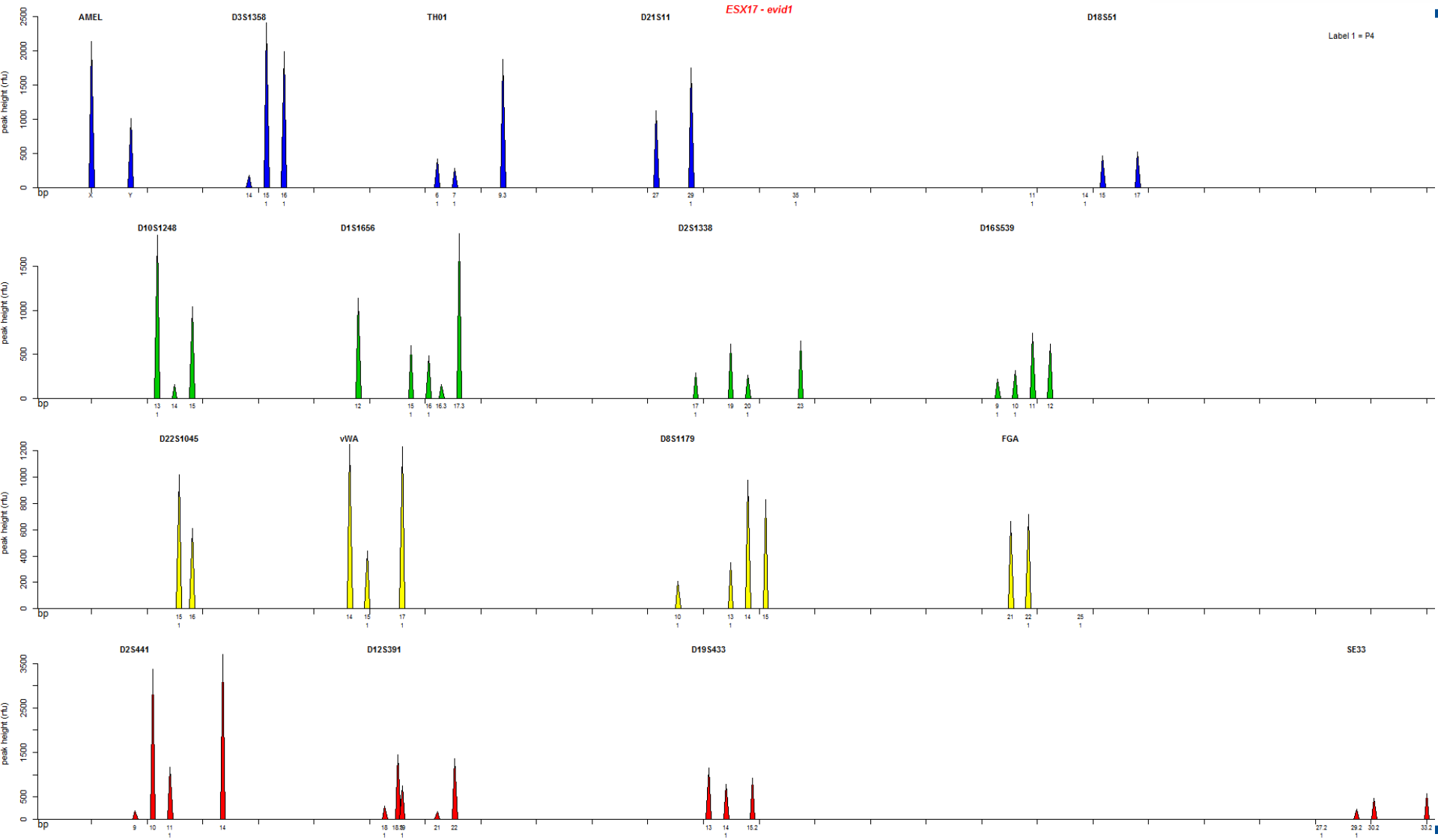
The GUI: View data



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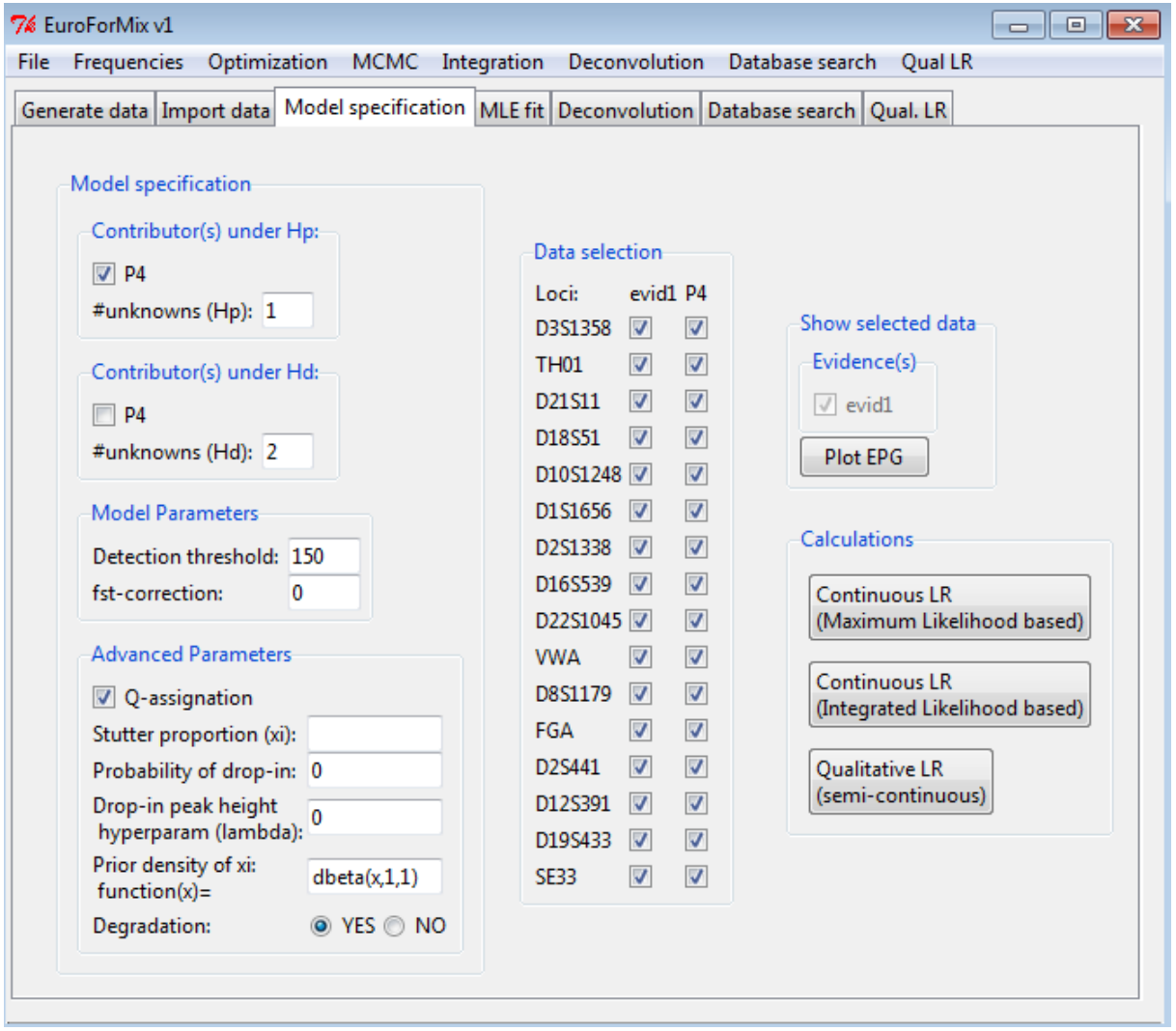
The GUI: Specify the model



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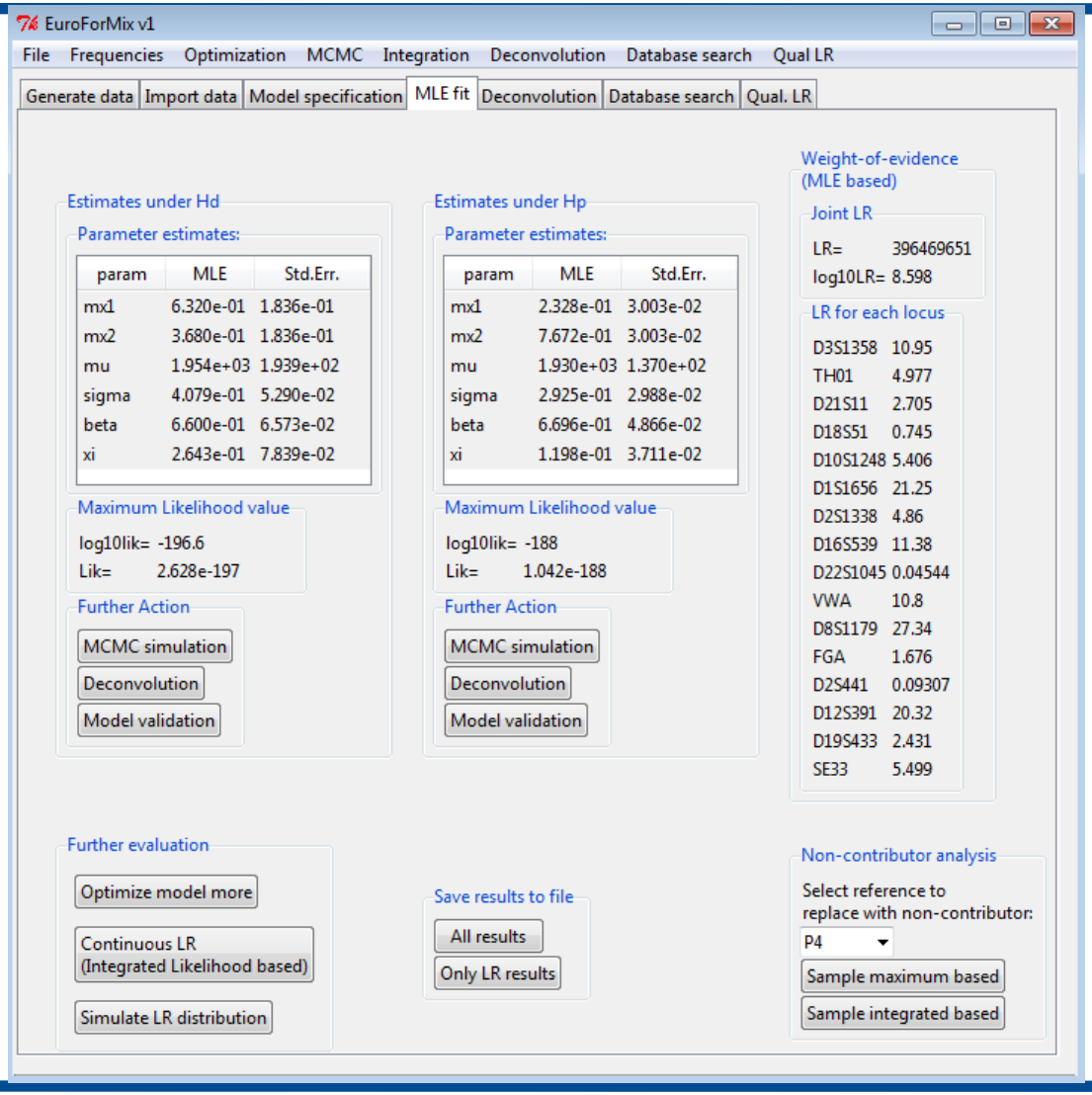
The GUI: Maximum Likelihood estimates



Using 3 random start points:

- Uses **4 seconds**!

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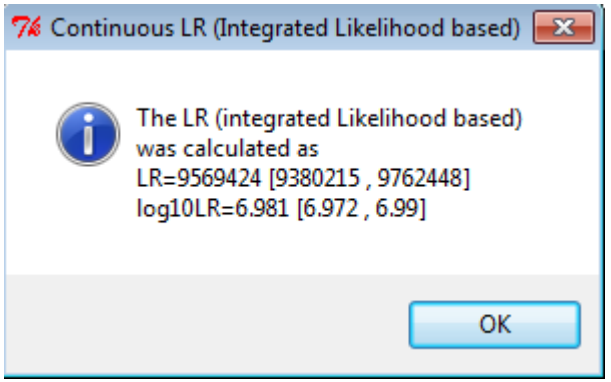
The GUI: Integrated Likelihood (Bayesian)

Using relative error 0.01:

Uses 3:45min receiving

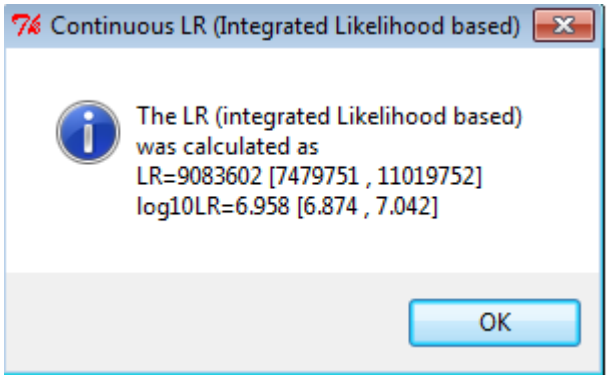
log10= 6.981 [6.972 , 6.99]



Using relative error 0.1:

Uses 0:32min receiving

log10= 6.958 [6.874 , 7.042]



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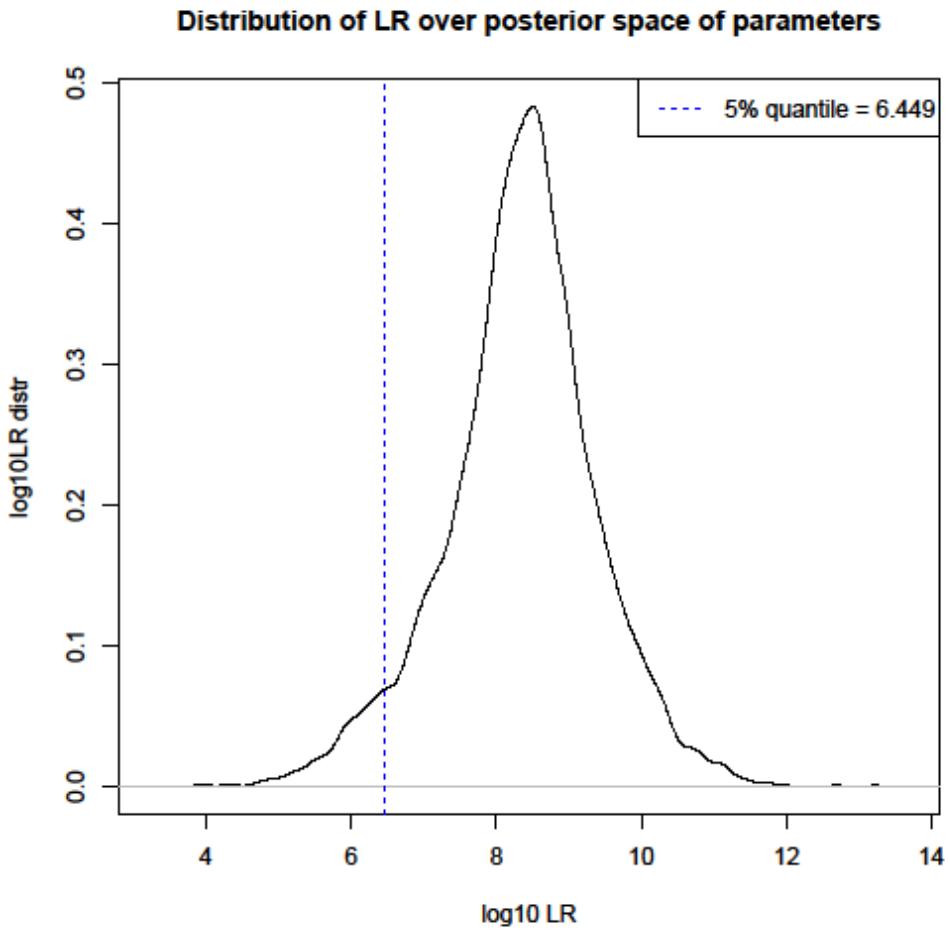
Plot from GUI: Sensitivity analysis



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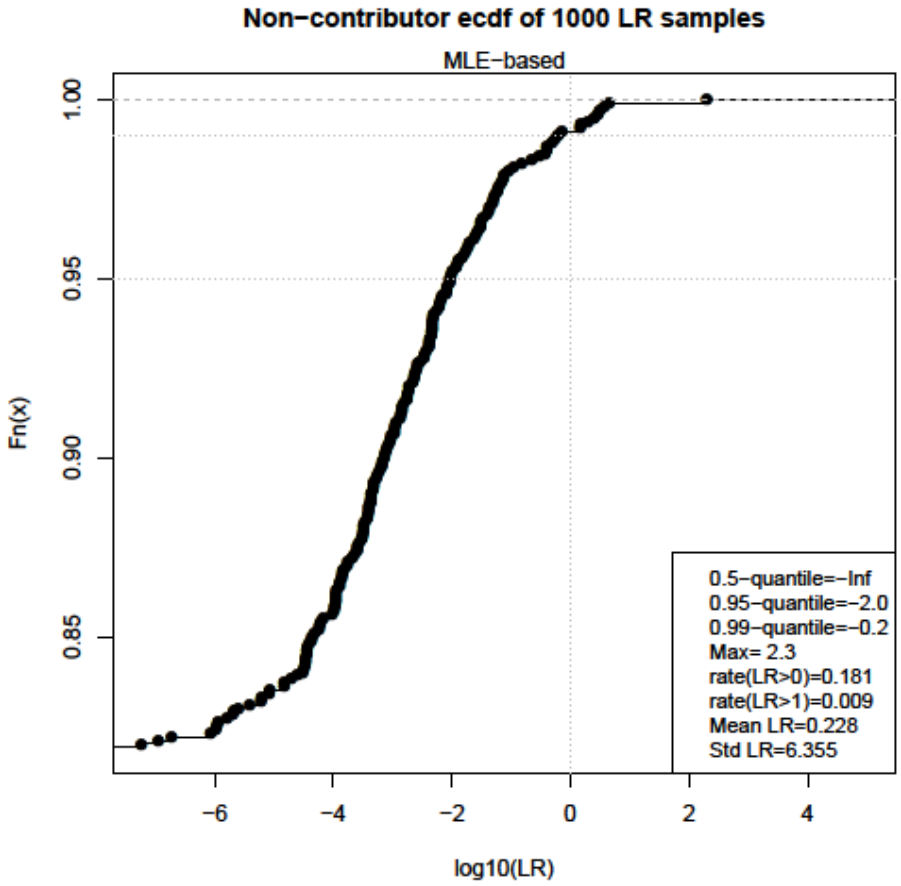
Plot from GUI: Non-contributor analysis

- Used to investigate that the specified model is not overfitting



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The GUI: Deconvolution

EuroForMix v1

File Frequencies Optimization MCMC Integration Deconvolution Database search Qual LR

Generate data | Import data | Model specification | MLE fit | Deconvolution | Database search | Qual. LR

rank	D3S1358	TH01_g2	D21S11_	D18S51_	D10S124	D1S1656_	D2S1338	D16S539	D22S104	VWA_g	D8S1179	FGA_g2	D2S441_	D12S391_	D19S433_	SE33_g2	posterior
1	15/16	9.3/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	14/15	21/22	10/14	18.3/22	13/15.2	30.2/33.2	0.929342065414233
2	15/16	9.3/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	14/15	22/22	10/14	18.3/22	13/15.2	30.2/33.2	0.0128107344949412
3	15/16	9.3/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	14/15	21/99	10/14	18.3/22	13/15.2	30.2/33.2	0.0124096304469715
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6	15/16	9.3/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	14/15	21/21	10/14	18.3/22	13/15.2	30.2/33.2	0.00595183876119175
7	15/16	9.3/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	14/15	22/99	10/14	18.3/22	13/15.2	30.2/33.2	0.00553505815273729
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9	15/16	9.3/9.3	27/29	15/17	13/15	12/17.3	20/23	11/12	15/16	14/17	14/15	21/22	10/14	18.3/22	13/15.2	30.2/33.2	0.00442000926011209
10	15/16	9.3/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	15/15	21/22	10/14	18.3/22	13/15.2	30.2/33.2	0.0013482060240806
11	15/16	6/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	14/15	21/22	10/14	18.3/22	13/15.2	30.2/33.2	0.00122981029737823
12	15/16	7/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	14/15	21/22	10/14	18.3/22	13/15.2	30.2/33.2	0.000820093065767984
13	15/15	9.3/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/17	14/15	21/22	10/14	18.3/22	13/15.2	30.2/33.2	0.000658253279872308
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19	15/16	9.3/9.3	27/29	15/17	13/15	12/17.3	19/23	11/12	15/16	14/15	14/15	21/22	10/14	18.3/22	13/15.2	30.2/33.2	0.000195828364561315

Save table

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

Accessibility

- EuroForMix is open-source and freely available through the R-package **euroformix** which is downloadable from R-forge.

- Homepage:
  - www.euroformix.com
  - Here is tutorial, manual and a vignette which explains all technical details.

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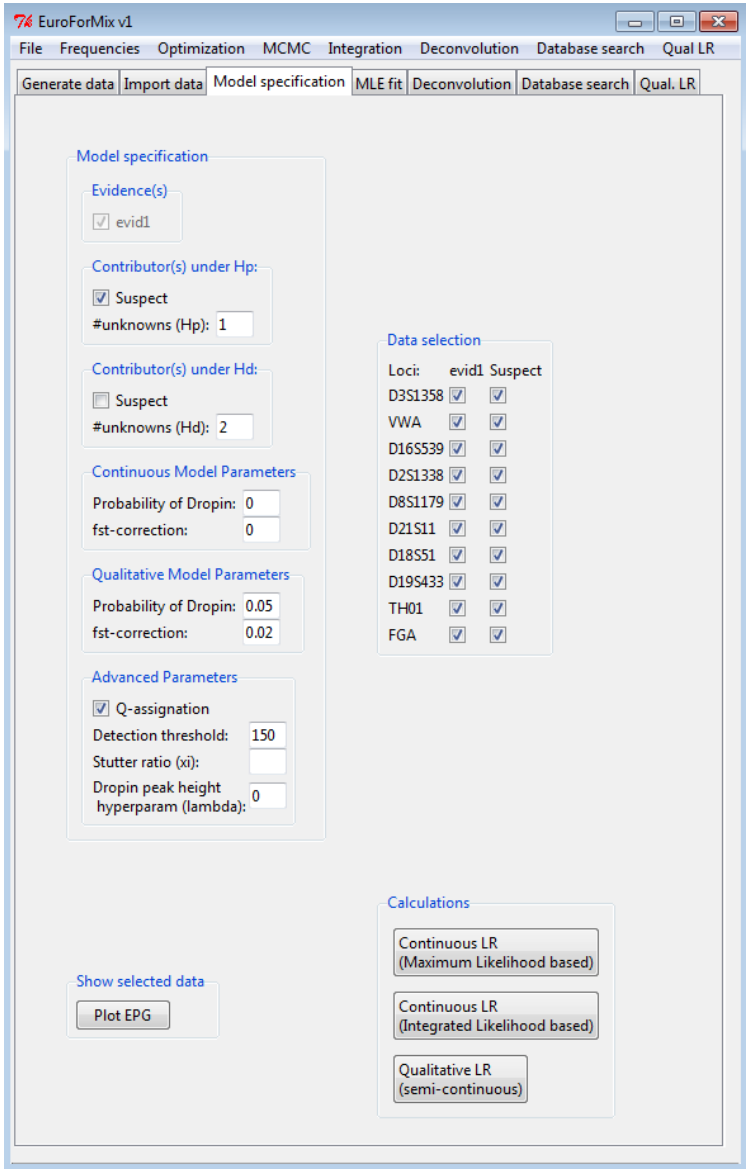
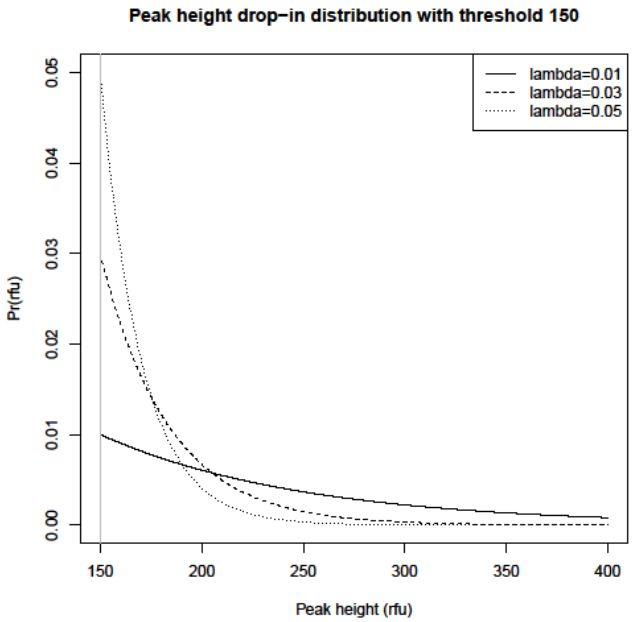
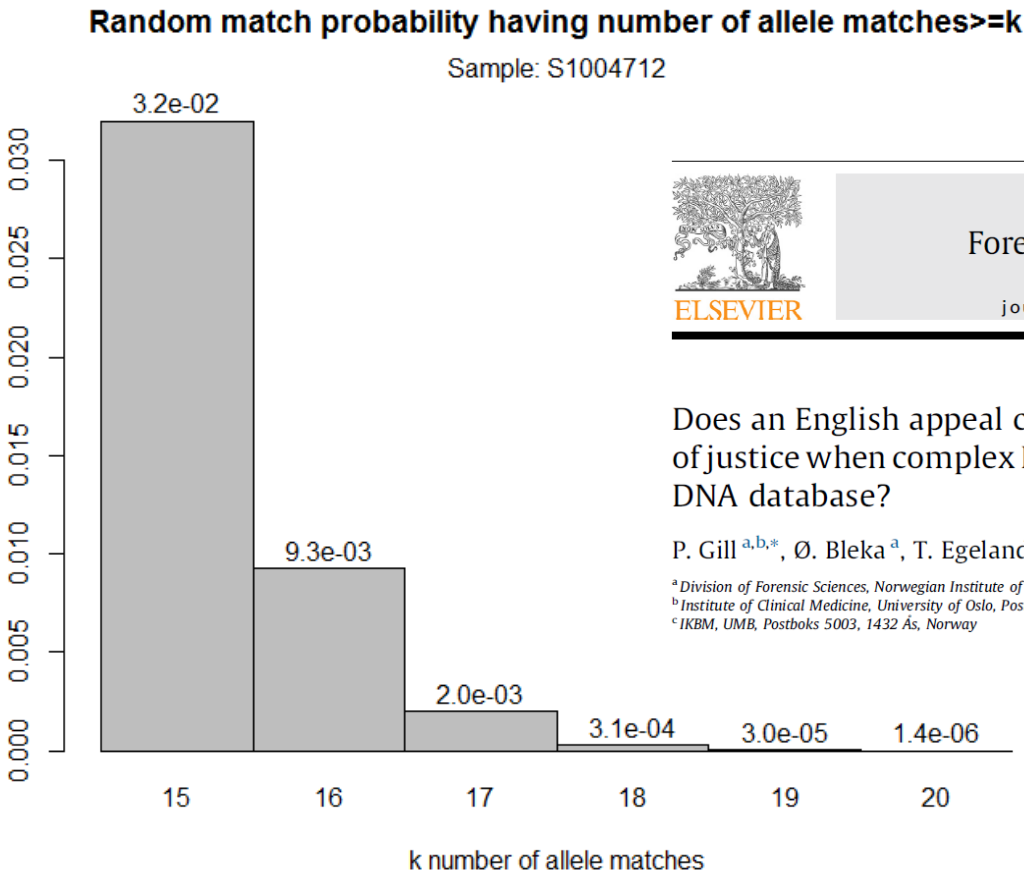
EFM

Model specification





Matching allele count method supported (as an exploratory tool)



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Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Does an English appeal court ruling increase the risks of miscarriages of justice when complex DNA profiles are searched against the national DNA database?

P. Gill [a,b,*], Ø. Bleka [a], T. Egeland [a,c]

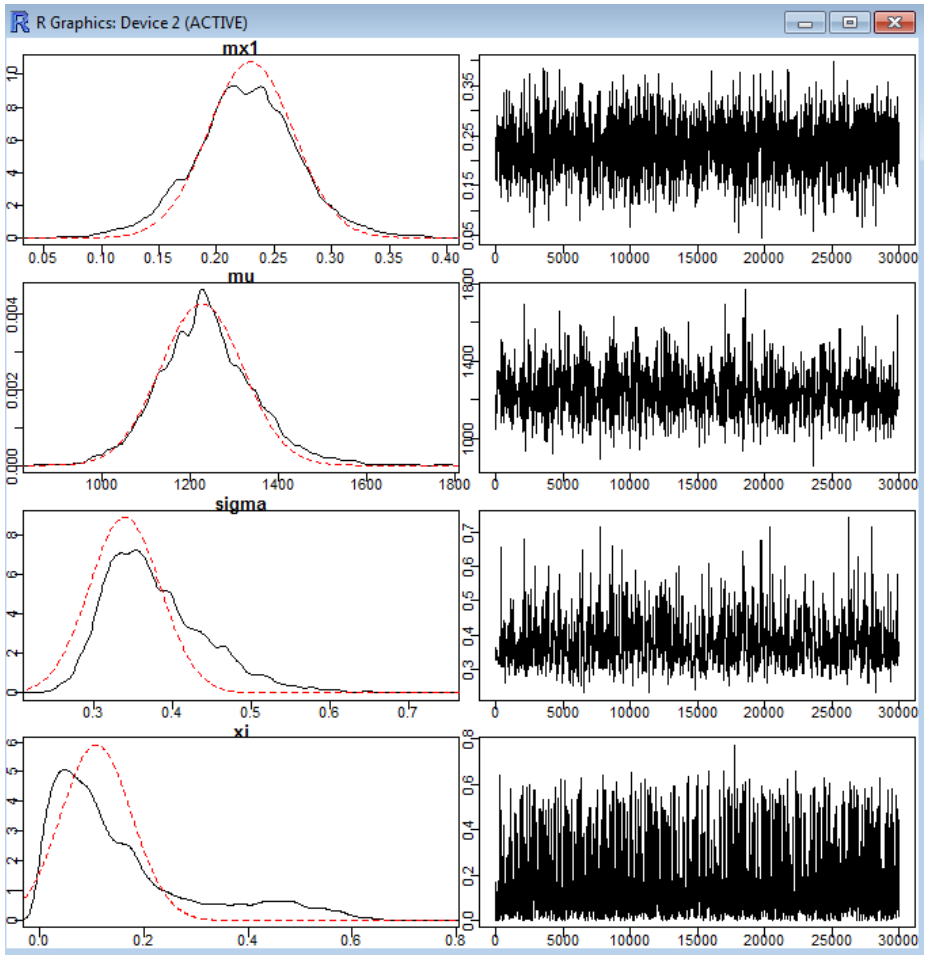
[a] Division of Forensic Sciences, Norwegian Institute of Public Health, Rikshospitalet, Sognsvannsveien 20, 0372 P.O. Box Nydalen, 0403 Oslo, Norway
[b] Institute of Clinical Medicine, University of Oslo, Postboks 4950 Nydalen, 0424 Oslo, Norway
[c] IKBM, UMB, Postboks 5003, 1432 Ås, Norway

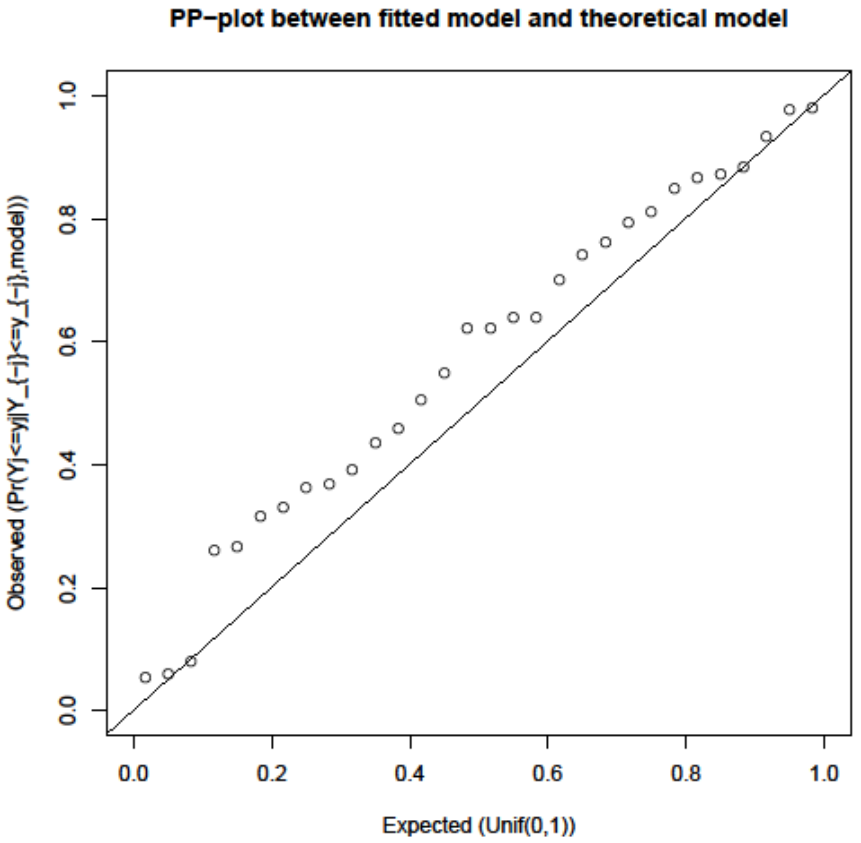
Quality indicators

The **purpose** of the MCMC simulation is to use it as an **exploratory tool** to show:

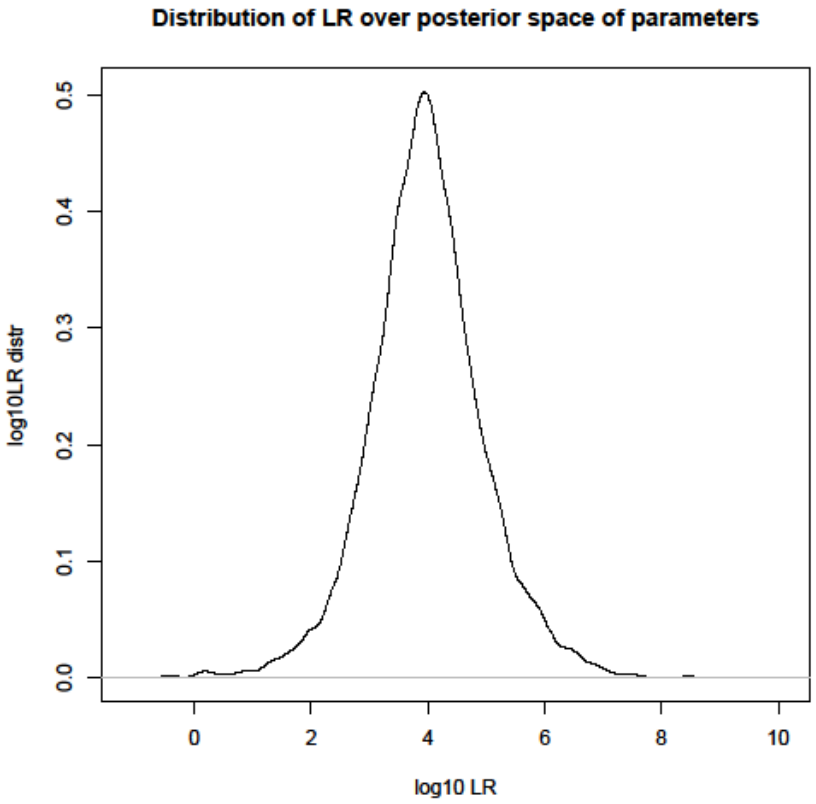
That the optimizer has found the global maximum.

The shape of the posterior distribution of the parameters.



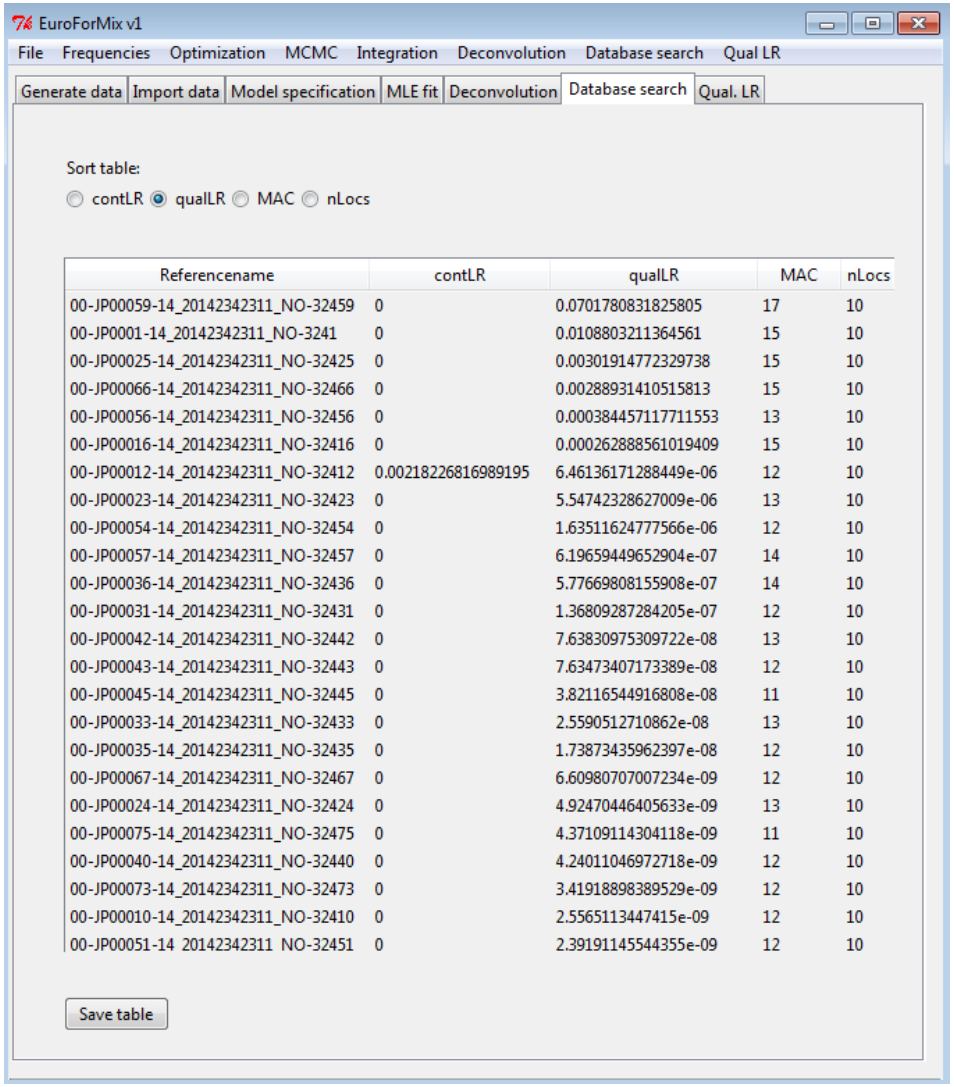


Distribution of the LR



Database search

Database search



Referencename	contLR	qualLR	MAC	nLocs
00-JP00059-14_20142342311_NO-32459	0	0.0701780831825805	17	10
00-JP0001-14_20142342311_NO-3241	0	0.0108803211364561	15	10
00-JP00025-14_20142342311_NO-32425	0	0.00301914772329738	15	10
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00-JP00016-14_20142342311_NO-32416	0	0.000262888561019409	15	10
00-JP00012-14_20142342311_NO-32412	0.00218226816989195	6.46136171288449e-06	12	10
00-JP00023-14_20142342311_NO-32423	0	5.54742328627009e-06	13	10
00-JP00054-14_20142342311_NO-32454	0	1.63511624777566e-06	12	10
00-JP00057-14_20142342311_NO-32457	0	6.19659449652904e-07	14	10
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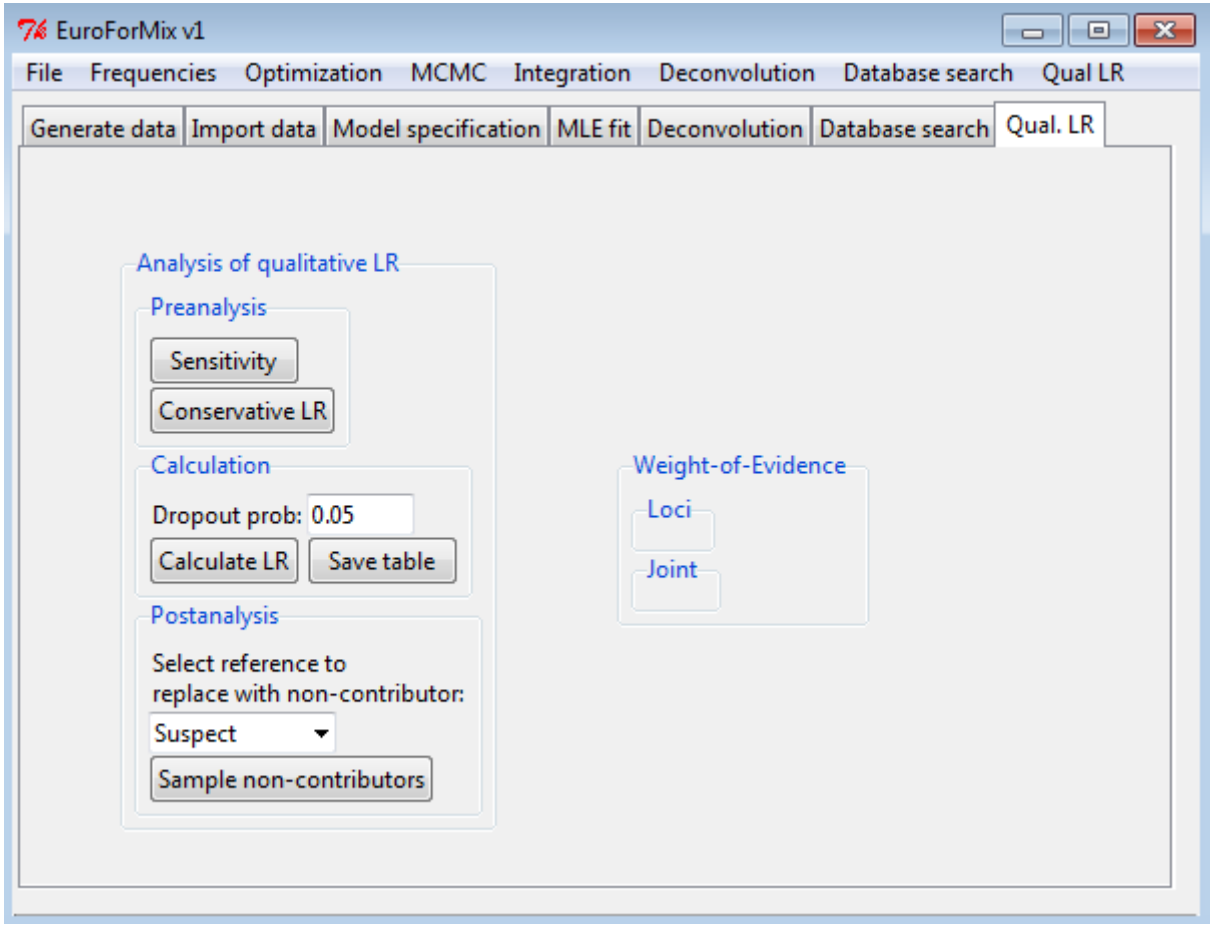
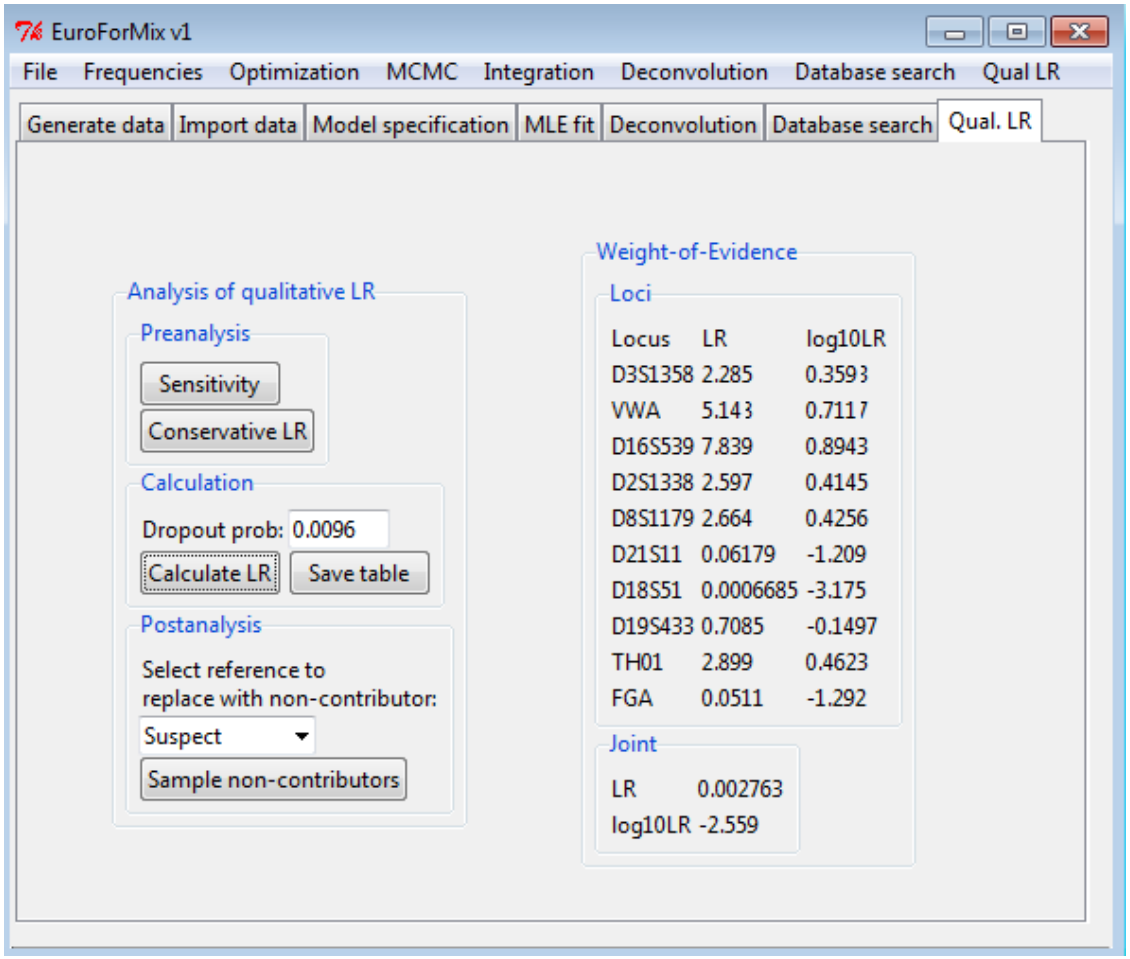
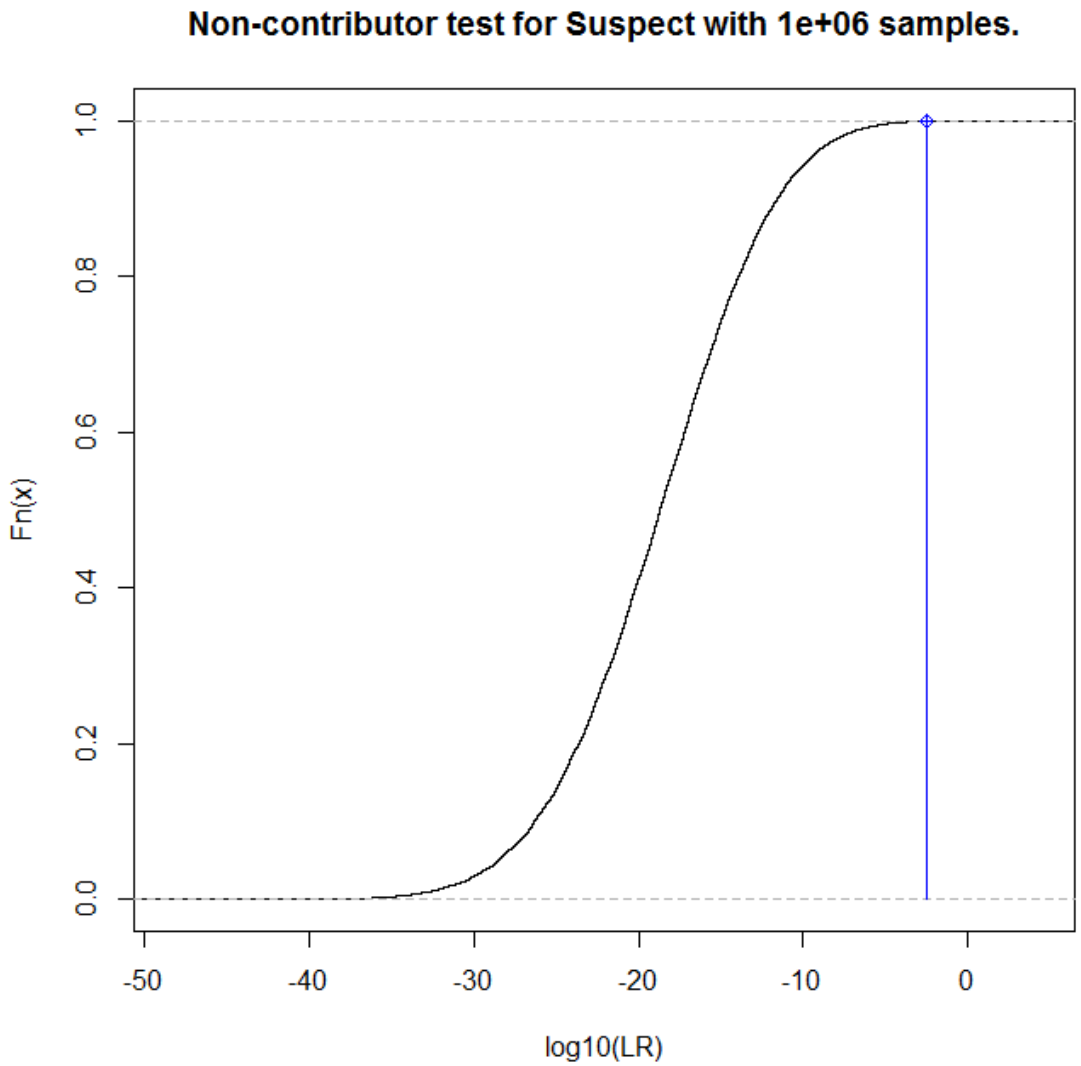
Qualitative LR (LRmix)



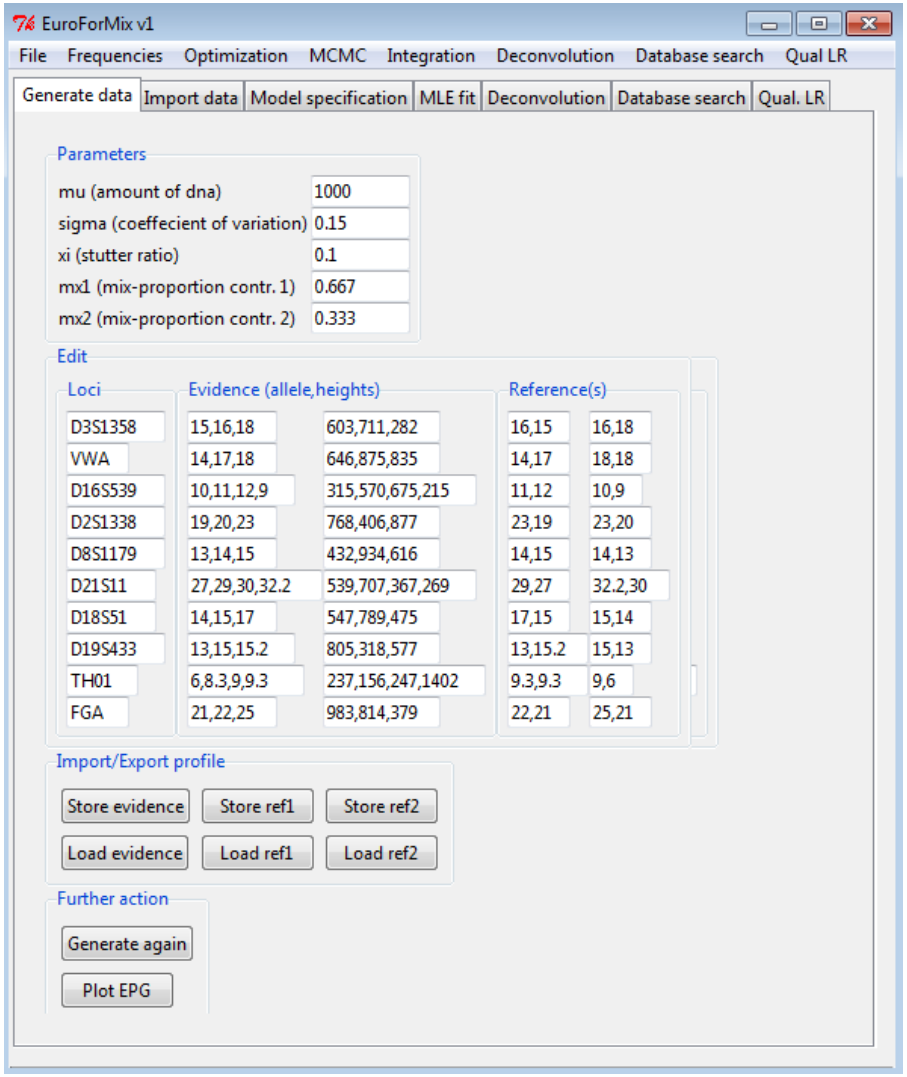
Lrmix output



Non-contributor tests supported for all modules

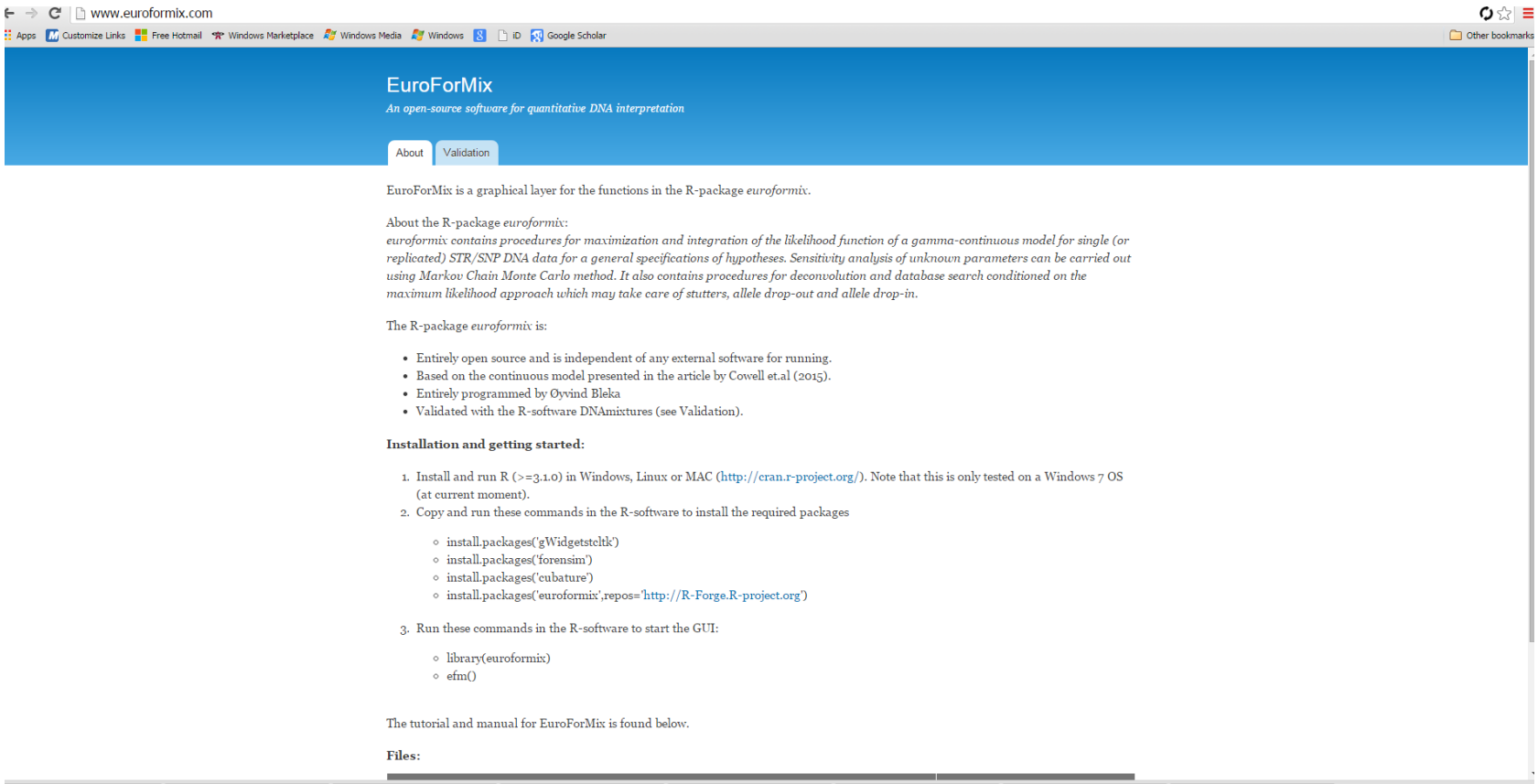


Simulation



Generates alleles using the population frequencies and simulates peak heights for a specified hypothesis (see Figure 32) using the continuous model.
The generation may simulate allele-dropout, drop-in (with a peak height model) and (n-1)-stutter. Allele-dropout is indirectly simulated if the peak height is below the defined threshold.

Home page


www.euroformix.com
Apps
Customize Links
Free Hotmail
Windows Marketplace
Windows Media
Windows
iD
Google Scholar
Other bookmarks
EuroForMix
An open-source software for quantitative DNA interpretation
About
Validation
EuroForMix is a graphical layer for the functions in the R-package euroformix.
About the R-package euroformix:
euroformix contains procedures for maximization and integration of the likelihood function of a gamma-continuous model for single (or replicated) STR/SNP DNA data for a general specifications of hypotheses. Sensitivity analysis of unknown parameters can be carried out using Markov Chain Monte Carlo method. It also contains procedures for deconvolution and database search conditioned on the maximum likelihood approach which may take care of stutters, allele drop-out and allele drop-in.
The R-package euroformix is:
Entirely open source and is independent of any external software for running.
Based on the continuous model presented in the article by Cowell et.al (2015).
Entirely programmed by Oyvind Bleka
Validated with the R-software DNAmixtures (see Validation).
Installation and getting started:
1. Install and run R (>=3.1.0) in Windows, Linux or MAC (http://cran.r-project.org/). Note that this is only tested on a Windows 7 OS (at current moment).
2. Copy and run these commands in the R-software to install the required packages
install.packages('gWidgetstcltk')
install.packages('forensim')
install.packages('cubature')
install.packages('euroformix',repos='http://R-Forge.R-project.org')
3. Run these commands in the R-software to start the GUI:
library(euroformix)
efm()
The tutorial and manual for EuroForMix is found below.
Files:

Research and Validation Efforts at the FBI Laboratory

EDNAP April 2015

Jodi Irwin
FBI Laboratory

Recent Reorganization of FBI DNA Units

- Former:
  - Nuclear DNA Unit
  - Mitochondrial DNA Unit
- Now:
  - DNA Caseworking Unit
  - DNA Support Unit
    - QC
    - Training
    - Validation
    - Research

Direct Support of Operational Efforts

- Standardization of workflows for nuclear DNA and mitochondrial DNA testing
  - Standardized calcified tissue extraction
  - New shed hair extraction protocol that yields 2-10X more DNA than previous method
- Validation of GlobalFiler and Fusion direct amplification kits for offender samples
- Performing validation of STRmix, using Identifiler Plus data from the 3130XL

Direct Support of Operational Efforts

- Streamlining and Improving mtDNA Casework
  - Automation
    - Extraction, qPCR, amplification, sequencing
    - We hope to do this for both questioned and known specimens
    - Starting with knowns
  - Once implemented, the changes are expected to save over $500,000/annually in mtDNA casework

Direct Support of Operational Efforts

- Development of reference population databases with expanded loci
  - 2011 samples typed with GlobalFiler and Fusion
  - New markers beyond the CODIS 13 will be required for NDIS as of January 2017

Population	N
Caucasian	202
African American	209
SW “Hispanic”	209
SE “Hispanic”	263
Filipino	91
Chamorro	95

Population	N
Navajo	235
Apache	196
Bahamian	159
Jamaican	177
Trinidadian	78
Alaska Native American	96

NGS Research

- Our focus, currently, is in developing NGS to *expand* institutional capabilities.
  - mtDNA – mtGenome recovery
  - mtDNA – mixtures aren't routinely interpreted now. Can we start teasing out contributors with NGS?
  - Can NGS help with our most difficult specimens?
  - When there's no hit for a crime scene profile in the CODIS database, can we can glean any other information from the sample? Phenotype? Ancestry?

NGS mtDNA data development

- A number of methods already published and tested for forensic application, not to mention numerous publications in other disciplines
- Technically, pretty low hanging fruit
  - Molecular biology largely in place
  - Paradigm and data type (sequence data) already in place and well-established in forensics
  - Data analysis and existing bioinformatic packages and pipelines really need only minor tweaks for basic casework functionality
- Development of detailed interpretation guidelines still required

Value of Complete mtGenome Data

U.S. Caucasians (n=263)

	HV1/HV2	CR	mtG
Unique Haplotypes	170 (65%)	196 (75%)	259 (>98%)

U.S. Hispanics (n=155)

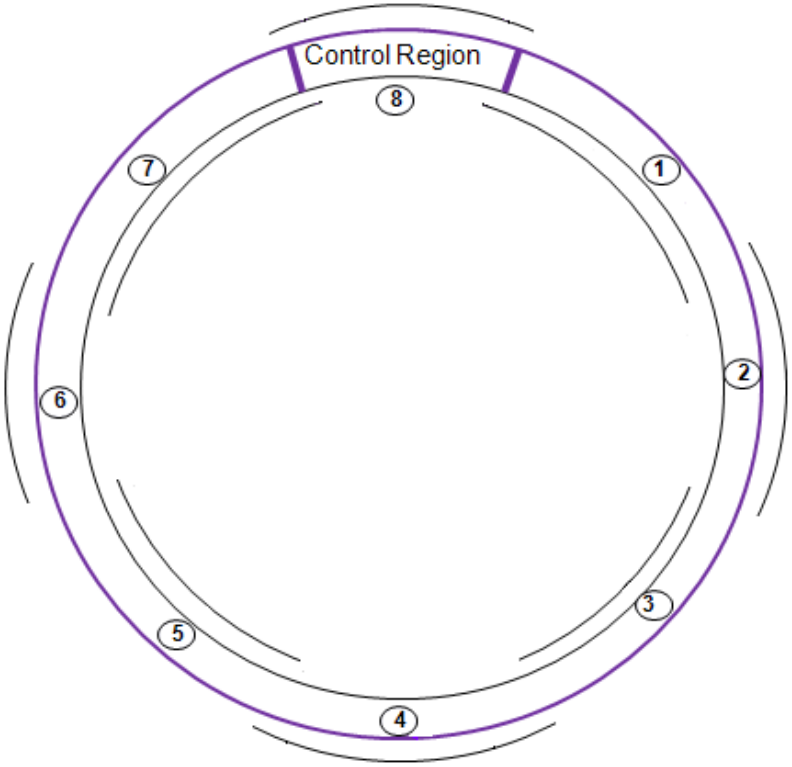
	HV1/HV2	CR	mtG
Unique Haplotypes	121 (78%)	124 (80%)	146 (94%)

African Americans (n=170)

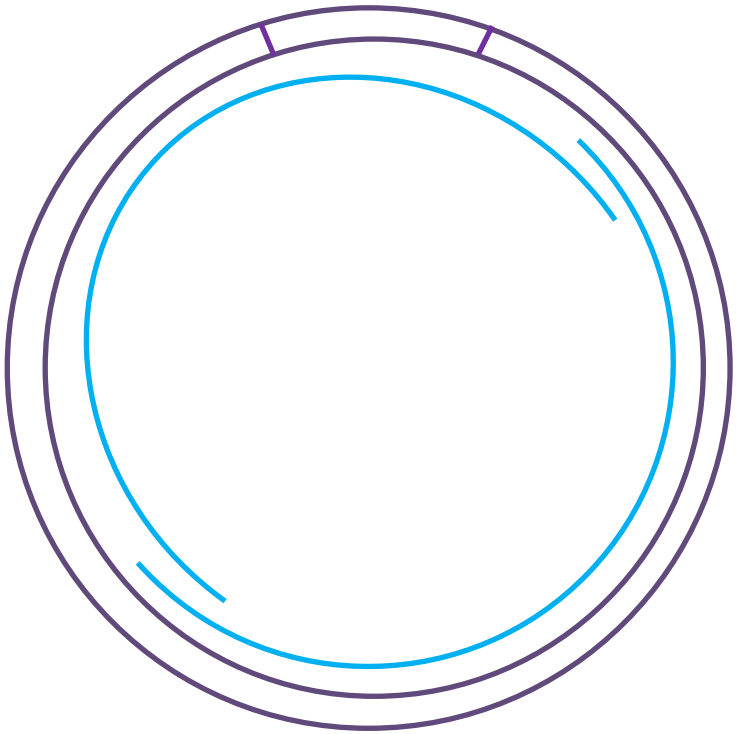
	HV1/HV2	CR	mtG
Unique Haplotypes	119 (71%)	126 (75%)	168 (99%)

Just et al. 2015, *FSI:Genetics*

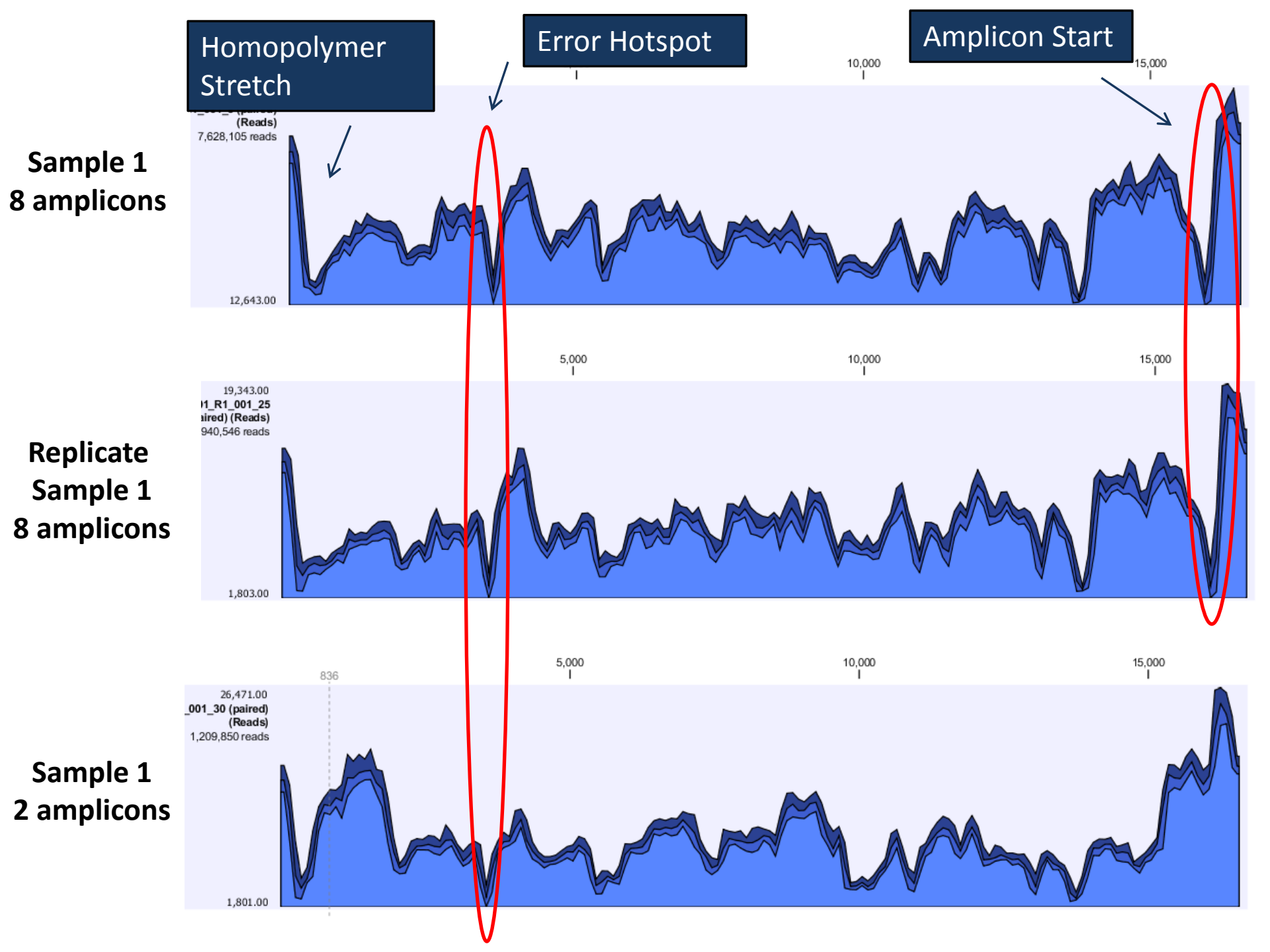
mtGenome PCR Strategies



8 amplicons per mtGenome

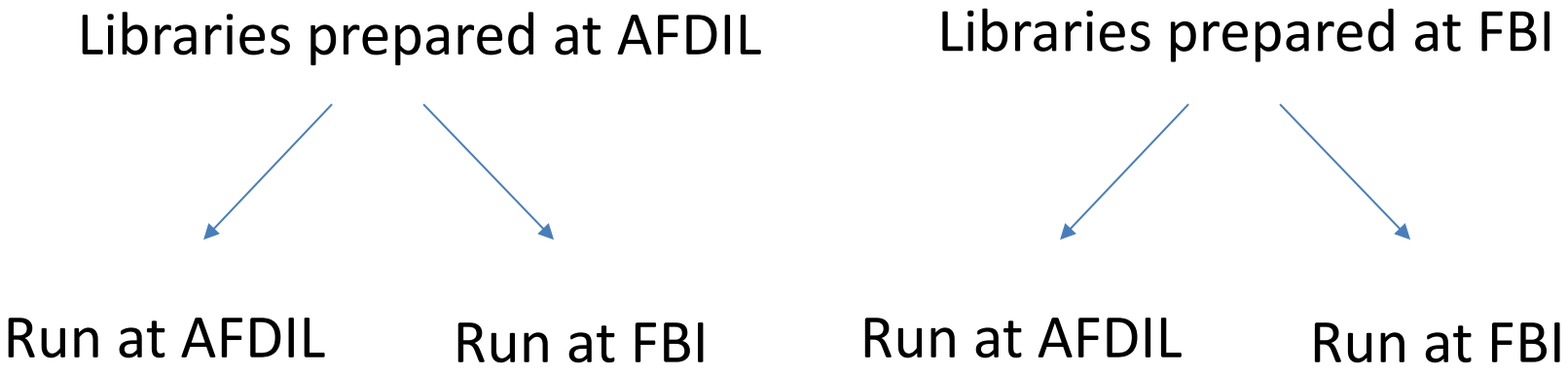


2 amplicons per mtGenome


Homopolymer Stretch
Error Hotspot
Amplicon Start
Sample 1
8 amplicons
Replicate
Sample 1
8 amplicons
Sample 1
2 amplicons
10,000
15,000
(Reads)
7,628,105 reads
12,643.00
5,000
10,000
15,000
19,343.00
940,546 reads
1,803.00
836
5,000
10,000
15,000
26,471.00
(Reads)
1,209,850 reads
1,801.00

NGS Concordance Study

- Collaboration with the Armed Forces DNA Identification Laboratory
- 90 high-quality population samples MP sequenced for the complete mtGenome
  - ***Sanger data available for comparison***



Replicate Data Analysis Performed in Both Laboratories

NGS Concordance Study

- Average read coverage ~2000X, 90 samples
- 99.9994% Concordance between Sanger and NGS
- 19 discordant sites out of ~3,000,000 positions analyzed
    - 6 point heteroplasmies not detected by Sanger
    - 13 due to misalignment or low read coverage
- In addition, 2 mixtures detected (~1:20 and ~1:50)

Lower Limits of Read Coverage

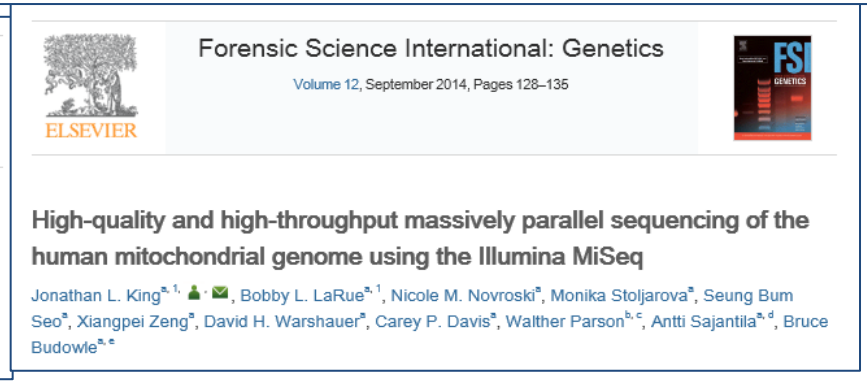
- What practical effect on data reliability does low read coverage have?
  - Compared coverage between libraries
  - Compared noise at 3195 variant positions

Site	Library 1 Cov	Library 1 Noise	Library 2 Cov	Library 2 Noise	Coverage Diff	Noise Level Diff
146	1033	0.2%	51	2%	95%	1.8%

  - Despite large differences in coverage, the level of background noise is not terribly different
  - In 99.5% of the cases (3148), the difference in noise level between low and high coverage sample was <1%
  - In 99.5% of the cases, the noise was less than 3% of the true signal

mtDNA Summary

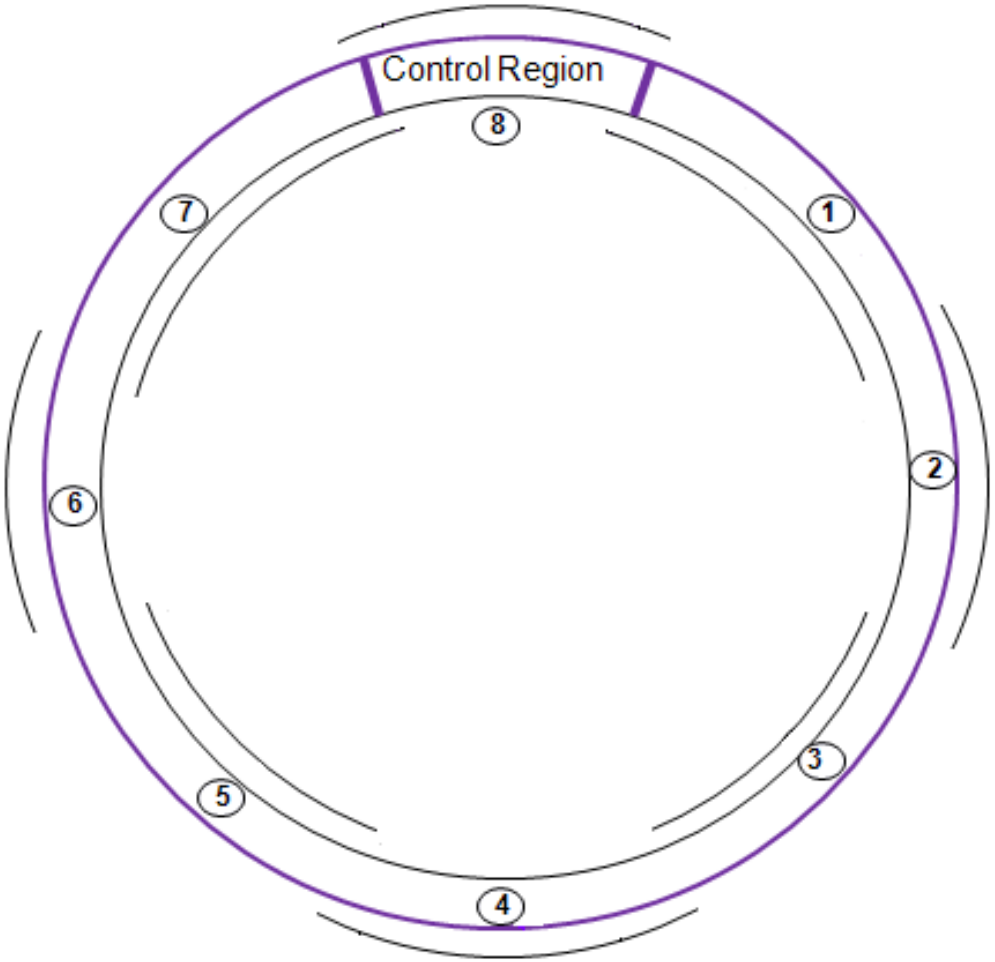
- Straightforward and robust targeted amplification protocols for entire mtGenome development from high-quality samples



Development of mtGenomes from challenging samples

- Recovery of large fragments unlikely in most mtDNA cases
- But, to cover the entire mtGenome in ~300bp amplicons would require 50 or 60 amplicons
- Can we efficiently, and cost-effectively, develop mtGenome profiles from challenging samples?

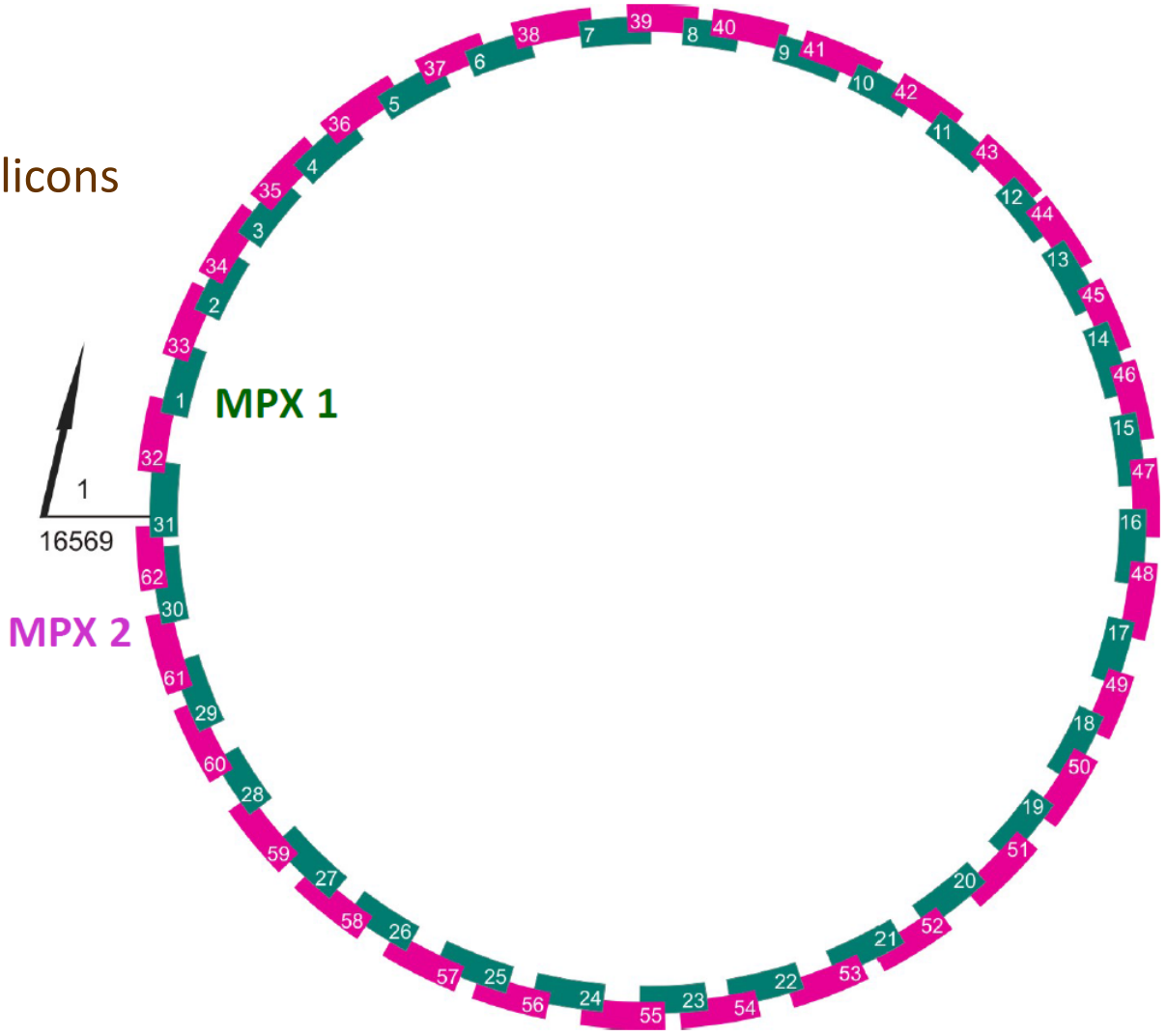
mtGenome PCR Strategy from shed hair



8 amplicons per mtGenome

mtGenome MPS of challenging samples

300-400 bp amplicons

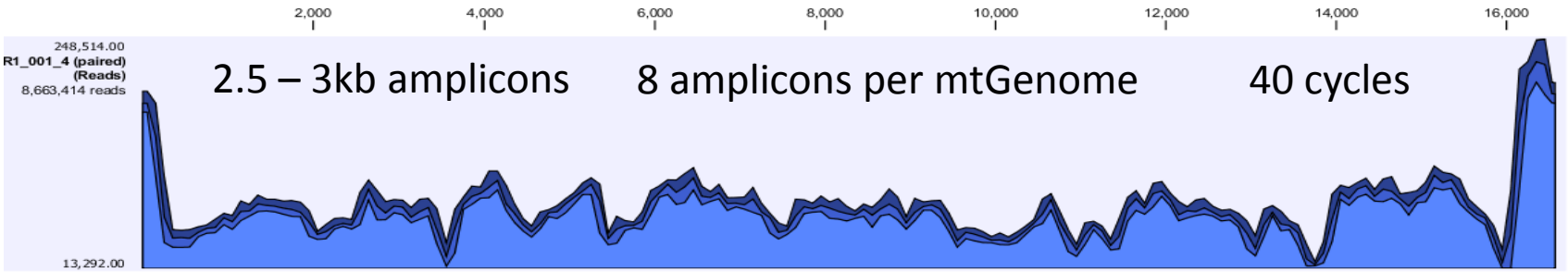


40-50% of hair samples yield amplicons of this size

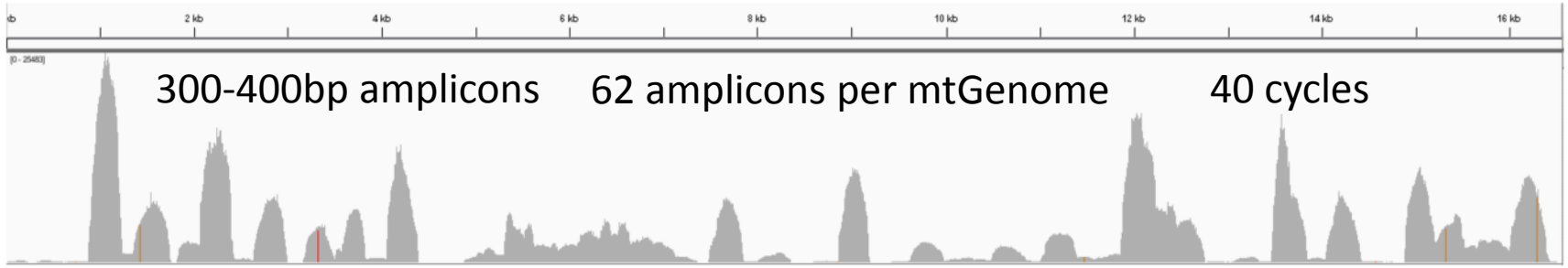
Slide used with permission of W. Parson, GMI

Entire mtGenomes from Single Hairs

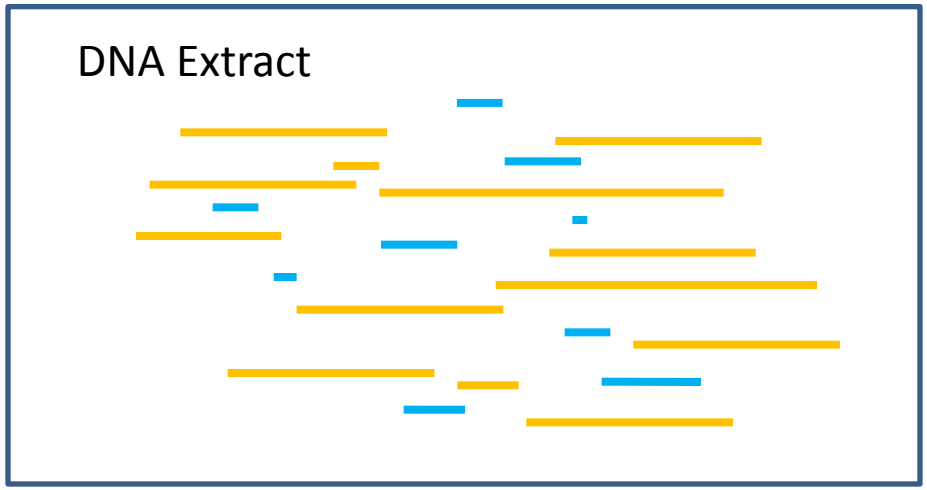
1.



2.



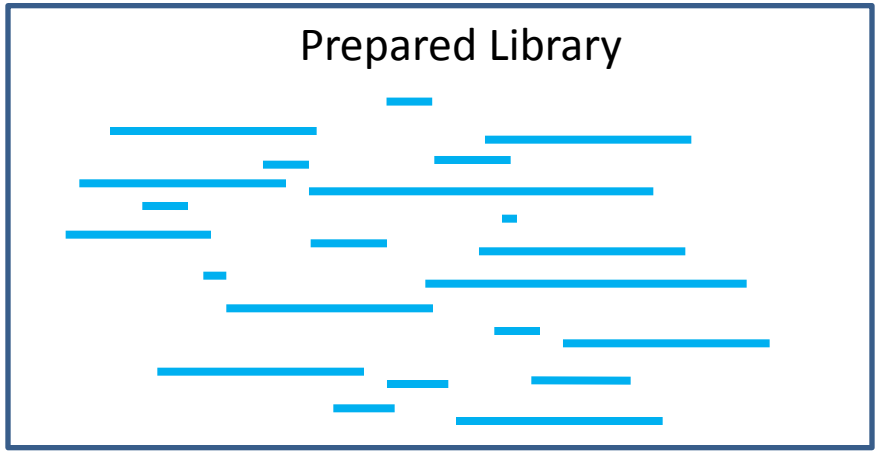
Target Enrichment



Targeted PCR

Non-Target DNA

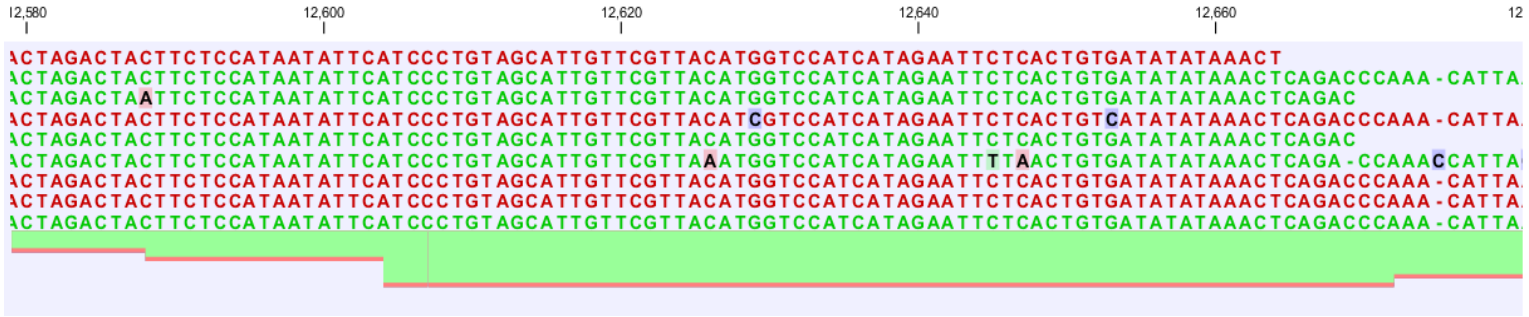
Target DNA



Assess quantity and quality of total DNA in the extract

Genomic Sequencing – Hair Sample

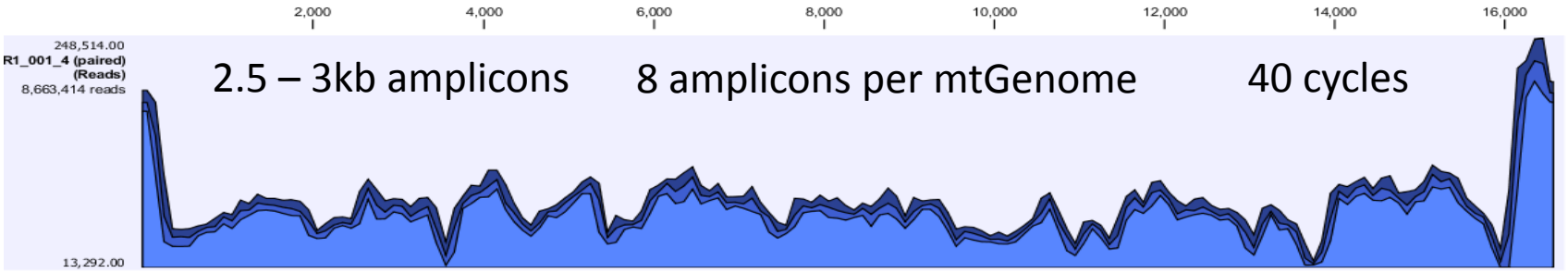
- Of the reads that mapped to the human genome:
  - mtDNA reads – 0.07% and 0.12%
  - complete mtGenome coverage – up to 139 reads



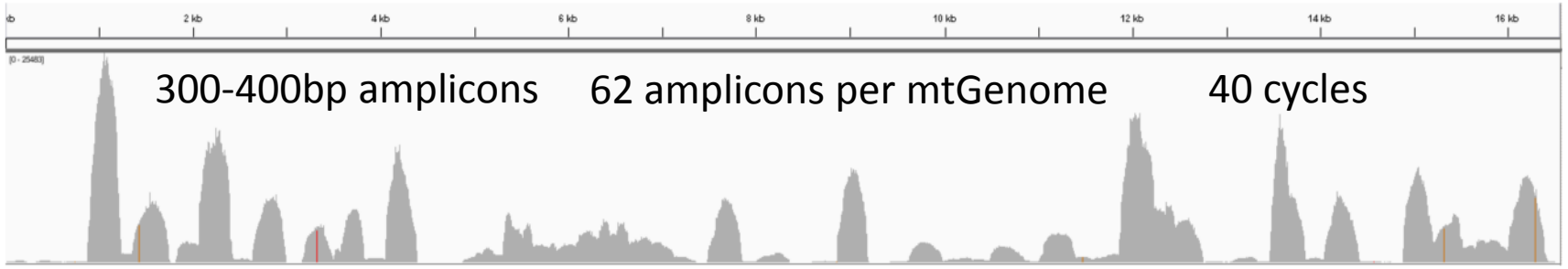
  - nucDNA reads – 99.93% and 99.88%

Entire mtGenomes from Single Hairs

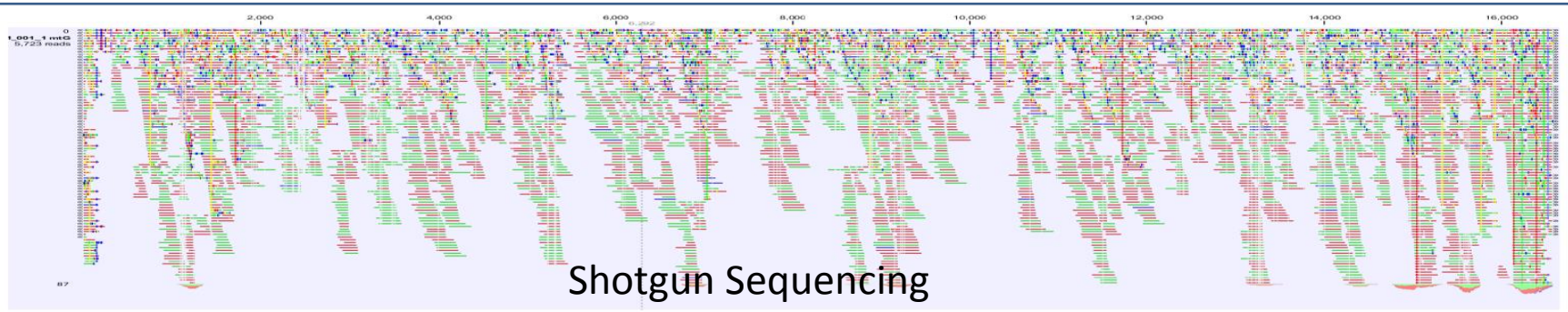
1.



2.



3.



mtDNA Summary

- Protocols for entire mtGenome development from low-quality samples

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

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

Application of next generation sequencing technologies to the identification of highly degraded unknown soldiers' remains

O. Loreille [a,*], H. Koshinsky [b], V.Y. Fofanov [b], J.A. Irwin [a]

[a] Armed Forces DNA Identification Laboratory, Rockville, MD, USA
[b] Eureka Genomics Corp., Hercules, CA, USA

Recovery of DNA fragments as small as 30bp

Templeton *et al. Investigative Genetics* 2013, 4:26
http://www.investigativegenetics.com/content/4/1/26

Investigative Genetics

METHODOLOGY Open Access

DNA capture and next-generation sequencing can recover whole mitochondrial genomes from highly degraded samples for human identification

Jennifer E L Templeton[1,2], Paul M Brotherton[1,3], Bastien Llamas[1], Julien Soubrier[1], Wolfgang Haak[1], Alan Cooper[1] and Jeremy J Austin[1,4*]

Massively parallel sequencing of complete mitochondrial genomes from hair shaft samples, *in press, FSI:Genetics*

Walther Parson, Gabriela Huber, Lilliana Moreno, Maria-Bernadette Madel, Michael D. Brandhagen, Simone Nagl, Catarina Xavier, Mayra Eduardoff, Thomas C. Callaghan, Jodi A. Irwin

mtDNA Summary

- When compared to mtDNA data currently generated via Sanger sequencing
  - 16X the data for known samples
  - 25X the data for questioned samples
  - Rough calculations suggest that we could obtain entire mtGenomes with NGS for half the current cost of HVI/HVII or CR

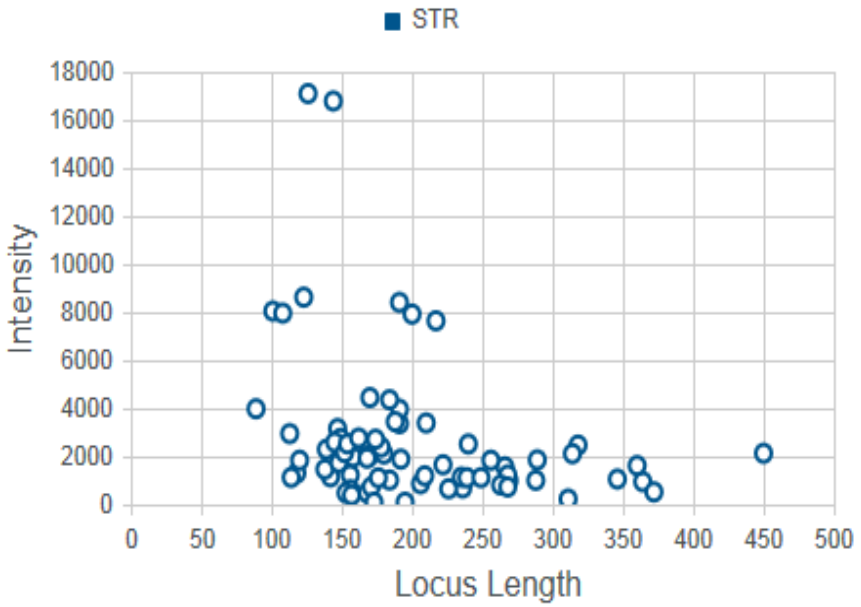
Promega PowerSeq

- All of the standard markers currently used in forensic casework – just in a single NGS assay
- Triplex – auSTRs, YSTRs and mtDNA control region
  - 24 autosomal STRs, 150-300bp amplicons
  - 21 Y-STRs, 150-300bp amplicons
  - mtDNA control region, 150-250bp amplicons
- Duplex – auSTRs and mtDNA control region
- Multiplexing 12 samples results in:
  - STR allele coverage between 3,000-10,000 reads
  - mtDNA control region coverage avg: 65,000

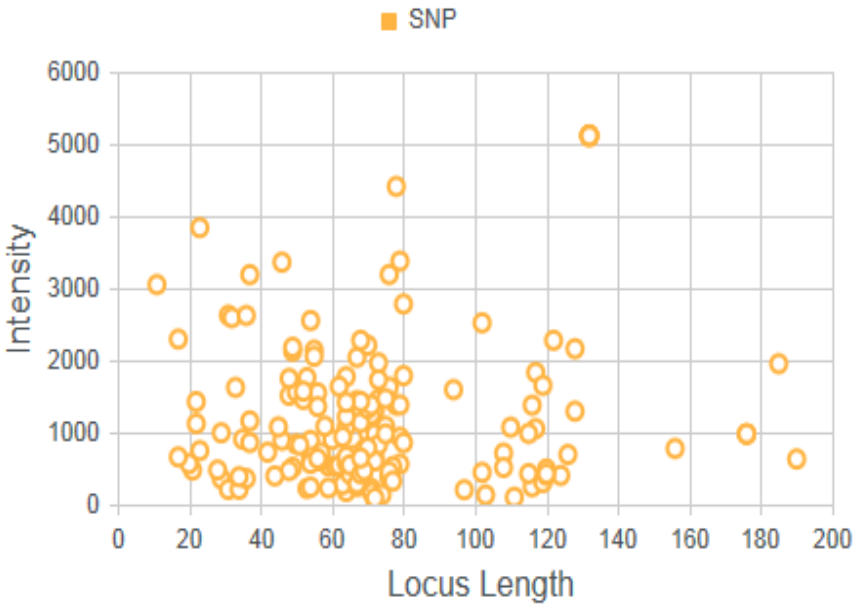
Illumina ForenSeq Beta Kit

- 63 STRs
  - 29 autosomal STRs, along with 9 X and 25 Y STRs
- In addition:
  - 95 identity-informative SNPs
  - 56 ancestry-informative
  - 22 phenotypically-informative SNPs

2800M Standard, 1ng



63/63 STRs
(25 Y markers)



95/95 SNPs

Profile Recovery from Low Quantity Samples

	STRs		SNPs	
50 pg 2800M Standard*	63/63	100%	94/95	99%
100 pg Backpack Swabbing	34/38	89%	92/95	97%
23 pg Bottle Cap Swabbing	30/38	79%	81/95	85%
61 pg Lanyard Swabbing	35/38	92%	87/95	92%
41 pg Computer Mouse Swabbing	33/38	92%	89/95	94%

* Average of triplicates

** A minority of loci had imbalance or interpretation threshold issues

Value for Other Sample Types

What about bone and hair samples that yield little or no nuclear DNA?

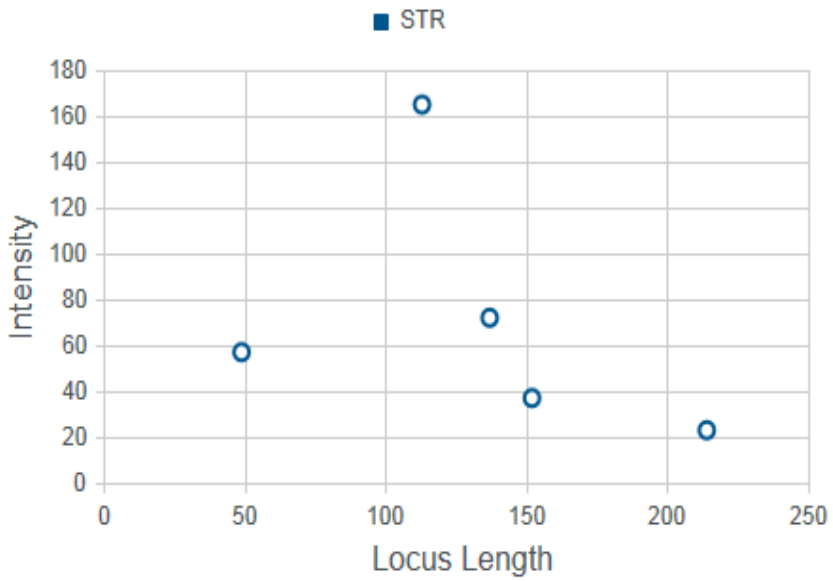
The vast majority of such samples tested at the FBI Laboratory yield nucDNA quantities (loosely inferred from mtDNA quantities) of 50pg or more

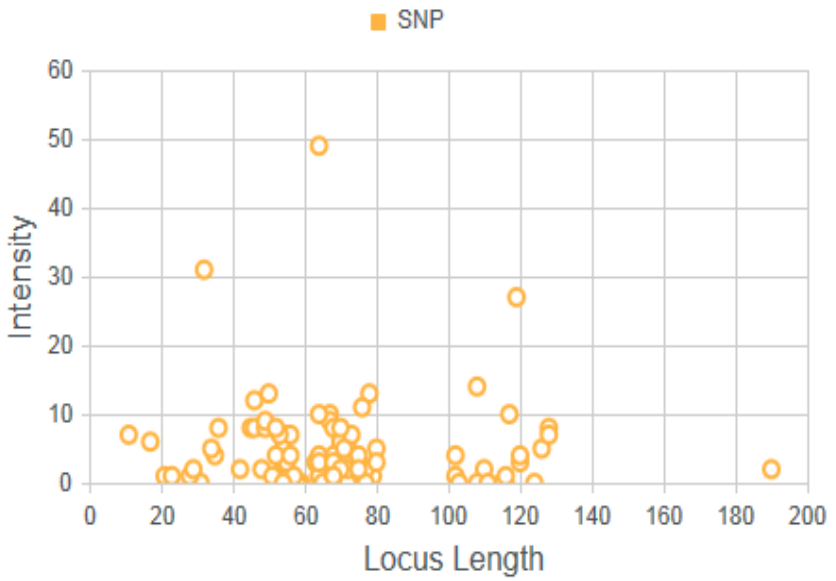
However, it is also a question of DNA quality…

Shed Hair

Not tested with Identifiler+

Estimated 60-100pg input





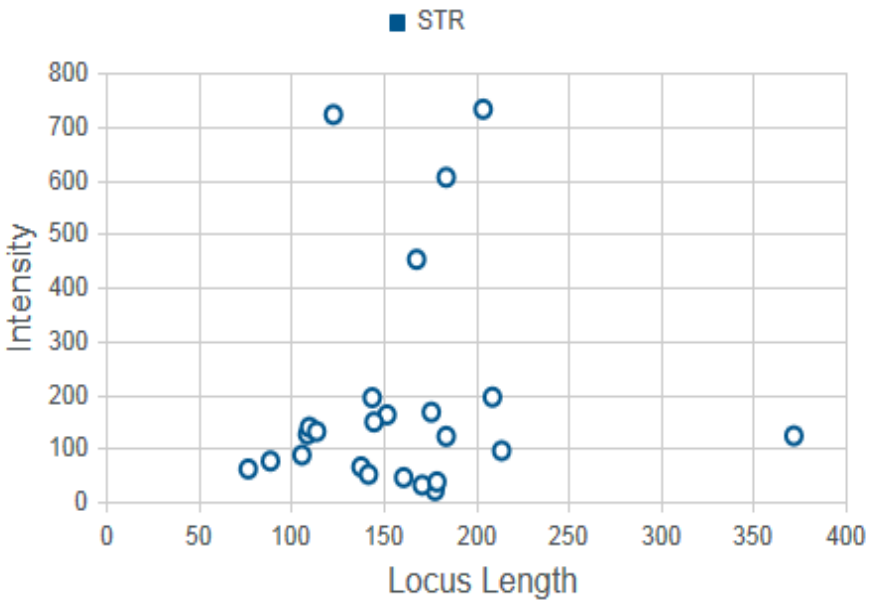
5 STRs

Most <150bp

3 Identity SNPs
Many AI/PI SNPs

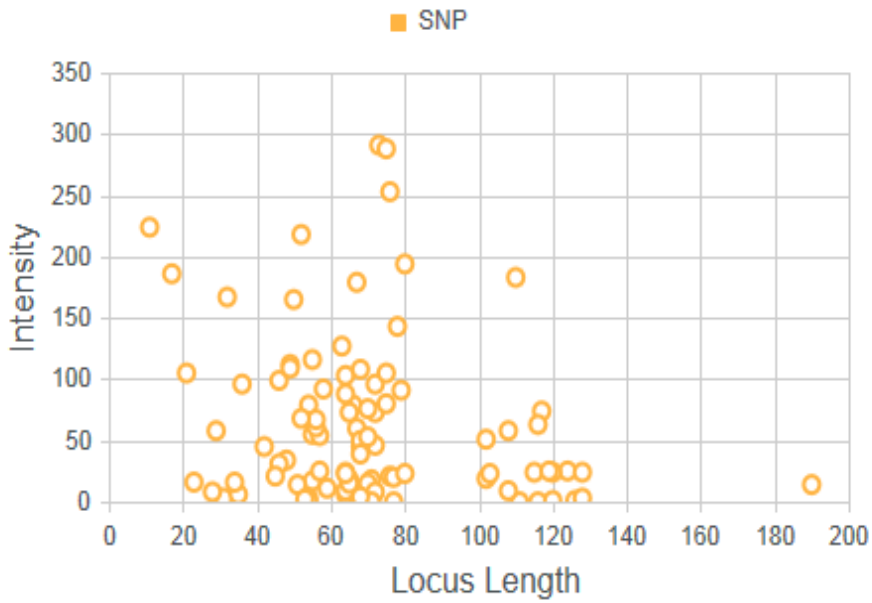
Degraded Bone Sample

No Data with Identifiler +
0.002ng/ul, 10pg total input



19 STRs

Vast majority <225bp



53 Identity SNPs

Hybridization Capture

- Recently purchased baits for both:
  - The entire mtGenome
  - All markers targeted in the Illumina ForenSeq kit
    - Baits for 63 STRs and 173 SNPs

- Testing of 4,000 year-old mummified remains at the request of a US museum, with the primary question one of gender.
- Ultimately, these efforts are intended to expand the lower range of sample quality from which probative DNA data may be recovered in forensic casework

Near Term NGS Goals

- Efforts are primarily geared towards developing NGS as a “rescue” technology for the worst specimens
- Initiate reference population databasing:
  - mtGenomes
  - STRs
  - SNPs

Acknowledgements

Federal Bureau of Investigation Laboratory:

- Lilly Moreno, Mike Brandhagen, Tom Callaghan, Tony Onorato, Tamyra Moretti, Eric Pokorak

Armed Forces DNA Identification Laboratory:

- Michelle Peck, Rebecca Just, Kimberly Sturk, Charla Marshall, Odile Loreille

Institute of Legal Medicine, Innsbruck, Austria

- Walther Parson

Cross-Technology/Platform Comparison on the lowest quality samples

(shed hair, degraded skeletal remains, other low quant/qual samples)

- Directly assess data recovery from the same extracts with:
  - Currently employed, CE-based assays:
    - Identifiler Plus
    - Minifiler
    - Sanger-based mtDNA control region sequencing
  - NGS/MPS
    - Commercially available assays
    - Shotgun sequencing
    - Hybridization capture

Presentation of evidence so that it can be understood

Peter Gill

Background

- Recently there have been some publicised appeal court rulings which show misunderstanding e.g. R v T; R v Dlugosz
- How much are we to blame for the miscommunications?
- What can we do?

- Lindsey et al (2003) Communicating statistical DNA evidence, 43 Jurimetrics J. 147-163
- Carried out an experiment with mock jurors and gave them two statements
  - There is only a 2% chance the defendant's hair would be indistinguishable from the perpetrator if he were innocent
  - In a city of 1,000,000 people there would be 20,000 such individuals
- Juries were less likely to convict with the second statement
- Also shown experimentally that understanding of probabilities was compromised compared to natural frequencies

Consider the two ‘equivalent’ statements below

- The probability that the suspect would match the blood specimen if he was not the source is one in 1 million.
- One in 1 million people in Manchester who are not the source would also match the blood specimen
- The latter statement is a direct cue to think about people other than the defendant ie *the number of false positives* in a relevant population

Doheny Adams court ruling supports natural frequencies

- “Members of the jury, if you accept the scientific evidence called by the Crown, this indicates that there are probably only four or five white males in the United Kingdom from whom that [crime] stain could have come. The defendant is one of them. If that is the position, the decision you have to reach, on all the evidence, is whether you are sure that it was the defendant who left that stain or whether it is possible that it some other individual”

Cognitive thinking affects interpretation

- People do not usually conform to Bayesian rules when reasoning with probability
- Expressions of probabilities that are mathematically equivalent are not psychologically equivalent
- However frequencies expressed as simple counts are more readily understood.
- Likelihood ratios are poorly understood (there is a body of literature which discusses this).

Complex DNA profiling

- Now we have reached a position where complex DNA profiles can be analysed
- We have to decide propositions
- We have to decide software
- We have to accept that there is no gold standard
- We have to accept different software give different answers

How will complex software analysis develop now

Forensic Science International: Genetics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Genotyping and interpretation of STR-DNA: Low-template, mixtures and database matches—Twenty years of research and development

Peter Gill [a,b,*], Hinda Haned [c], Oyvind Bleka [a], Oskar Hansson [a], Guro Dørum [d], Thore Egeland [a,d]

[a] Norwegian Institute of Public Health, Department of Forensic Biology, PO Box 4404 Nydalen, 0403 Oslo, Norway
[b] Department of Forensic Medicine, Sognsvannsveien 20, Rikshospitalet, 0372 Oslo, Norway
[c] Netherlands Forensic Institute, Department of Human Biological Traces, The Hague, The Netherlands
[d] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Aas, Norway

A diversity of methods

- I conclude: «While there can be incorrectly calculated LRs, finding a perfect LR solution for all situations is not possible. Balding [65] states: ‘‘Likelihoods depend on modeling assumptions, and there can be no ‘‘true’’ statistical model for a phenomenon as complex as an LTDNA profile’’ Consequently, there is no agreement within the forensic community on the best approach, and it is unrealistic to suppose that any single method will be universally adopted. This means that in practice a diversity of methods will be used for the foreseeable future. In principle, there is nothing wrong with this. It will encourage research. An inevitable outcome, to be encouraged, is that court-reports will be routinely prepared and challenged by different software that use different modeling assumptions. Typically, commercial software will not be available to defence experts and they will default to open source or non-commercial software. However, if similar answers are obtained, then confidence in results should increase. Here, we follow, Steele and Balding [37], and suggest that a difference in the order of one ban (one unit in log10 scale) is negligible.”

Positioning commercial vs open-source software

- There needs to be an debate on how commercial vs open source software interact
- There is a particular problem for the defence re availability of commercial software
- The obvious alternative is for defence to use open-source
- Open source is also available to the prosecution for counter argument so it should represent a level playing field
- But different answers are expected between different software that may be several orders of magnitude difference
- Will courts be confused?
- A possible way forward is to position ourselves to recommend testing with alternative software. The expert then recommends the court to accept the most conservative answer.

The importance of non-contributor tests to qualify the LR

- It is difficult to simplify the likelihood ratio construct itself
- But maybe we can think in terms of a two stage process:
  - The experts agree on the model to be used
  - Agree propositions to be tested (in particular)
  - Then non-contributor tests can be applied to place the evidence into perspective
  - A large LR cannot necessarily assumed to be probative
  - How likely is it that a random man will give a probative LR

LRmix studio non-contributor tests

- Easy to simulate a million non-contributors in LRmix studio

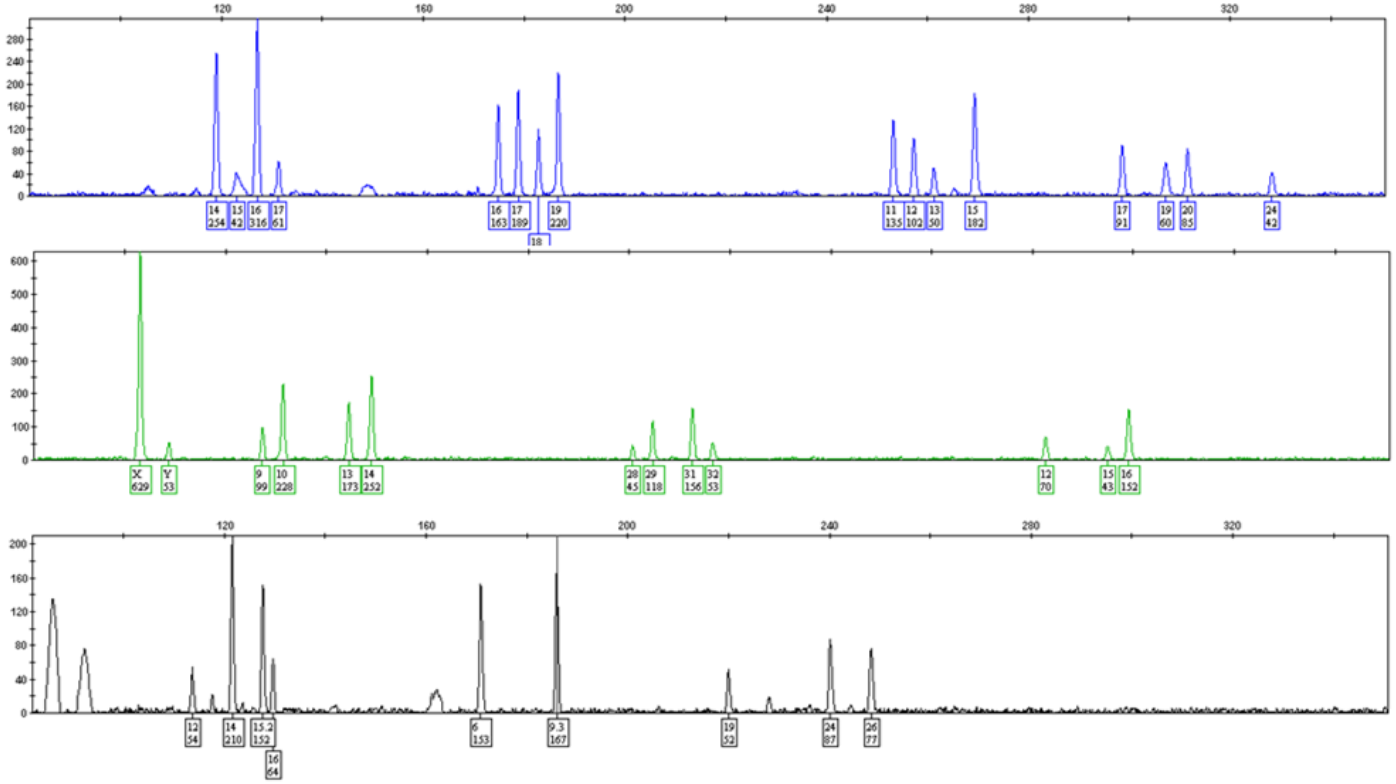
Case example

Step 1

- The crime-stain is from an epithelial swab taken from the female victim
- There are two suspects accused of sexual assault, S_1 and S_2 respectively; both deny the offence.

- This epg is classified as a low template of three or more individuals since there are multiple alleles per locus that fall within the criterion of the low template zone (between the LDT and the stochastic threshold (*T*))– we expect dropout may occur, but the profiles appear to be well represented.

Epg

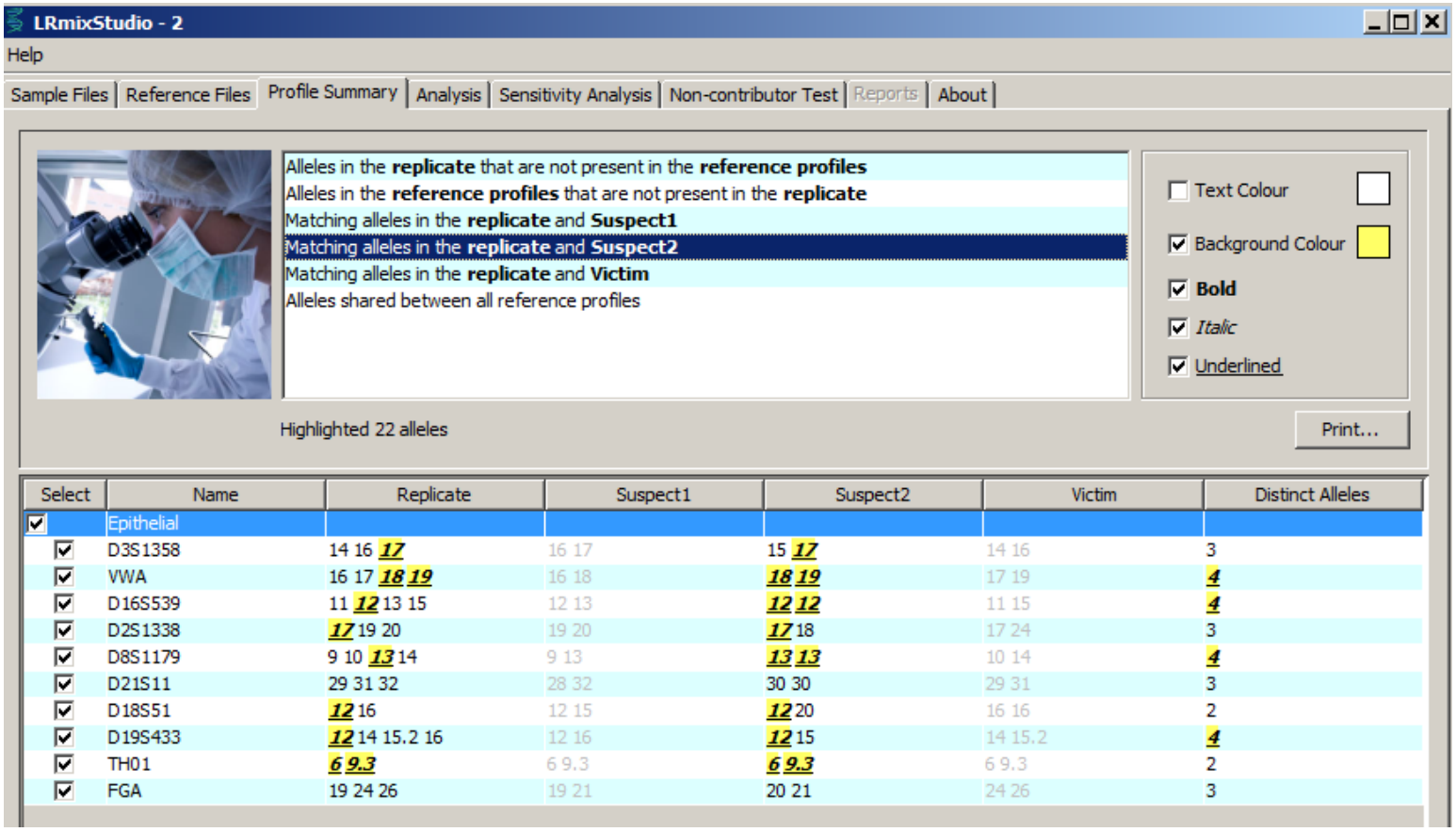

120
160
200
240
280
320
14 254
15 42
16 316
17 61
16 163
17 189
18
19 220
11 135
12 102
13 50
15 182
17 91
19 60
20 85
24 42
X 629
Y 53
9 99
10 228
13 173
14 252
28 45
29 118
31 156
32 53
12 70
15 43
16 152
12 54
14 210
15.2 152
16 64
6 153
9.3 167
19 52
24 87
26 77

Step 2: List the alleles with informative formatting

	Crime-stain alleles								
Marker	Allele1	Allele2	Allele3	Allele4	S1	S1	S2	S2	Unique alleles
AMEL	X	Y			X	Y	X	Y	2
D3S1358	14	16	17	(15)	16	17	15	17	4
VWA	16	17	18	19	16	18	18	19	4
D16S539	11	12	13	15	12	13	12	12	4
D2S1338	17	19	20	(24)	19	20	17	18	4
D8S1179	9	10	13	14	9	13	13	13	4
D21S11	29	31	32		28	32	30	30	5
D18S51	12	16	(15)		12	15	12	20	4
D19S433	12	14	15.2	16	12	16	12	15	5
TH01	6	9.3			6	9.3	6	9.3	2
FGA	19	24	26		19	21	20	21	5

Key:
Alleles that are shared between victim and S_1 or S_2 (green background).
Alleles that are found in the crime stain and not observed in any known individual (blue background, not applicable in this case).
Alleles that are below the detection threshold but appear to be distinct (bracketed).
Alleles that are found in the crime stain that match a known individual under *Hd* (victim) (red typeface).

LRmix Studio summary output



Select	Name	Replicate	Suspect1	Suspect2	Victim	Distinct Alleles
☑	Epithelial					
☑	D3S1358	14 16 ***17***	16 17	15 ***17***	14 16	3
☑	VWA	16 17 ***18*** ***19***	16 18	***18*** ***19***	17 19	***4***
☑	D16S539	11 ***12*** 13 15	12 13	***12*** ***12***	11 15	***4***
☑	D2S1338	***17*** 19 20	19 20	***17*** 18	17 24	3
☑	D8S1179	9 10 ***13*** 14	9 13	***13*** ***13***	10 14	***4***
☑	D21S11	29 31 32	28 32	30 30	29 31	3
☑	D18S51	***12*** 16	12 15	***12*** 20	16 16	2
☑	D19S433	***12*** 14 15.2 16	12 16	***12*** 15	14 15.2	***4***
☑	TH01	***6*** ***9.3***	6 9.3	***6*** ***9.3***	6 9.3	2
☑	FGA	19 24 26	19 21	20 21	24 26	3

Step 3: Establish the minimum number of contributors for the 'preliminary' propositions

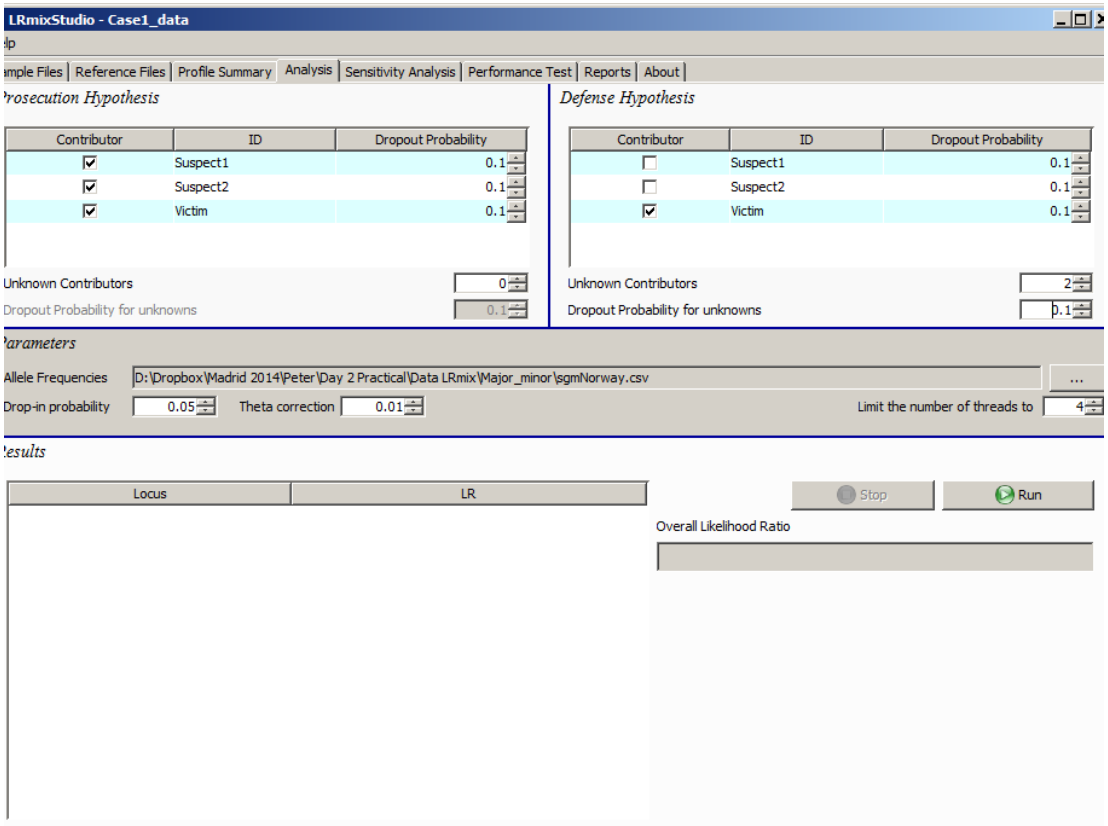
a) The swab is from a victim (*V*). There are two suspects (S_1,S_2) under *Hp*,

- b) In this example, some loci have 5 unique alleles across sets hence there is a minimum of three individuals present under *Hp*.

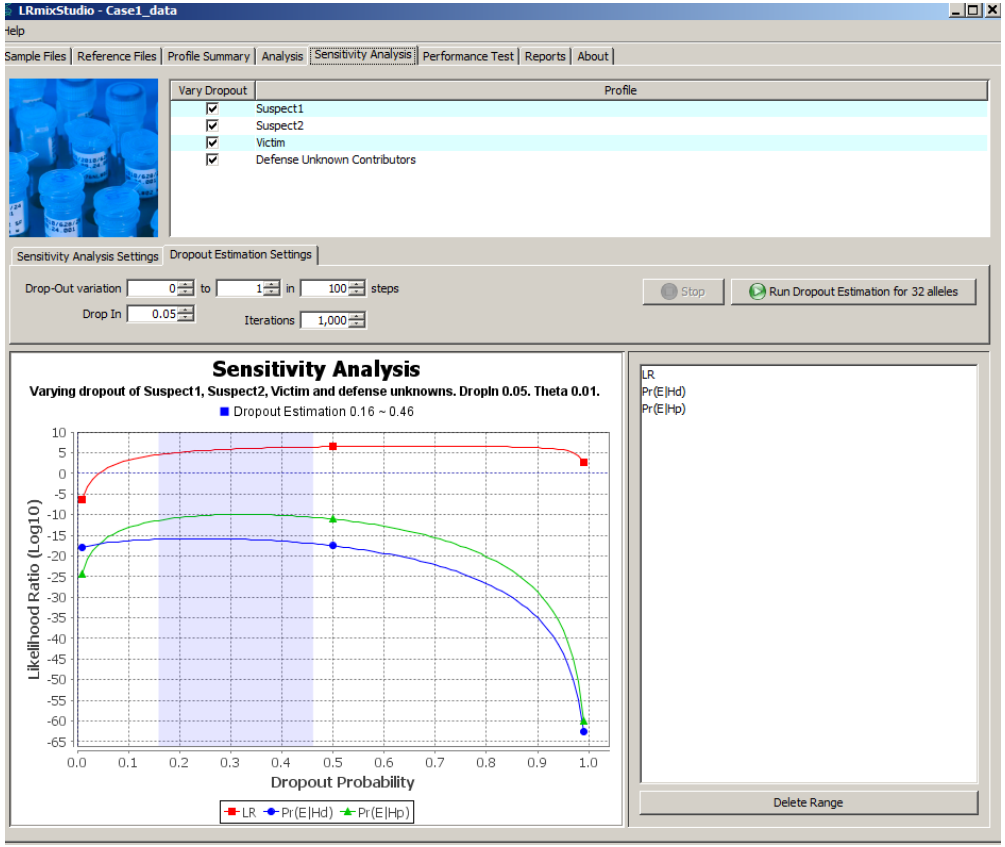
- c) A similar calculation can be made under *Hd* where the sets of genotypes formed by S_1,S_2 are not used, but in our rationale, it is convenient to anchor the minimum number of contributors on *Hp* and to assume equivalence (this is revisited later in the procedure).

- d) Consequently, the preliminary propositions are formulated as *Hp=V,*S_1,S_2 and *Hd=V,U,U*

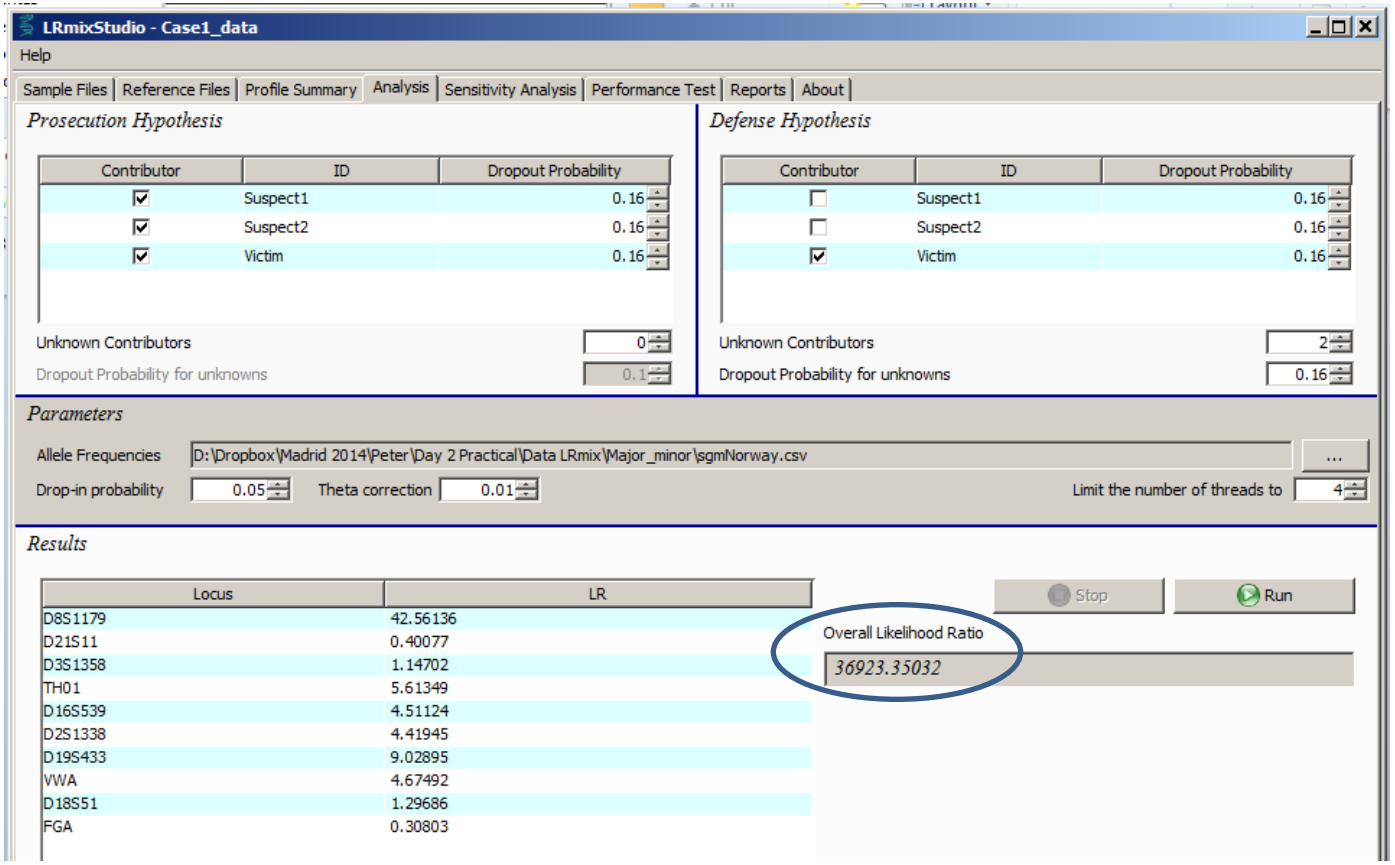
Set Propositions in Analysis Tab



Sensitivity analysis



Analysis


LRmixStudio - Case1_data
Help
Sample Files
Reference Files
Profile Summary
Analysis
Sensitivity Analysis
Performance Test
Reports
About
Prosecution Hypothesis
Contributor
ID
Dropout Probability
Suspect1 0.16
Suspect2 0.16
Victim 0.16
Unknown Contributors 0
Dropout Probability for unknowns 0.1
Defense Hypothesis
Contributor
ID
Dropout Probability
Suspect1 0.16
Suspect2 0.16
Victim 0.16
Unknown Contributors 2
Dropout Probability for unknowns 0.16
Parameters
Allele Frequencies
D:\Dropbox\Madrid 2014\Peter\Day 2 Practical\Data LRmix\Major_minor\sgmNorway.csv
...
Drop-in probability 0.05
Theta correction 0.01
Limit the number of threads to 4
Results
Locus
LR
D8S1179 42.56136
D21S11 0.40077
D3S1358 1.14702
TH01 5.61349
D16S539 4.51124
D2S1338 4.41945
D19S433 9.02895
VWA 4.67492
D18S51 1.29686
FGA 0.30803
Stop
Run
Overall Likelihood Ratio
36923.35032

Step 4: LRmix Studio analysis

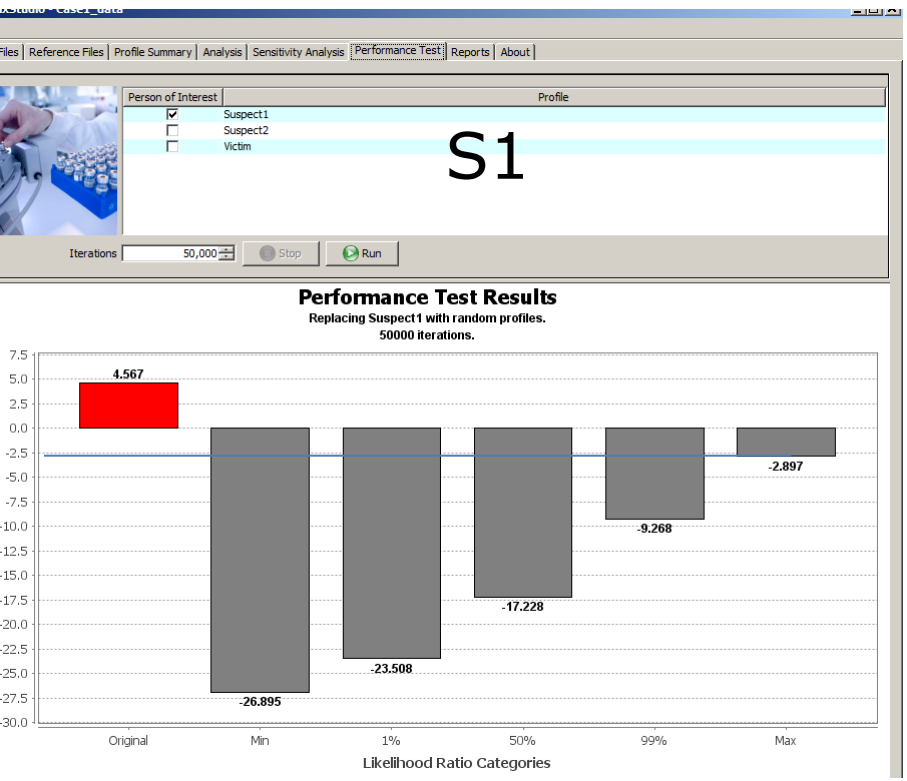
- *Hp=V,S*$_1$*,S*$_2$ and *Hd=V,U,U*

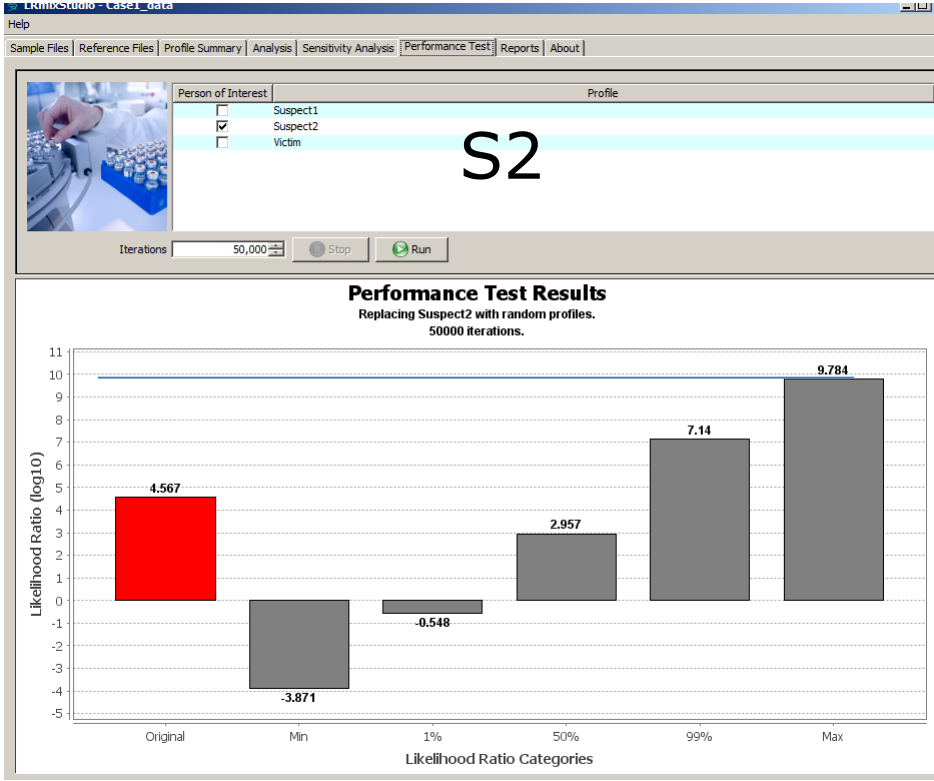
- Note 2 Suspects in numerator

- The $\log_{10}(LR_{min})$= 4.56 is derived for a drop-out probability *Pr*(*D*)=0.16.

- Pr(D) value is in fact the 5 percentile calculated from an empirical distribution of the drop-out probability conditioned on the expected number of alleles observed relative to the genotype of the hypothesised contributors, the procedure is described by Haned et al (FISG 2012)

Performance plot (evaluate one suspect at a time)





Run the same number of non-contributors
As the LR=c.50,000 in this case. Is the Max value <LR?

Rule for any complex analysis software

- Never evaluate two or more ‘known’ individuals in the numerator, unless mirrored in the denominator
- Eg. Hp= V,S1,S2 vs V,U,U is not OK
- However Hp=V,S1,U vs V,U,U is OK

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

A new methodological framework to interpret complex DNA profiles using likelihood ratios

P. Gill [a,b,*], H. Haned [c]

[a] Norwegian Institute of Public Health, Oslo, Norway
[b] University of Oslo, Oslo, Norway
[c] Netherlands Forensic Institute, Department of Human Biological Traces, The Hague, The Netherlands

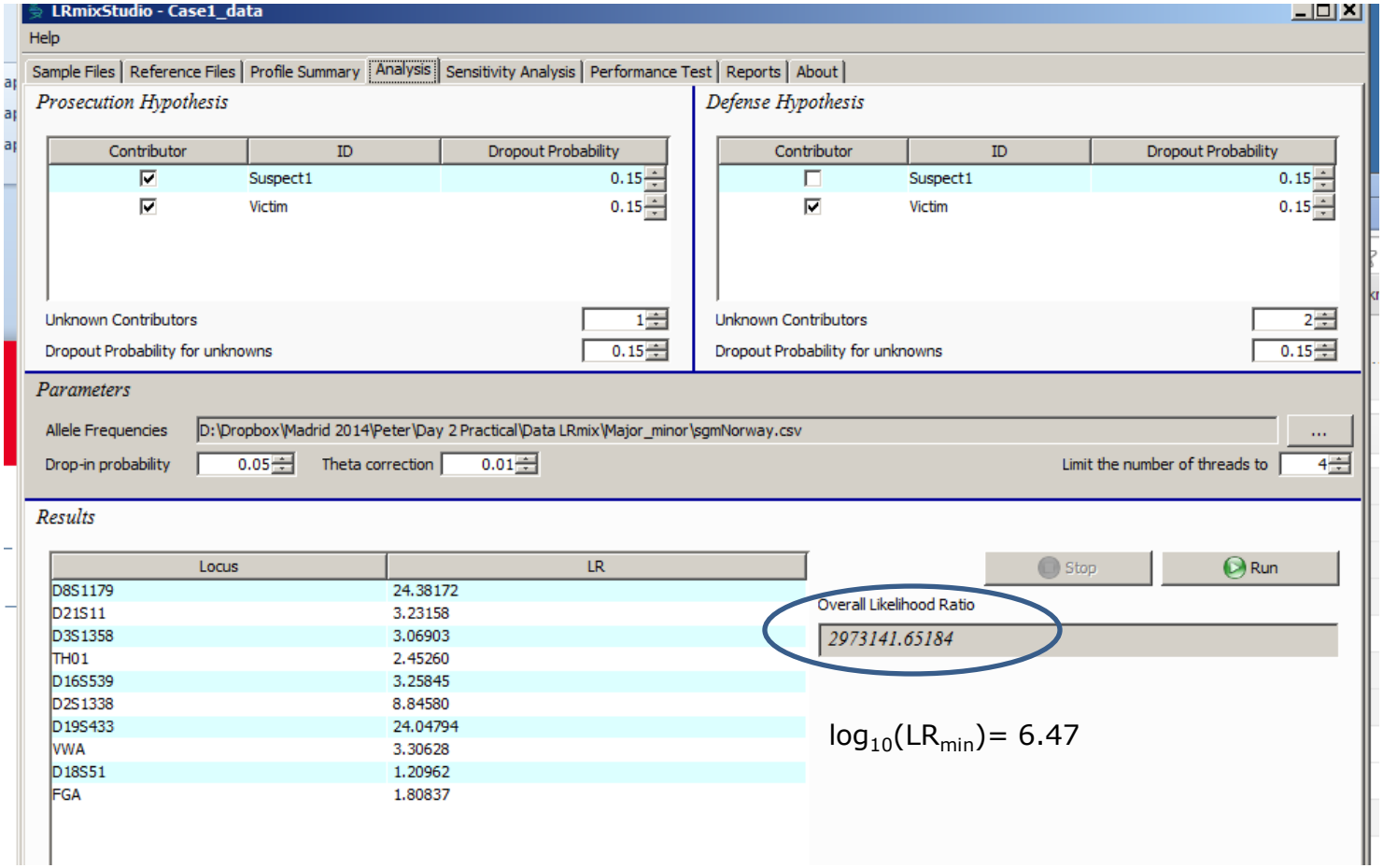
Limitations of Complex software

Never combine conditions in the numerator (unless duplicated in the denominator) as the LR is not meaningful

Always simplify the propositions

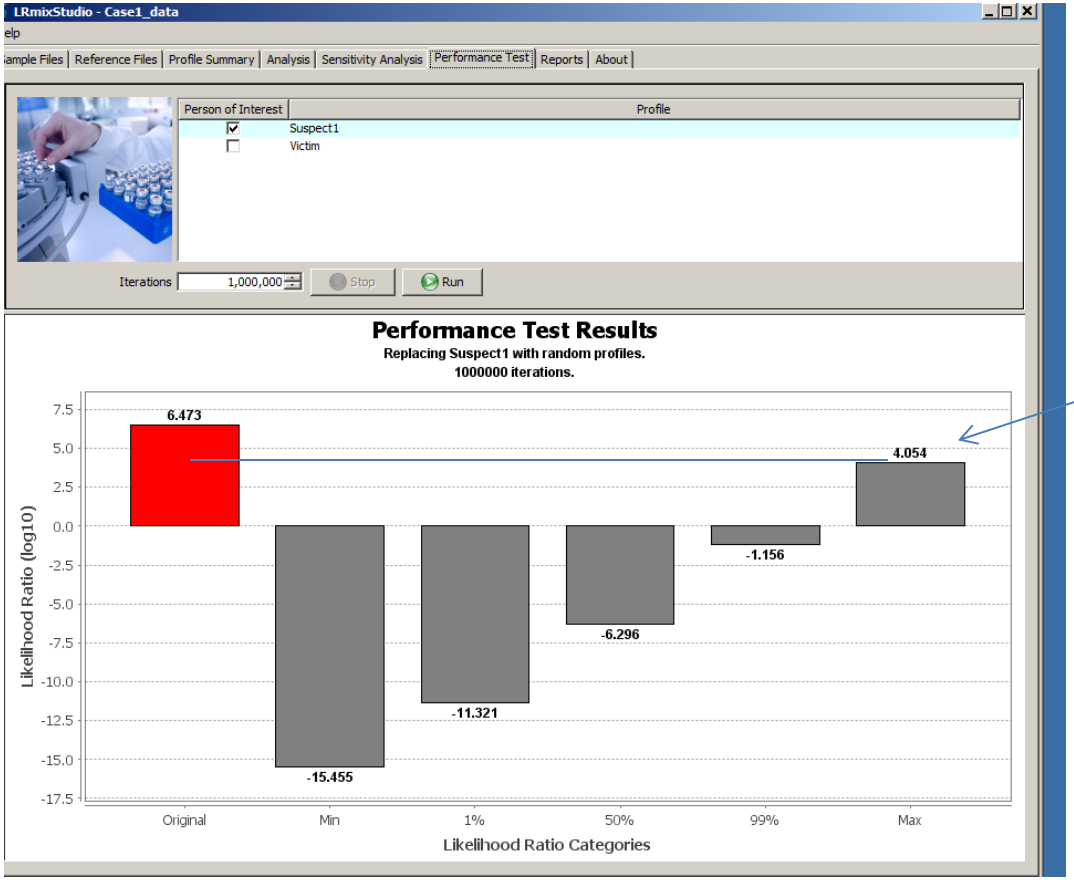
Suspect 1 calculation

Hp=S1,V,U and Hd=V,U,U



$\log_{10}(LR_{min})= 6.47$

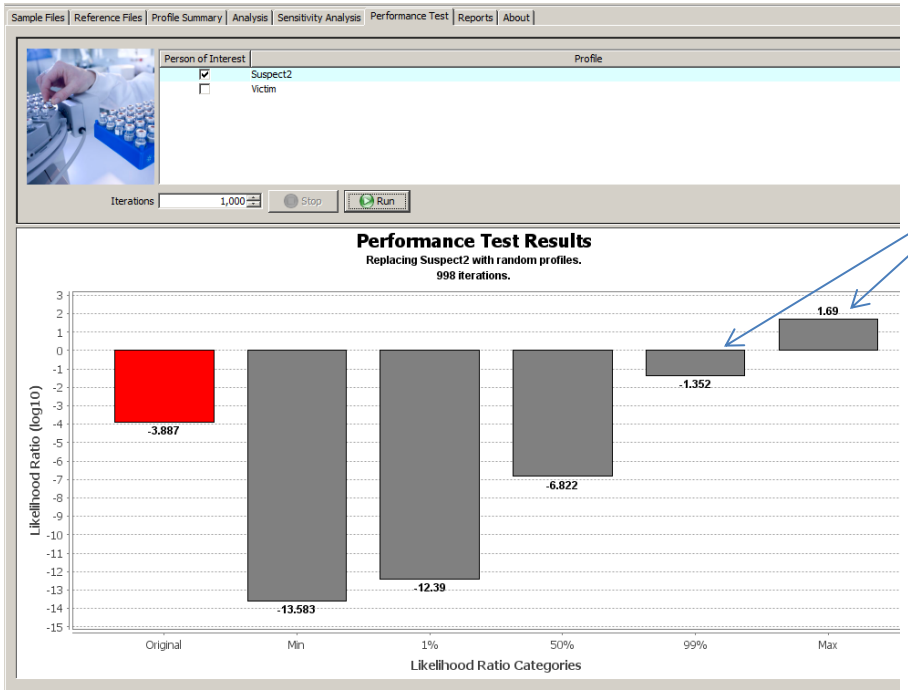
LRmix Studio S1 effect (1 million iterations)



Note this value is less than the LR

Now determine the S2 effect

- *Hp=S_2,V,U; Hd=V,U,U.*
- Pr(D_{min})=0.1, $\log_{10}(LR_{min})$= -3.8 which is clearly 'exclusionary'



Note this value is greater than the LR

Step 6: Non-contributor performance (Np) tests

•*Np* tests can be used to support the conclusion that evidence supporting S_1 is 'inclusionary' whereas evidence supporting S_2 is 'exclusionary'

	Three person mixture			Non-contributor performance
Hp	*Hd*	Random man substituted	$\log_{10}$(LR)	percentiles
S_1,S_2,*V*	*V*,*U*,*U*	S_1	4.5	(-23,-17,-9)
S_1,S_2,*V*	*V*,*U*,*U*	S_2	4.5	(-3,+2.9,+7)
S_1,*V*,*U*	*V*,*U*,*U*	S_1	6.4	(-11,-6,-1)
S_2,*V*,*U*	*V*,*U*,*U*	S_2	-3.8	(-12,-6,-1)

Conclusion

- It is not sufficient to provide a LR without the assurance of non-contributor analysis, especially for complex propositions
- There is a temptation to use software as black box, but this is dangerous
- Danger that there is insufficient defence challenge
- The software is meant to be used as a dialogue between prosecution and defence to decide propositions, models etc

Now we can start to think of explaining evidence in a different way

I have evaluated the proposition that Mr X is a contributor to the crime stain Y compared to the alternative proposition that Mr X is not a contributor to crime stain Y using the conditions defined in the LRmix model. These conditions are as follows:

a) Mr X and the victim are both contributors to the sample
b) An unknown person and the victim are both contributors to the sample

The evidence is 1 million times more likely if the first proposition (a) is true, compared to the alternative described by (b).

Qualification: This figure can be qualified with a test of robustness. To do this we replace Mr X with a random unrelated individual and we repeat the measurement of the likelihood ratio. We do this a total of 1 million times, with a different random individual each time.
When this was carried out the greatest likelihood ratio observed was of the order of 10,000

Extract from a statement based on a court report

In this case I calculated a likelihood ratio of 1 million (page X, supplement Y); if the answer is robust, then we would expect to observe that random individuals (non-contributors) would be expected to give a very low (exclusionary) likelihood ratio.
I have simulated 1 million individuals in a computer and measured the likelihood ratio of each calculation. From page X (supplement Y), I observed that non-contributors gave very low LRs. The maximum LR observed out of 1 million random individuals was equal to 10,000 and 99 percent of results were less than LR=0.01.
Discriminatory metric can be used to measure the distance of the observed LR – 99 percentile
This is not a RMNE but it simplifies the explanation in a similar way.

Provided that the LRmax was less than the LRobserved , we can also convert into natural frequencies which is consistent with Adams/Doheny court ruling:

"In a population of 1 million random people I would expect approximately two individuals, unrelated to the defendant, to give a LR that equals or exceeds the observed LR provided by the defendant this case"

Or in the UK of 30 million men I would expect 30 individuals, unrelated to the defendant, that equal or exceed the LR

DISCUSS

Quantifiler® Trio DNA Quantification Kit & PCR Setup Optimization: Results of Internal Validation

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Aikaterini Kondili, PhD
Penelope Miniati, PhD

Scientific Support Section,

Subdivision of Biological And Biochemical Examinations And Analyses,

Forensic Sciences Division,

Hellenic Police

Quantifiler® Trio is DNA Quantification Kit by Life Technologies
It utilizes two autosomal targets and a Y target

Target	Amplicon length	Ploidy	Copy Number	Dye/Quencher
Human Target, small autosomal	80 bases	Diploid	multicopy	VIC® dye with MGB quencher
Human Target, large autosomal	214 bases	Diploid	multicopy	ABY® dye with QSY® quencher
Human Male Target†	75 bases	Haploid	multicopy	FAM™ dye with MGB quencher
Internal PCR Control	130 bases	NA	Synthetic IPC template is included in the primer mix	JUN® dye with QSY® quencher

Degradation Index = [Small Target] / [Large Target]

The Degradation Index is used as a general indicator of whether large DNA fragments may perform more poorly relative to small DNA fragments in STR reactions.

DI ≤ 1 → Good Quality, Robust Sample

DI > 1 → Degradation Increases the Further Away from 1.

Trio was validated according to:

- **SWGDAM Revised Validation Guidelines**: www.fbi.gov/hq/lab/fsc/backissu/

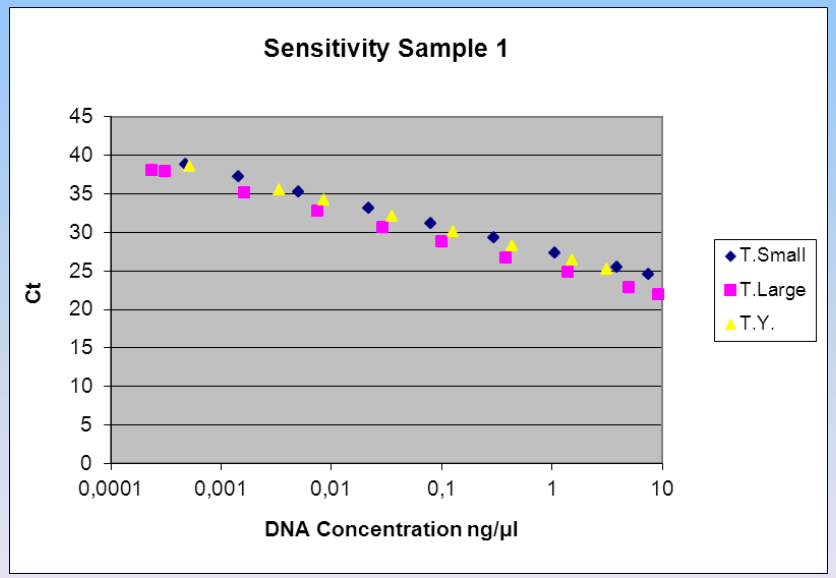
- Document Type: POLICY, Ref. Code: **ENFSI DNA WORKING GROUP**, Issue No: 001, Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process (2012)

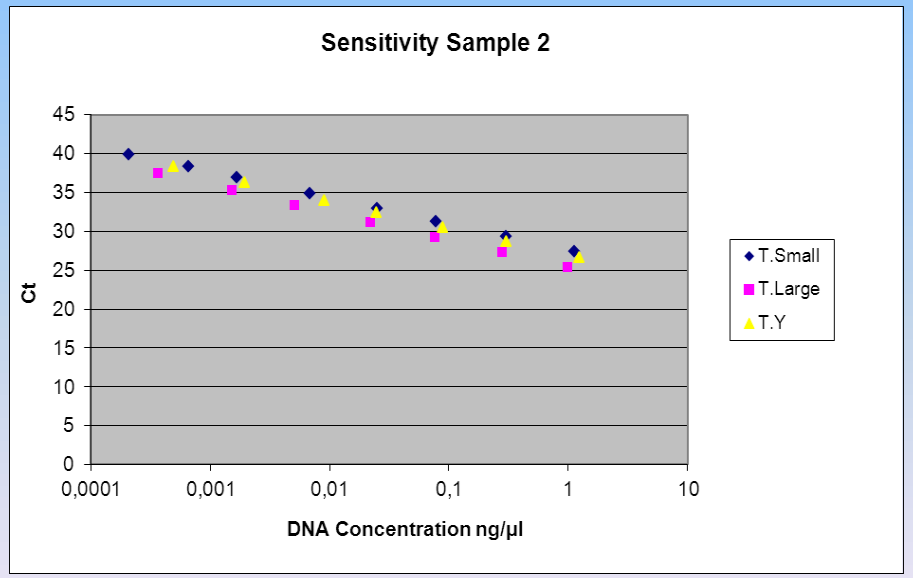
Experiments designed to assess:

- Sensitivity – Stochastic Effects
- Repeatability – Reproducibility - Precision
- Mixture Studies (Male : Female)
- Contamination Studies
- Stability of Standards

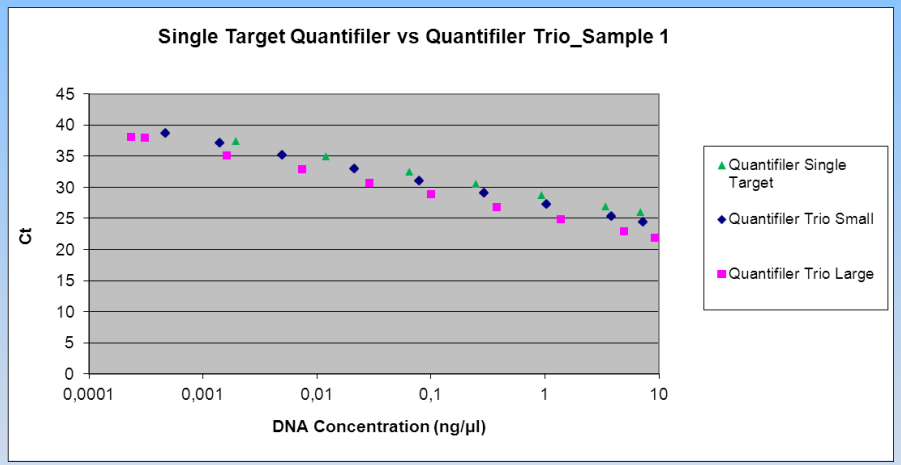
Sensitivity – Stochastic Effects

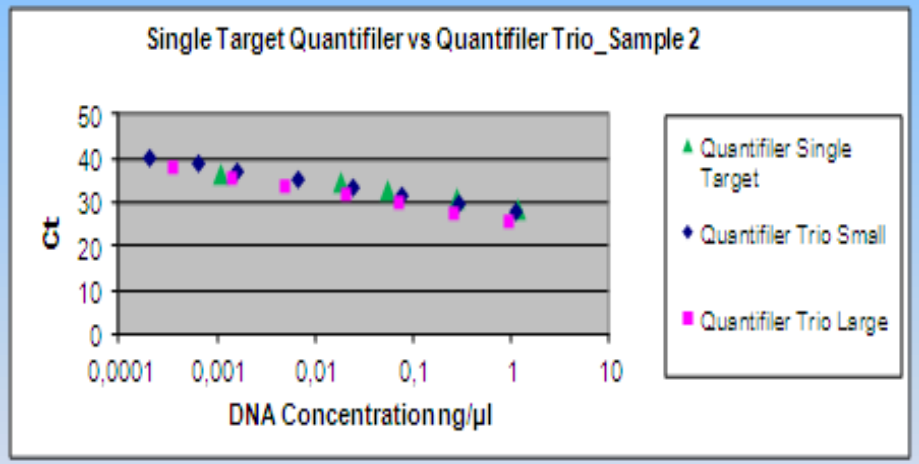
- Sample 1: M to F mixture – 10 concentrations ranging from 10 ng/µL down to 0,8 pg/µL x3
- Sample 2: a Gednap single source male DNA - 8 concentrations ranging from 1,25 ng/µL to 0,8 pg/µL x3





Samples used in Sensitivity Studies were also cross checked with the original single target Quantifiler® kit.





Repeatability – Reproducibility - Precision

❖ Repeatability

- 2 standard DNA samples: Quantifiler Trio THP Standard DNA plus Promega 2800 PC
- The two samples used for the above sensitivity studies

In total 15 different concentrations were analysed x3.

Concentration Values are Repeatable down to 5pg/µL

❖ Reproducibility

The samples from the sensitivity experiments were also tested at different times and/or different users

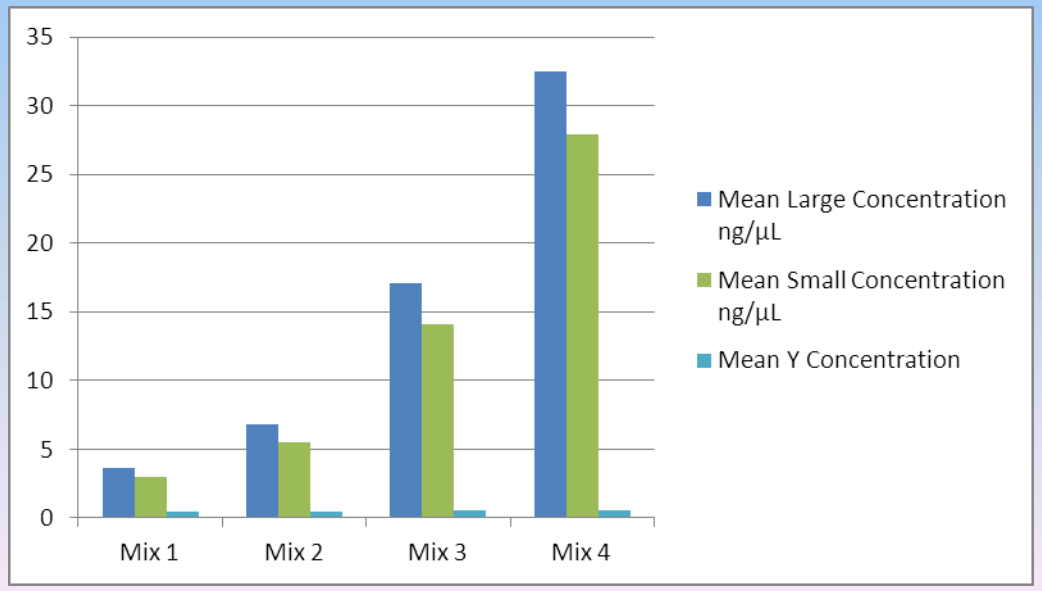
Results were consistent throughout the experiments

❖ Precision

Evaluation of Ct results from above experiments demonstrated the System's Precision

Mixture Studies

Sample	Male DNA ng/µL	Female DNA ng/µL	M:F
Mix 1	0,5	2,5	1:05
Mix 2	0,5	5,0	1:10
Mix 3	0,5	12,5	1:25
Mix 4	0,5	25,0	1:50

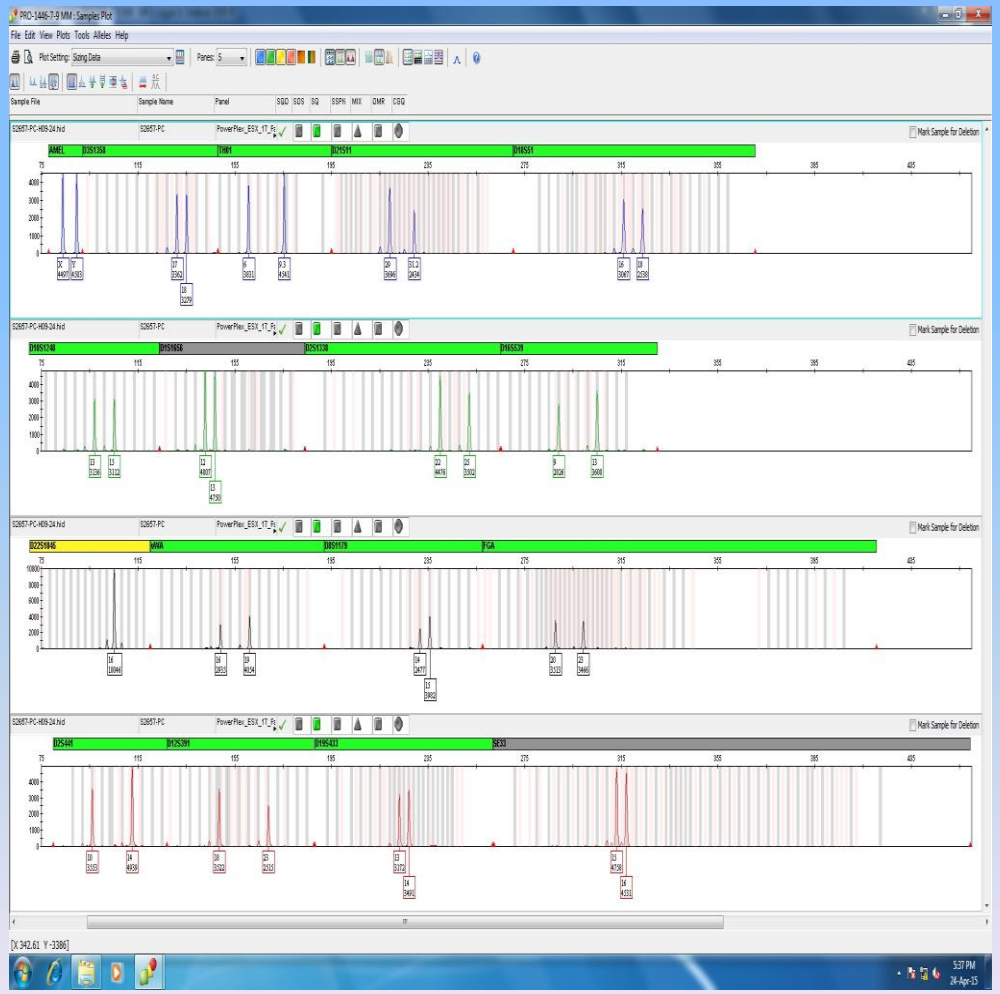


A Question About The Primary Quantification Target

Assess quantity

Purpose	After viewing the results and assessing the quality of the results, determine whether sufficient DNA is present to proceed with a short tandem repeat (STR) assay. **Note:** The primary quantification value is from the small autosomal target. Use this value for determination of STR input amount.

PowerPlex® ESX 17 profile of 2800M Control DNA generated in an ABI 3500*xl* genetic analyzer



A Novel Experiment:
Small vs Large for Autosomal PCR Setup?

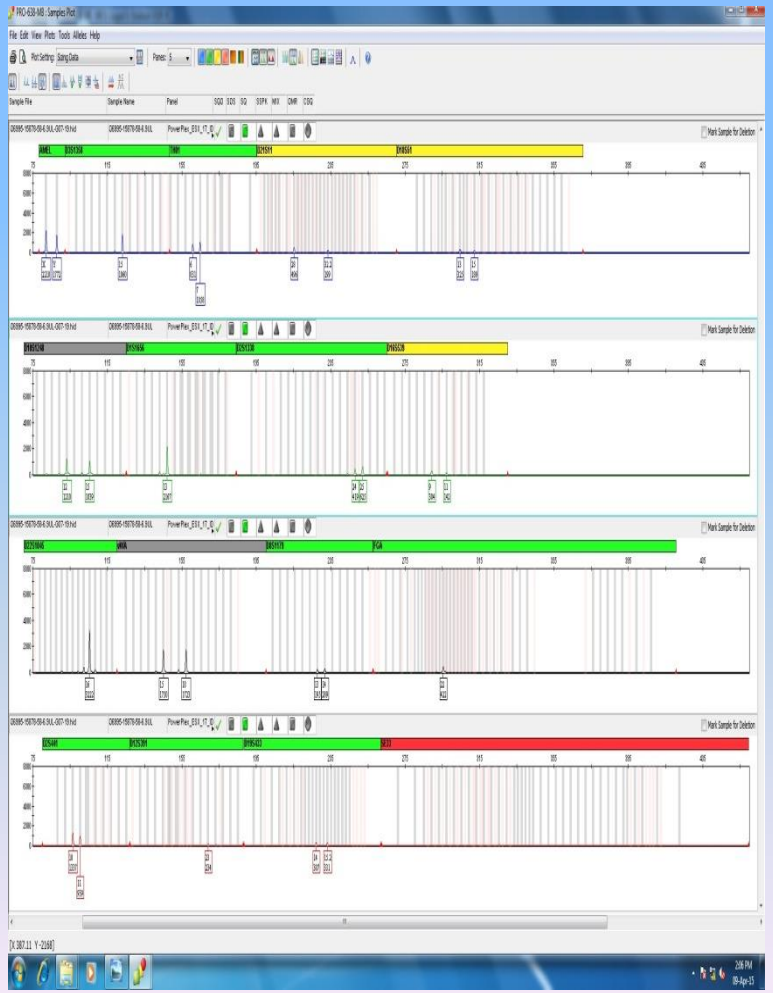
- 5 Mock Samples
- 8 Gednap Samples
- 34 Non Probative Casework Samples

were set up for PowerPlex® ESX 17 PCR with two values:

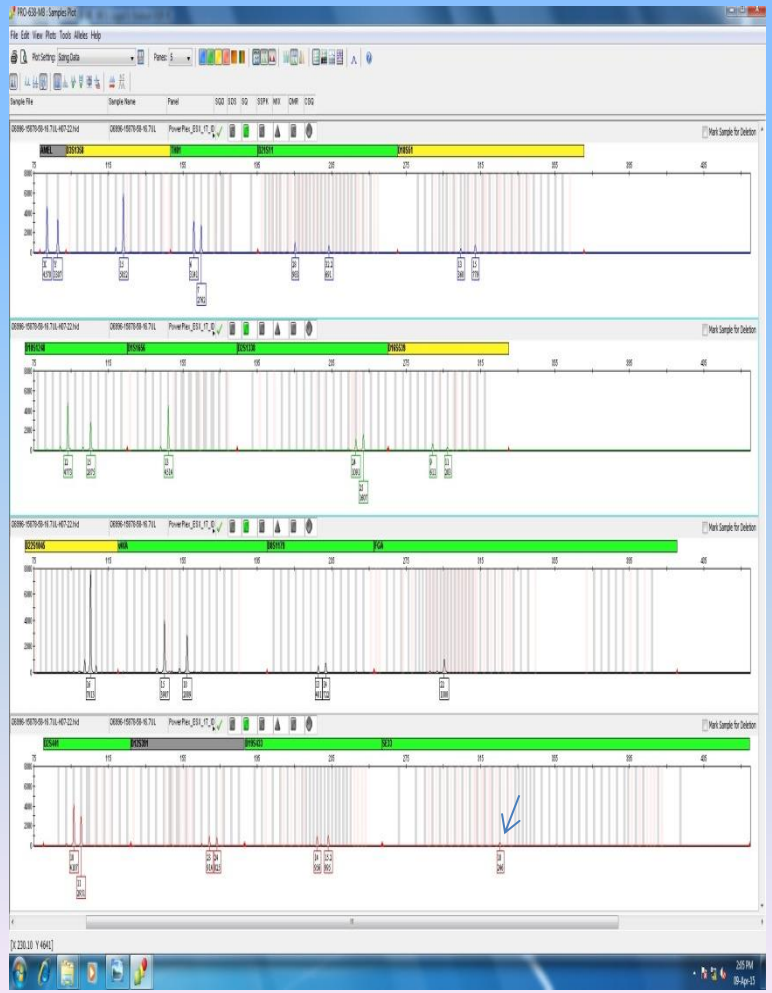
1. Small Target Concentration
2. Large Target Concentration

PowerPlex® ESX 17 profiles
Razor Swab - DI:3.3

Small: 0.1 ng/µL, V_{PCR}:4.9 µL

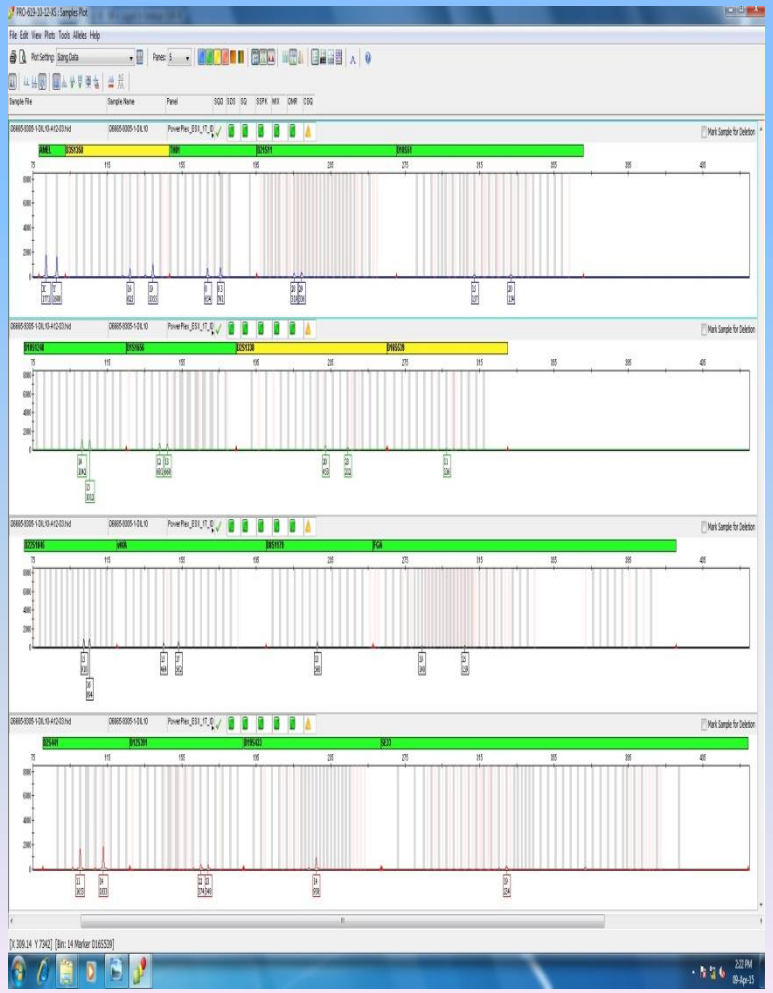


Large: 0.03 ng/µL, V_{PCR}:16.7 µL

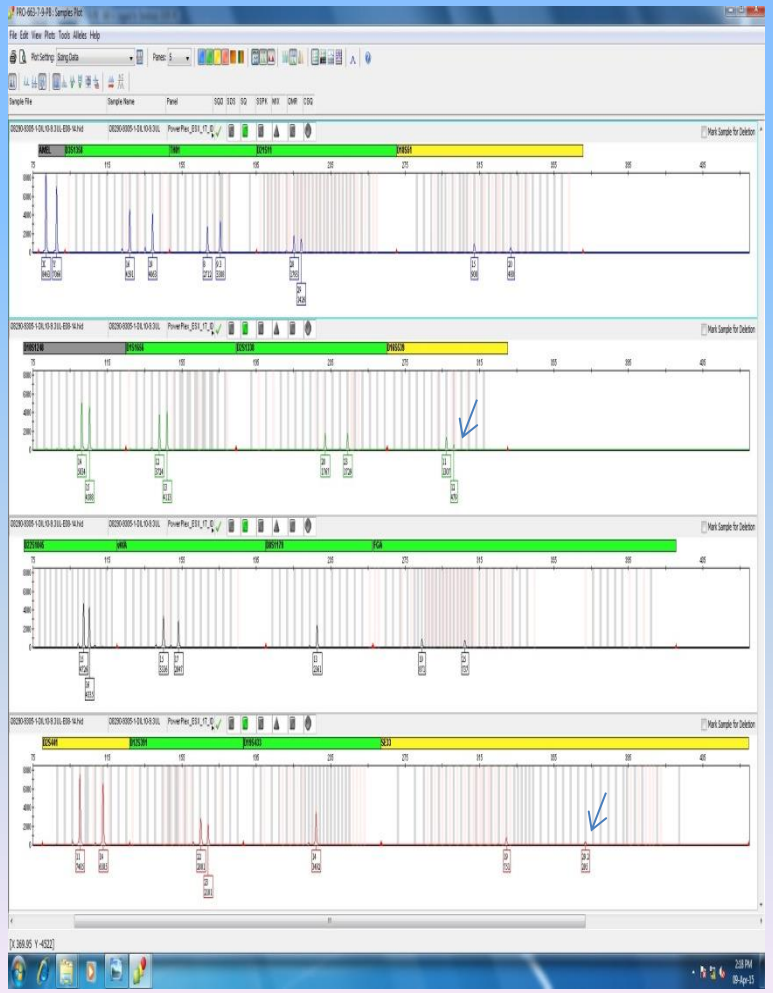


PowerPlex® ESX 17 profiles
Blood Swab - DI:3.5

Small: 1.9 ng/µL, V_{PCR}:0.26 µL

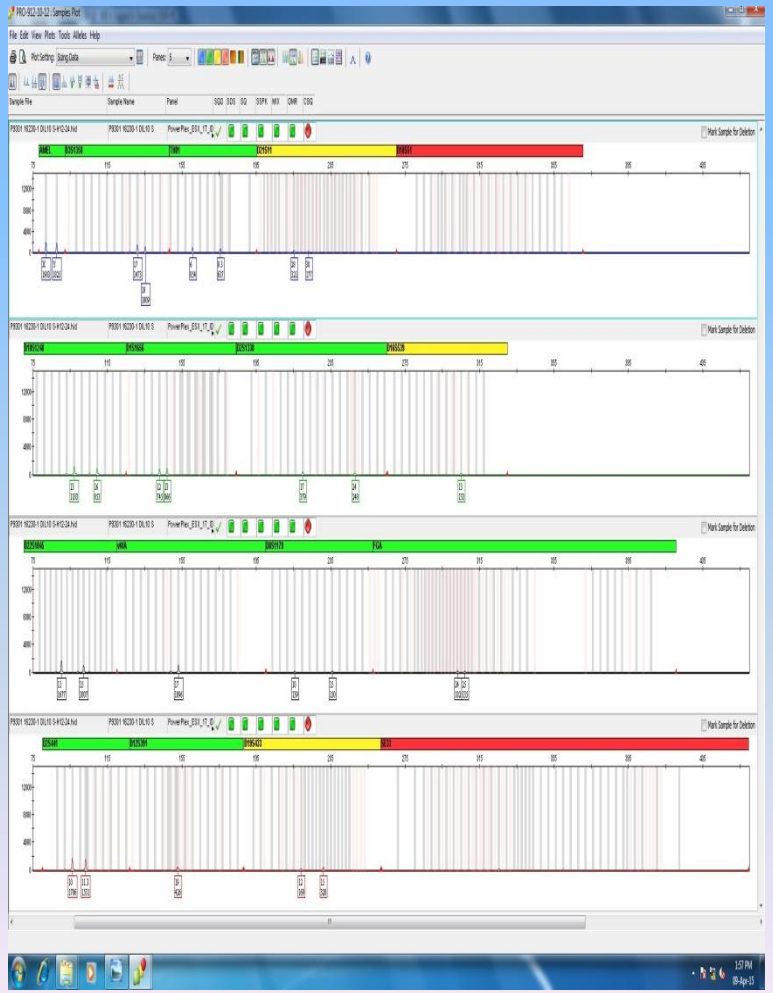


Large: 0.54 ng/µL, V_{PCR}:0.93 µL

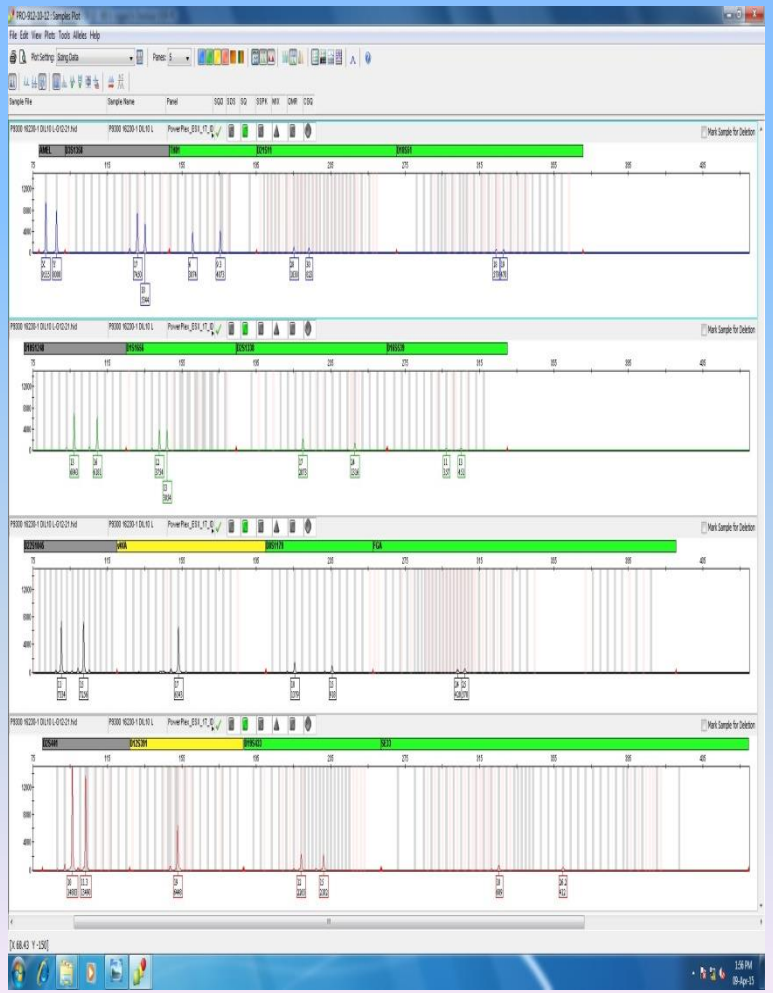


PowerPlex® ESX 17 profiles
Tooth Sample - DI:4.8

Small: 1.78 ng/µL, V_{PCR}:0.28 µL

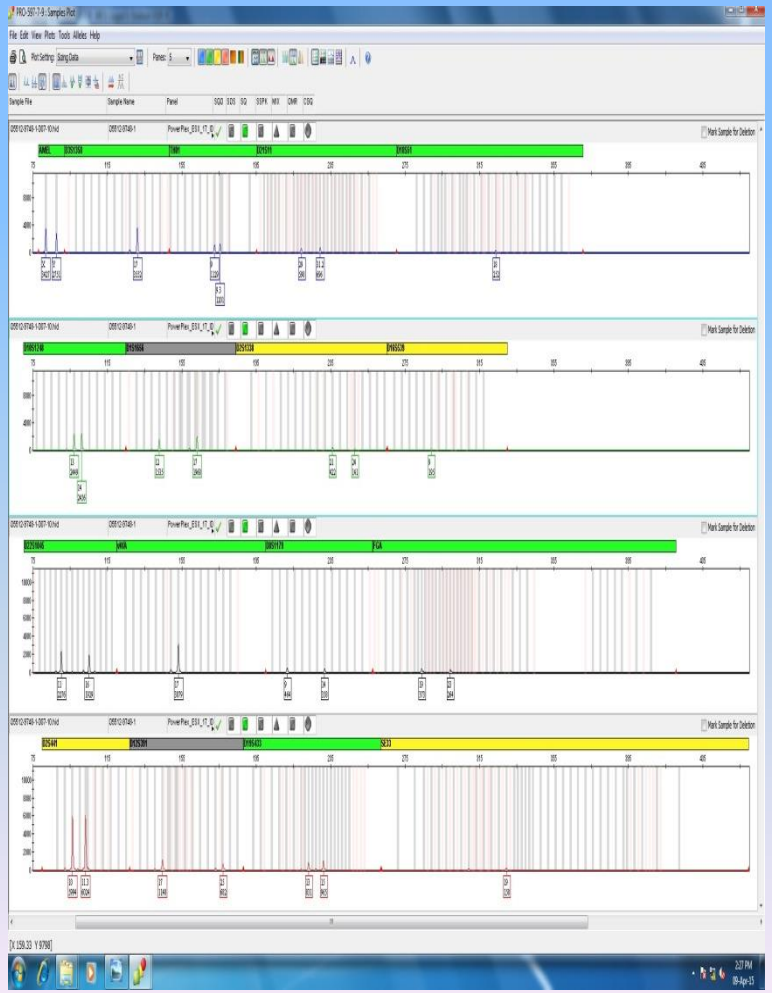


Large: 0.37 ng/µL, V_{PCR}:1.4 µL

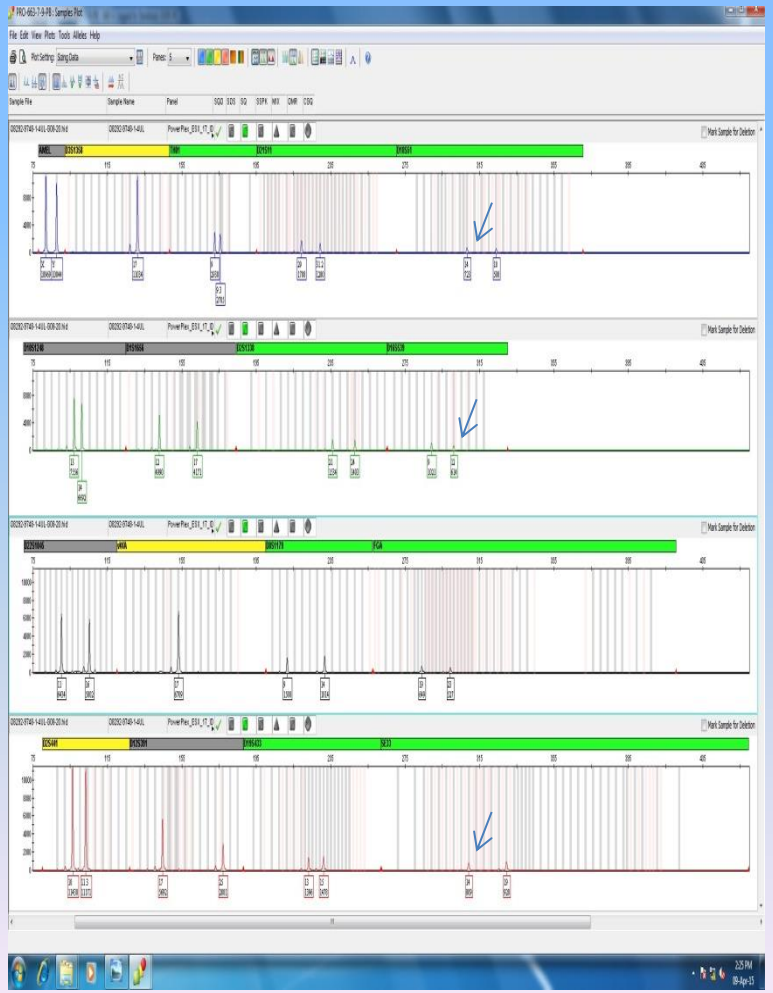


PowerPlex® ESX 17 profiles
Cigarette Butt - DI:6.4

Small: 0.77 ng/µL, V_{PCR}:0.65 µL

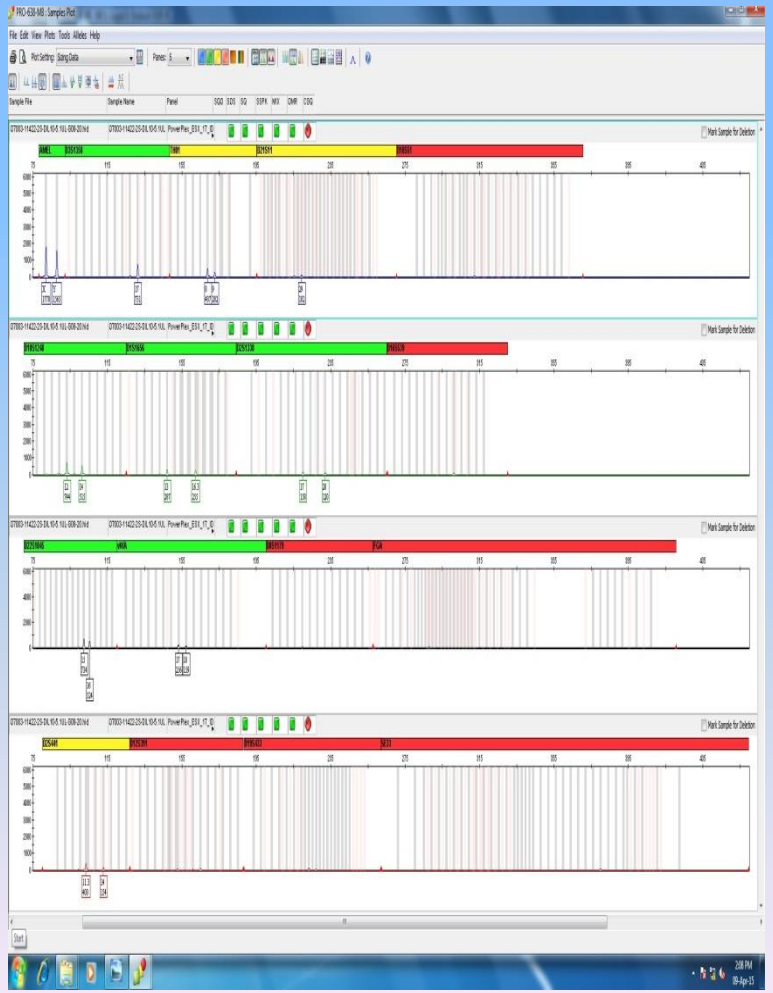


Large: 0.12 ng/µL, V_{PCR}:4.1 µL

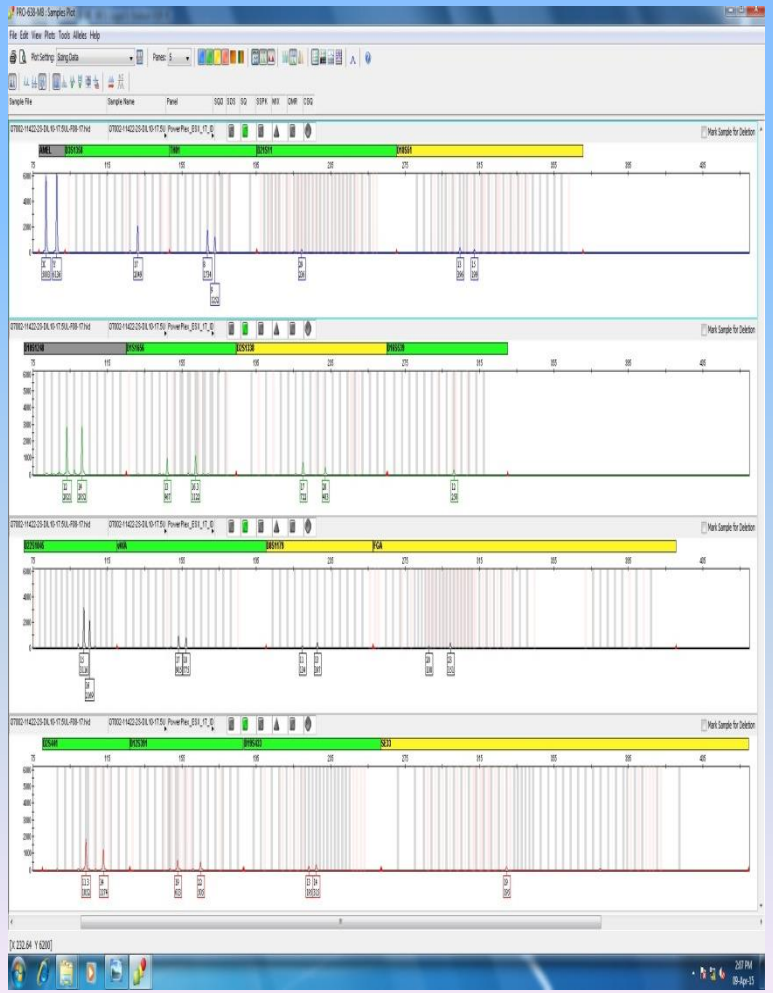


PowerPlex® ESX 17 profiles
Saliva Stain - DI:14.4

Small: 0.098 ng/µL, V_{PCR}:5.1 µL

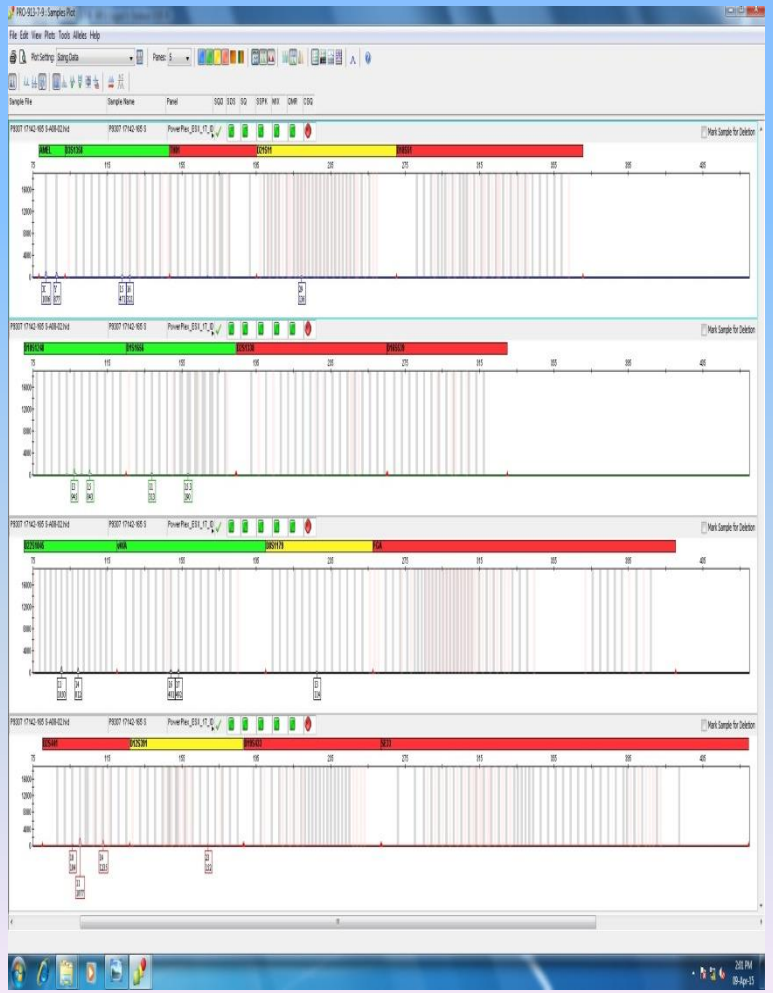


Large: 0.0068 ng/µL, V_{PCR}:73.5 µL

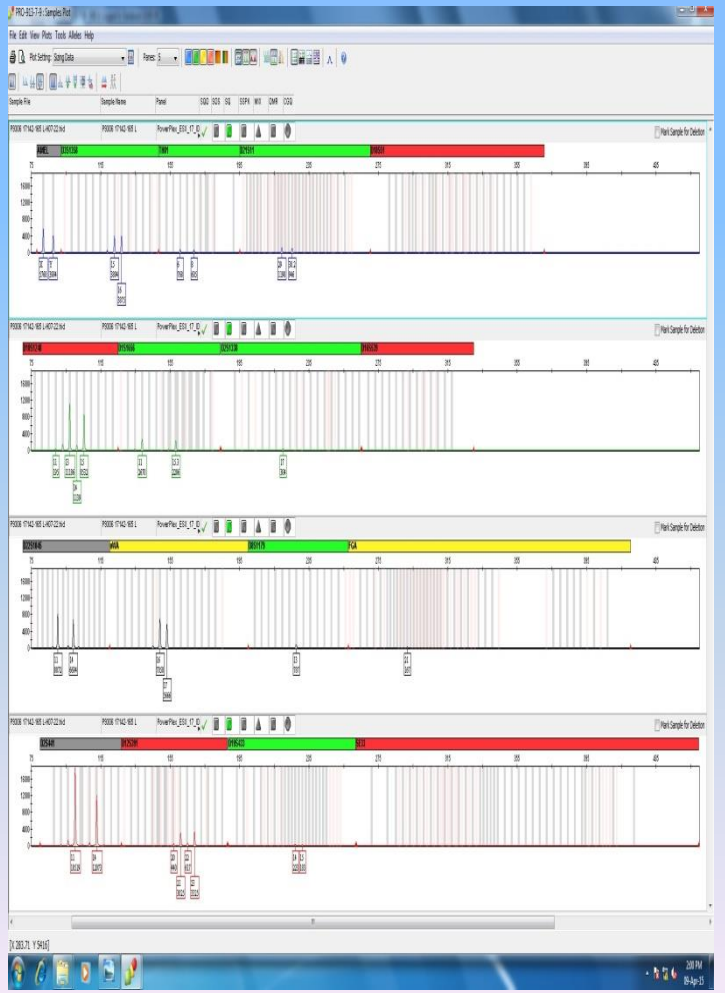


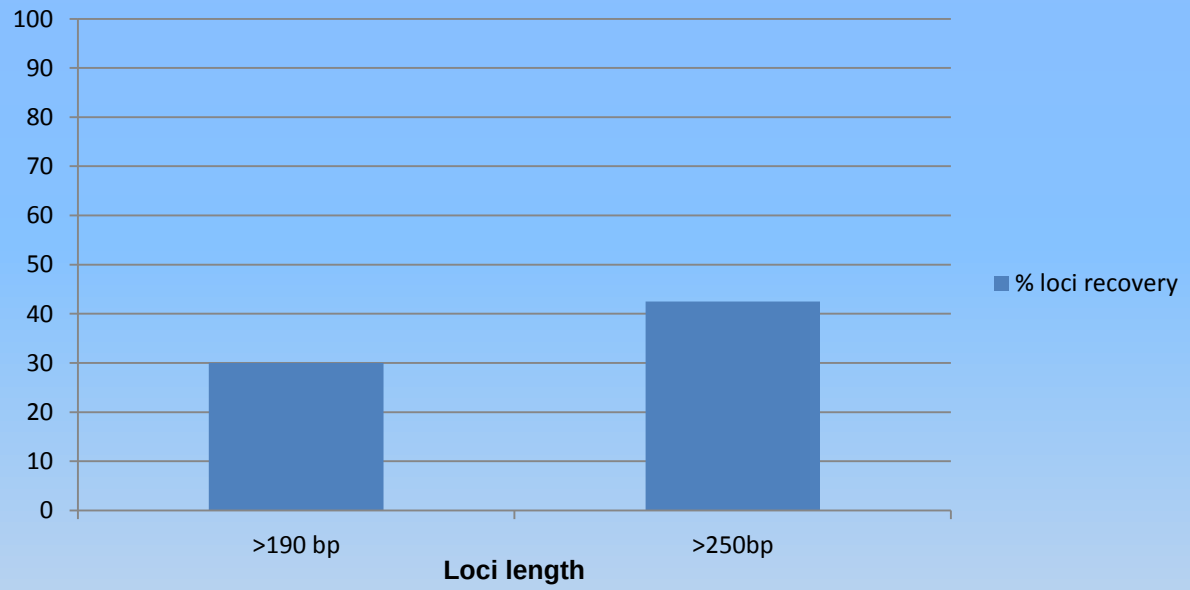
PowerPlex® ESX 17 profiles
Swab from Mobile Phone - DI: 20

Small: 0.22 ng/µL, V_{PCR}:2.4 µL



Large: 0.011 ng/µL, V_{PCR}:45.0 µL

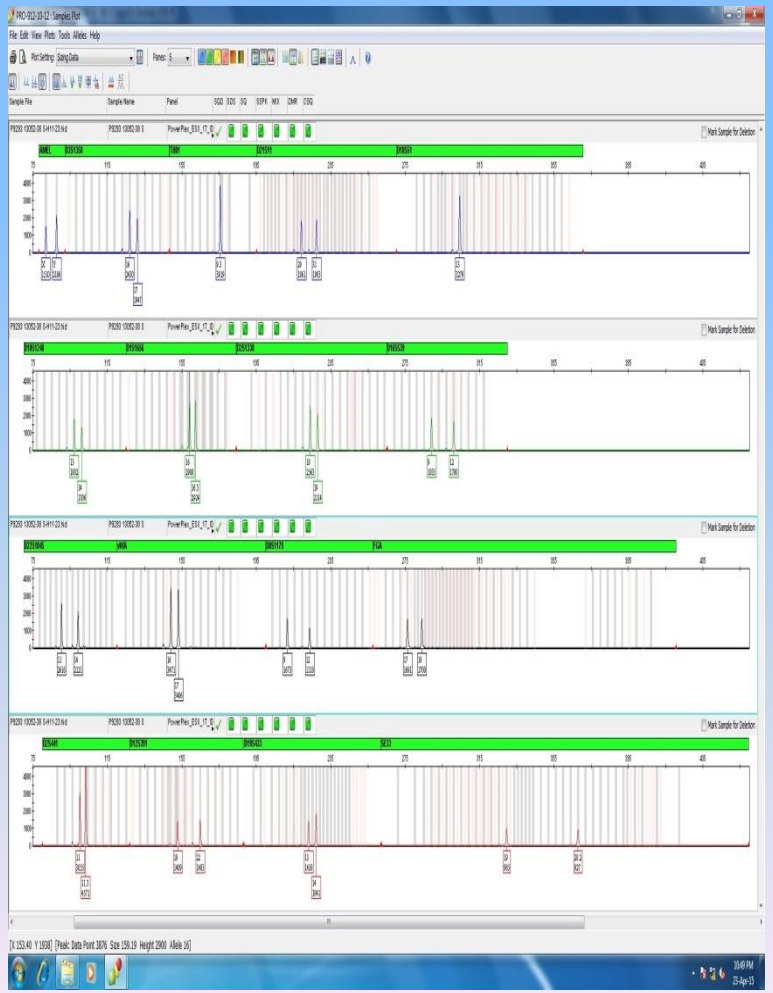




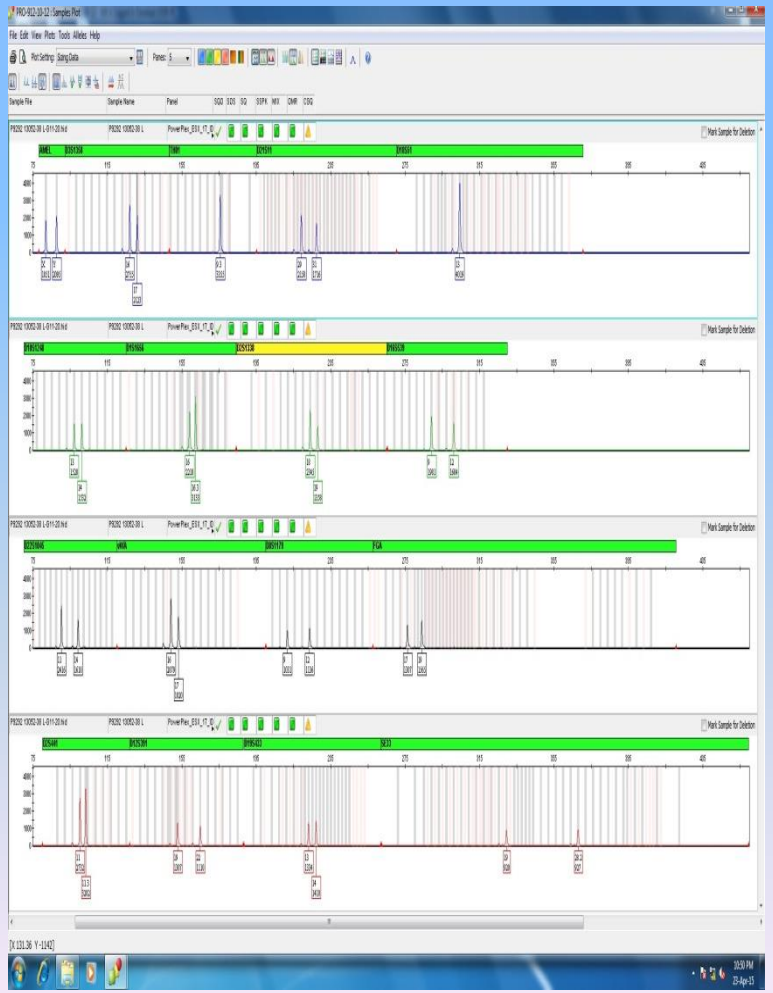
Loci recovery from single source casework samples with DI > 1

PowerPlex® ESX 17 profiles
Gednap Sample - DI: 0.79

Small: 0.083 ng/µL, V_{PCR}:6.1 µL

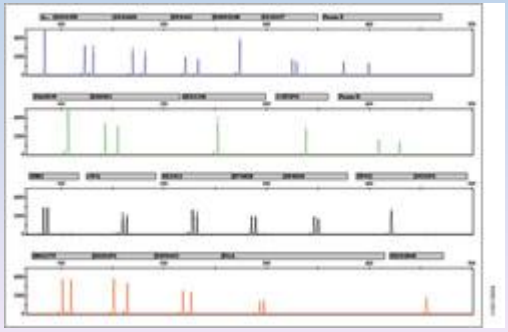


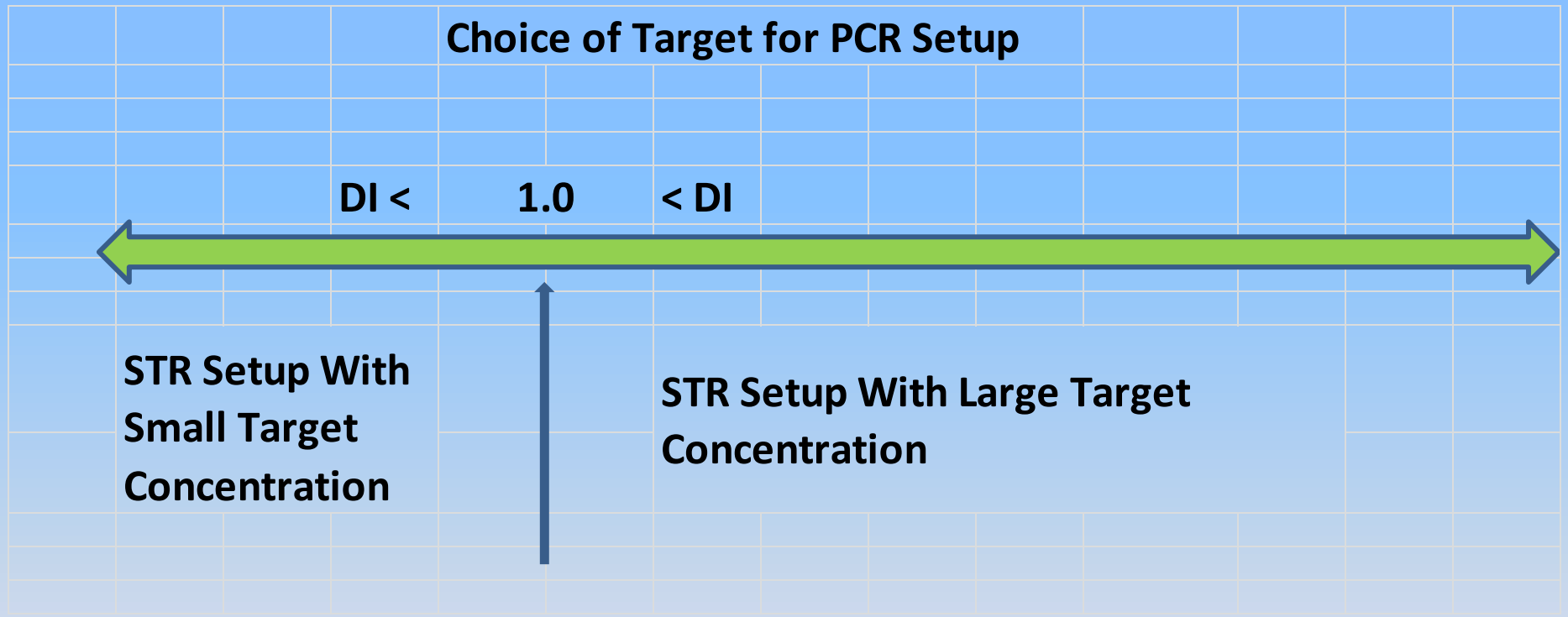
Large: 0.104 ng/µL, V_{PCR}:4.8 µL



Steps in Forensic DNA Analysis

?




Choice of Target for PCR Setup
DI <
1.0
< DI
STR Setup With Small Target Concentration
STR Setup With Large Target Concentration


Thank You!