EUROPEAN DNA PROFILING GROUP (EDNAP) MEETING

Bratislava, The Slovak Republic

23 April 2013

Host: Livia Zatkalikova.
Chairman: Niels Morling.

A list of participants is attached.

Welcome
Deputy director, Ing. Jozef Mĺkvikĺ, PhD, welcomed members to Bratislava.

Update on exercises

mRNA exercise no 6 Cordula Haas
Cordula Haas presented considerations concerning mRNA markers that may identify skin cells. Preliminary results of investigations with these markers were presented. Cordula Haas offered to organise a collaborative exercise. Critical information, reagents and samples will be sent from Zürich before the summer holidays. Results are expected to be submitted to Zürich in September 2013 so that the preliminary results can be presented and discussed at the EDNAP meeting in October/November 2013 (see attached presentation).

The IrisPlex exercise on genetic prediction of eye colour Manfred Kayser
Manfred Kayser presented the results of the collaborative EDNAP exercise for the prediction of eye colour with the IrisPlex (presentation attached). A manuscript will be prepared soon.

Updates from other groups

EMPOP Walther Parson
Walther Parson gave an update on the developments in EMPOP (presentation attached). Since the last meeting three EMPOP/mtDNA related papers were published in the Encyclopedia of Forensic Sciences series and a case study on ancient remains. EMPOP developments were presented at international conferences including the annual CODIS conference in Oklahoma (Nov 2012), the 100th Science Meeting in Kolkata (Jan 2013) and the HUGO Meeting in Singapore (Apr 2013). WP reported about mtGenome Next Generations Sequencing using the Ion Torrent PGM (Life Technologies). EMPOP Release 9 was issued including 29,444 haplotypes (presentation attached).

Interpol Richard Scheithaur

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

Ingo Bastisch mentioned that the Interpol DNA MEG will prepare a publication with recommendations related to a worldwide practice for the identification of missing persons in daily policing practice. Whereas DVI has undergone several instances of evaluation and is being dealt with in international working groups the daily practice in missing person

identification lacks such standards. The possibility of identifying MPs by means of DNA has a high potential for success when good working practice and standardisation is in place. The MEG recommendations are intended to close that gap. Especially, the standard set of STR markers must be extended so that successful searches of STR profiles can be expected when searching in national and international missing persons/unidentified bodies databases.

Tom Parsons discussed the role of the Interpol DVI Steering Group and Interpol DVI Standing Committee, and the upcoming release of a new version of the Interpol AM/PM forms. Issues concerning the inclusion of DNA profile data on the AM/PM forms were discussed, and reference was made to developing plans to establish an Interpol "DVI Platform" to assist with global DVI preparedness and innovation.

NIST Niels Morling
Niels Morling informed that the previous position of John Butler as chief of the Applied Genetics Group at NIST has been taken over by Peter Vallone. John Butler now has another position in NIST. Pete/John sent a pdf with a description of the present work at NIST (presentation attached).

Orlando, Florida Niels Morling
Jack Ballantyne had to cancel his attendance. Instead he sent the attached pdf with presentation of the present work (presentation attached).

Australia John Scheffer
John Scheffer presented the latest forensic genetic news from the laboratory in Melbourne and from Australia in general (presentation attached).

Euroforgen - NoE Peter Schneider
Peter Schneider gave an update on the EUROFORGEN Network of Excellence project that started 1 January 2012 (presentation attached).

EDNAP web site update (www.isfg.org/EDNAP) Peter Schneider
Members are encouraged to visit the website.

Future activities Niels Morling
Chris Phillips offered to organise a collaborative exercise on the use of ancestry informative markers (AIMs). A detailed suggestion will be presented at the next EDNAP meeting.

Any other business
There was no other business.

Closing of the meeting
The meeting closed with sincere thanks to Livia Zatkalikova and her colleagues at the laboratory in Bratislava.

Amendment
After the meeting, it was decided to ask the colleagues in Athens to organise the next EDNAP meeting as well as the steering group meeting of the DNA Working Group of ENFSI in October/November 2013. Members will receive further information as soon as possible.

Attachments are found at the EDNAP website

- List of participants
- Presentations by
  - Cordula Haas: Suggestion for a skin cell mRNA exercise
  - Manfred Kayser: Presentation of IrisPlex results
  - Walther Parson: EMPOP report
  - John Butler/Peter Vallone: NIST report
  - Jack Ballantyne: Report from Orlando
  - John Scheffer: Presentation from Melbourne/Australia
  - Peter Schneider: EUROFORGEN-NoE report.

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EDNAP
mRNA profiling exercise 6

Cordula Haas / Erin Hanson / Jack Ballantyne

April 23, 2013, Bratislava

Tasks from last EDNAP meeting

- Prepare manuscript on EDNAP RNA exercises 4+5 (menstrual blood, vaginal secretion, housekeeping genes)

- Explore the possibility of identifying epithelial cells by means of mRNA investigations and, hopefully, present a plan at the next EDNAP meeting for a collaborative exercise

EDNAP mRNA profiling exercise 6: Identification of skin

- Skin mRNA markers
- Specificity
- Extraction methods
- Skin RNA dilution series
- Skin stains
- Suggestion mRNA exercise 6

Evaluated mRNA markers

Skin markers + Multiplexes

- LCE1C
- IL1F7
- LCE1D
- LCE2D
- CCL27

Hanson, Ballantyne
(FSI Genetics, 2012)

- LOR
- KRT9
- CDSN

Visser, Kayser
(Int J Legal Med, 2011)

Evaluated mRNA markers: Specificity

(Hanson et al., FSI Genetics, 2012 + unpublished data)

4 ul or 0.25-10 ng insert into RT — singleplexes

Samples: (10 ul or 1/4 swab)	LCE1C	IL1F7	LCE1D	LCE2D	CCL27	LOR	KRT9	CDSN
Blood	0/4	0/4	0/5	0/4	0/4	0/3	0/2	0/3
Saliva/buccal	0/4	0/4	0/5	0/4	0/4	3/5	0/3	0/5
Semen	0/4	0/4	0/5	0/4	0/4	0/3	0/2	0/3
Vaginal secretions	0/4	0/4	0/5	0/4	0/4	3/3	0/2	1/3
Menstrual blood	0/4	0/4	0/5	0/4	0/4	2/3	0/2	0/3
Skin	10/11	13/13	12/15	10/13	6/6	3/3	only >25ng	3/3

Tissues (~1ng)	LCE1C	IL1F7	LCE1D	LCE2D	CCL27	LOR	KRT9	CDSN
Adipose	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Bladder	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Brain	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Cervix	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Colon	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Esophagus	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Heart	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Kidney	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1
Liver	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Lung	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Ovary	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Placenta	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Prostate	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Skeletal Muscle	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Small Intestine	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Spleen	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Testes	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Thymus	0/1	0/1	0/1	0/1	0/1	1/1	1/1	0/1
Thyroid	0/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1
Trachea	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
H2O	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Evaluated mRNA markers: Specificity

(Lindenbergh et al., FSI Genetics, 2012)

			Housekeeping			Blood			Mucosa			Saliva		Semen		Menstrual Secretion		Skin		Vaginal Mucosa		
	Sample sets	n	18S-rRNA	ACTB[a]	GAPDH	HBB	AMICA1	CD93	KRT4	SPRR2A	KRT13	STATH	HTN3	PRM1	SEMG1	MMP7	MMP11	CDSN	LOR	MUC4	HBD1	HBD1[b] 1plex
1	Blood	8																				n.d[c]
2a	Semen-fertile	6																				n.d
2b	Semen-sterile	2																				n.d
3	Saliva	8																				n.d
4	Menstrual Secretion	8																				n.d
5	Vaginal Mucosa	8																				
6	Blood-skin	8																				n.d
7	Skin-cotton	8																				n.d
8	Skin-cotton-stub	8																				n.d
9	Skin-washed	8																				n.d
10	Skin-unwashed	8																				n.d
11	Skin-foot	8																				n.d
12	Skin-back	8																				n.d
13	Skin + 10µl Saliva	8																				n.d
14	Drink Simulation	8																				n.d
15	Tonque Samplings	8																				n.d

	0%
	12,5%
	25%
	37,5%
	50%
	62,5%
	75%
	87,5%
	100%

[a] For all the skin samplings, drink simulation and tongue samplings,0.2 µM ACTB was used instead of 0.6 µM.

[b] For vaginal mucosa samples the HBD1 primer set was also tested in singleplex.

[c] n.d.: not determined

Extraction methods

Pinpoint™ Slide RNA Isolation System II
Catalog No. **R1007**

Arcturus® PicoPure® RNA Isolation Kit

Samples

- Dilution series of commercial RNA, on swabs
- Swab from skin
- Hand print on paper
- touched objects (computer mouse, keyboard, etc.)
- Finger prints on glass

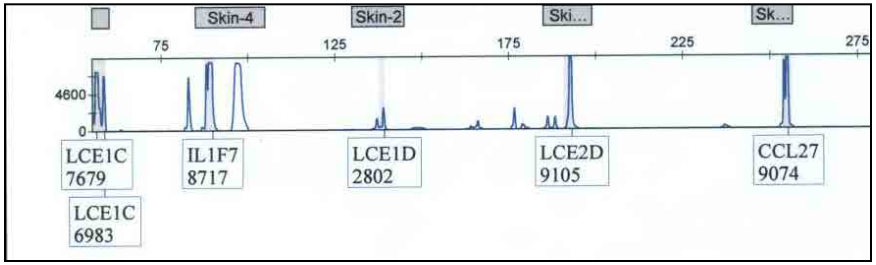
RNA results: Dilution series

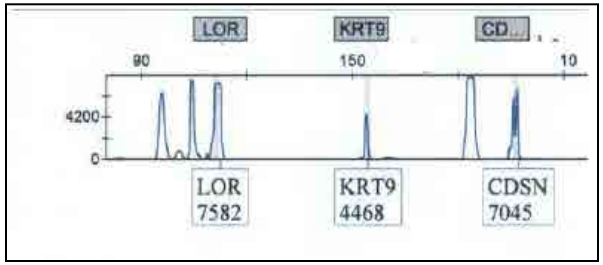
Commercial skin RNA on swabs, immediate analysis:

original amount on swab	5plex (FAM) - Qiagen Multiplex PCR kit						Visser marker - multiplex		
	LCE1C (RFU)		IL1F7 (RFU)	LCE1D (RFU)	LCE2D (RFU)	CCL27 (RFU)	CDSN (RFU)	KRT9 (RFU)	LOR (RFU)
500ng	7679	6983	8717	2802	9105	9074	7582	4468	7045
250ng	7761	7157	8726	2052	8917	8911	7944	2574	8158
125ng	7836	7410	8643	1687	8928	8975	8591	573	7983
62.5ng	8326	5372	8674	663	1345	5184	8932	0	3990
31.25ng	8381	6767	9088	954	7742	8635	8948	0	3832
Skin pos. Contr.	7875	4117	8744	7425	8983	8985	8068	8850	8524

Hanson/Ballantyne (5plex)

Visser et al (3plex)





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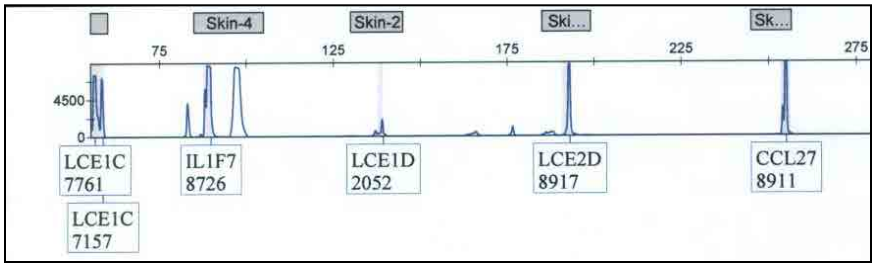

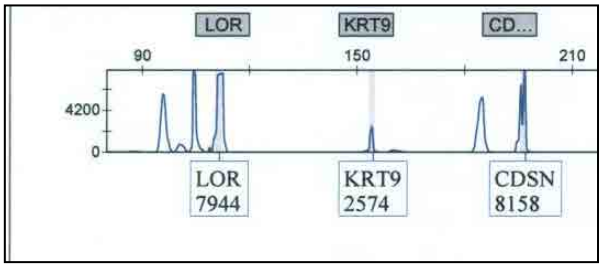

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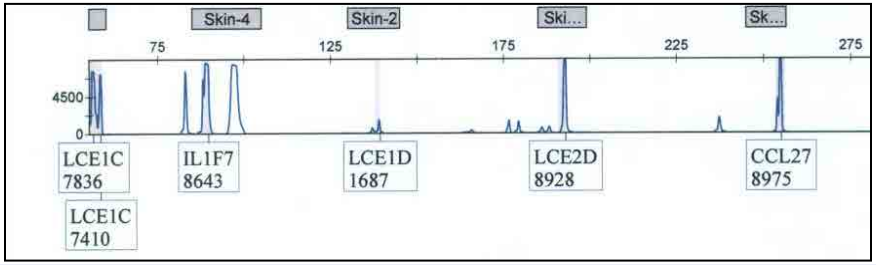

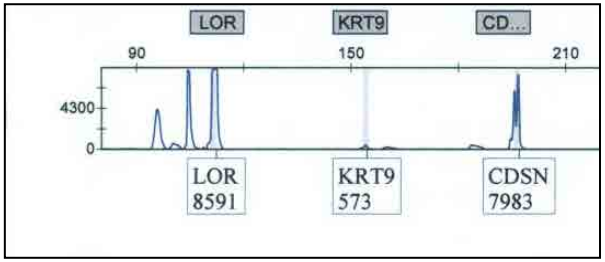

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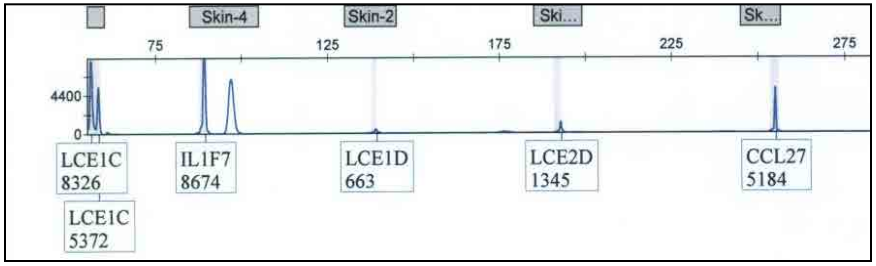
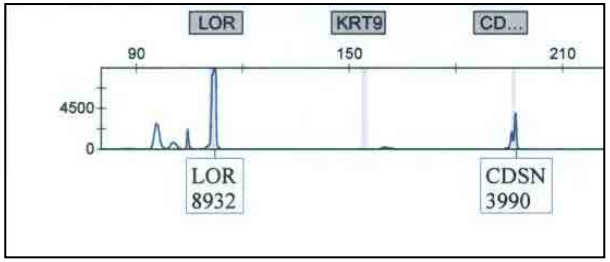
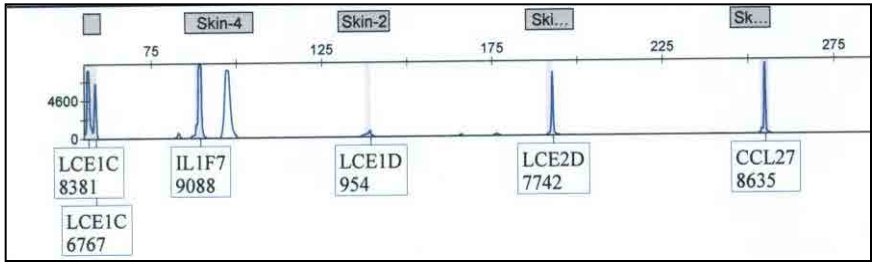
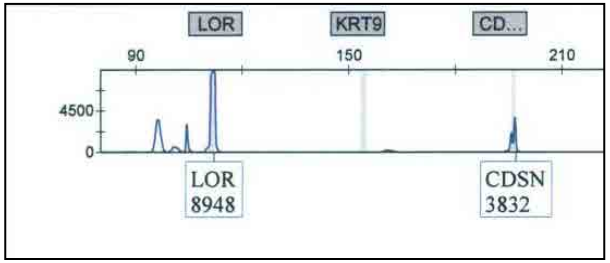




62.5ng



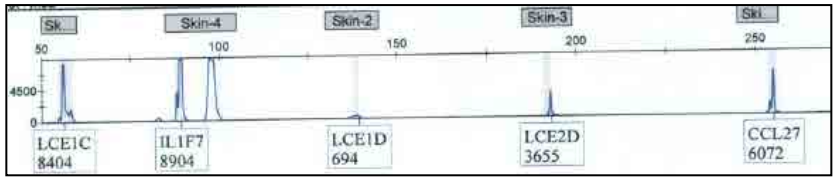
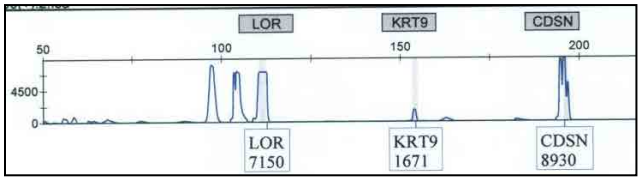
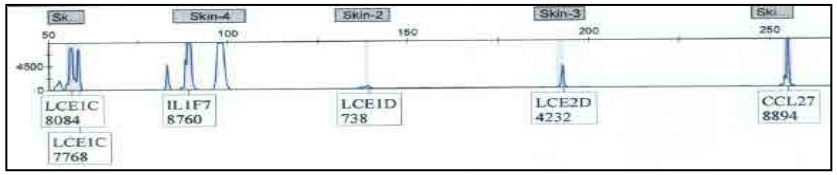
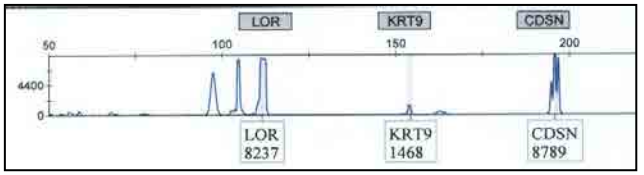
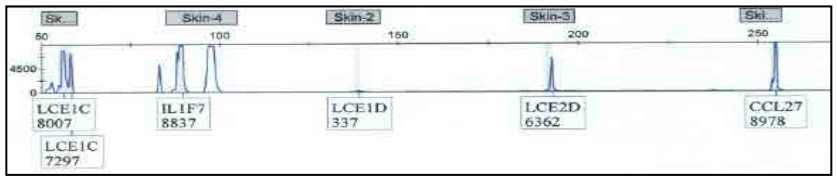
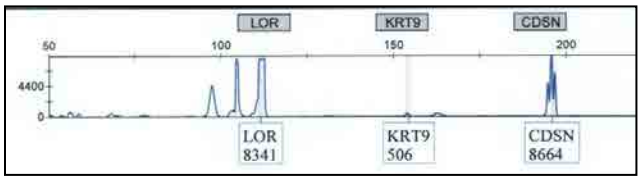
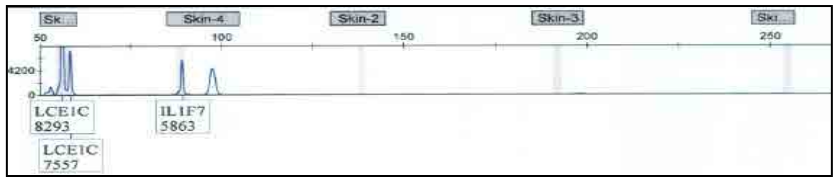
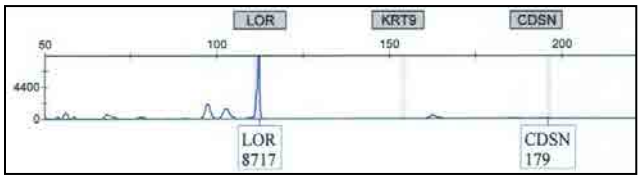
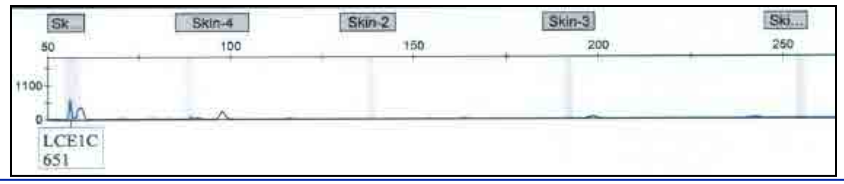
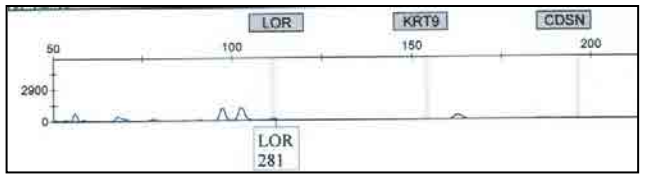
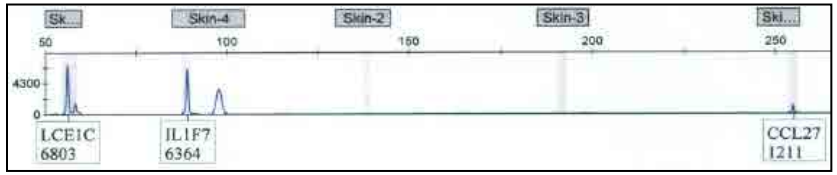
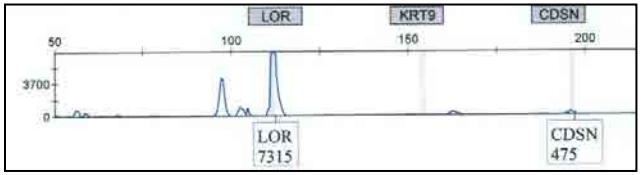
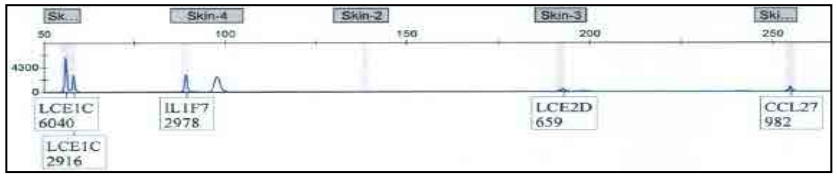
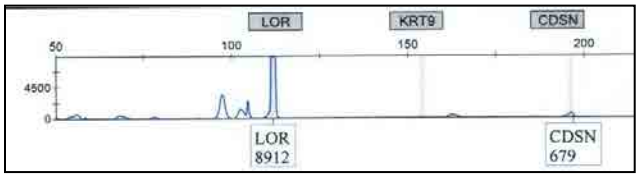
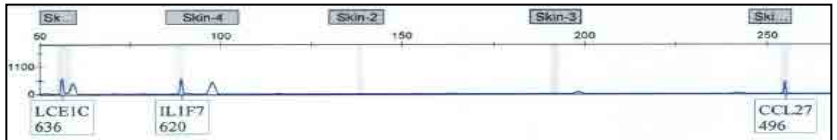


31.25ng

RNA results: Dilution series

Commercial skin RNA on swabs, analysis after 4 months:

original amount on swab	5plex (FAM) - Qiagen Multiplex PCR kit						Visser markers - multiplex		
	LCE1C (RFU)		IL1F7 (RFU)	LCE1D (RFU)	LCE2D (RFU)	CCL27 (RFU)	CDSN (RFU)	KRT9 (RFU)	LOR (RFU)
500ng	8404		8904	694	3655	6072	7150	1671	8930
250ng	8084	7768	8760	738	4232	8894	8237	1468	8789
125ng	8007	7297	8837	337	6362	8978	8341	506	8664
62ng	8293	7557	5863	0	0	0	8717	0	179
36ng	651	0	0	0	0	0	281	0	0
15ng	6803	0	6364	0	0	1211	7315	0	475
7ng	6040	2916	2978	0	659	982	8912	0	679
3ng	636	0	620	0	0	496	0	0	0
Skin positive control	8235	2775	8783	8094	8960	8758	6970	5444	8722

500ng			500ng
250ng			250ng
125ng			125ng
62ng			62ng
36ng			36ng
15ng			15ng
7ng			7ng
3ng		No detection	3ng

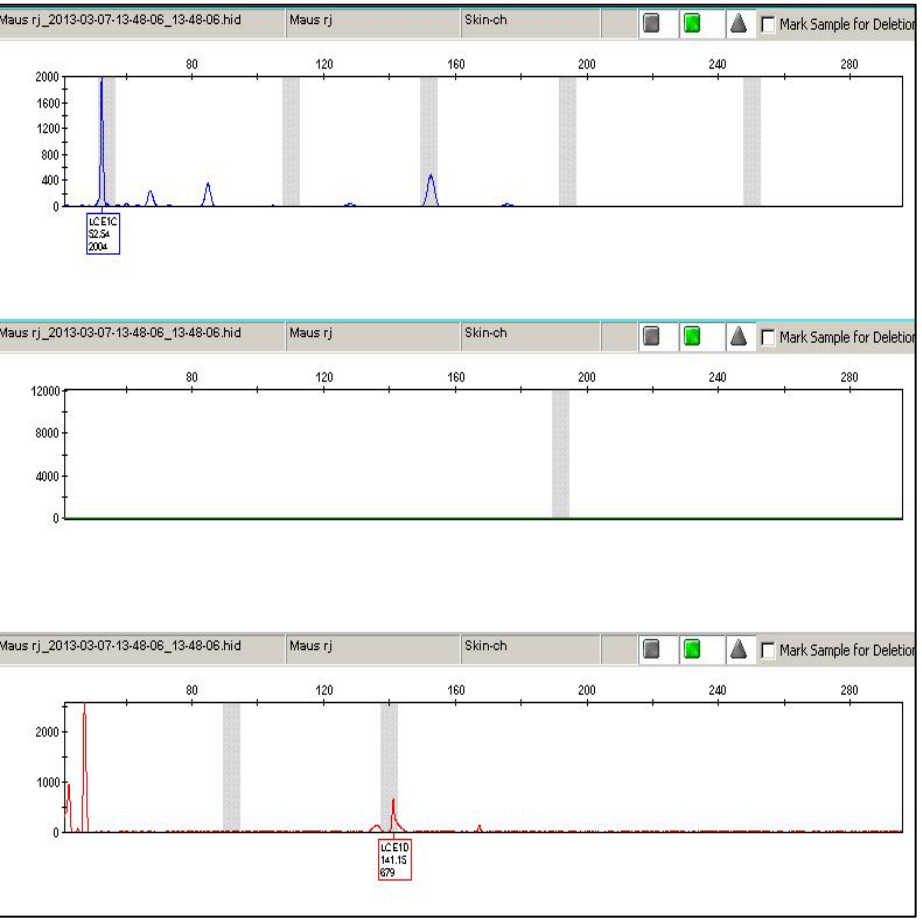
RNA results: stains I

Method	Stain	5plex - Hanson markers					3plex Visser markers		
		LCE1C 56/58 bp	**IL1F7** 92 bp	**LCE1D** 142 bp	**LCE2D** 193 bp	**CCL27** 254 bp	**LOR** 109 bp	**KRT9** 152 bp	**CDSN** 193 bp
Qiagen Allprep RNA/DNA	swab from left palm rj pressed on paper	3726	-	-	-	-	-	-	-
Qiagen Allprep RNA/DNA	swab from right palm rj pressed on paper	1325	-	-	-	-	2503	-	-
Qiagen Allprep RNA/DNA	swab from PC-mouse rj	2004	-	679	-	-	-	-	-
Qiagen Allprep RNA/DNA	swab from PC-mouse ro	4918	-	-	-	-	-	-	-
Qiagen Allprep RNA/DNA	swab from PC-mouse jb	5374	-	-	677	-	4125	-	-
Pinpoint	fingerprint fr on glass	272	-	-	-	-	-	-	-
Pinpoint	fingerprint bn on glass	3865	-	-	-	-	-	-	-
Pinpoint	fingerprint ro on glass	10501	-	1386	-	-	-	-	-
Pinpoint	empty glass	-	-	-	-	-	-	-	-
Pinpoint	shift-button rj 1cm2	28593	979	7631	-	-	3487	-	-
Pinpoint	shift-button jb 1cm2	27009	-	947	-	-	-	-	-
Arcturus	swab from left palm rj	16789	8719	18930	32557	-	31024	13414	7800
Arcturus	swab from right palm rj pressed on paper	25638	-	2768	3337	-	8249	-	-
Arcturus	swab from PC-mouse vc	17149	-	-	-	-	4325	-	-
Arcturus	swab from PC-mouse fr	8273	-	-	-	-	3136	3107	-
Arcturus	swab from PC-mouse mf	16346	-	-	-	-	4412	-	-
Arcturus	swab from fingerprint rj on glass	14811	-	-	2603	-	3178	-	-
	pos.control	27442	4080	15236	22693	-	30876	-	18144
	10 ng DNA (2800M)	1 peak at 56.34 bp, blue					n.d.	n.d.	n.d.

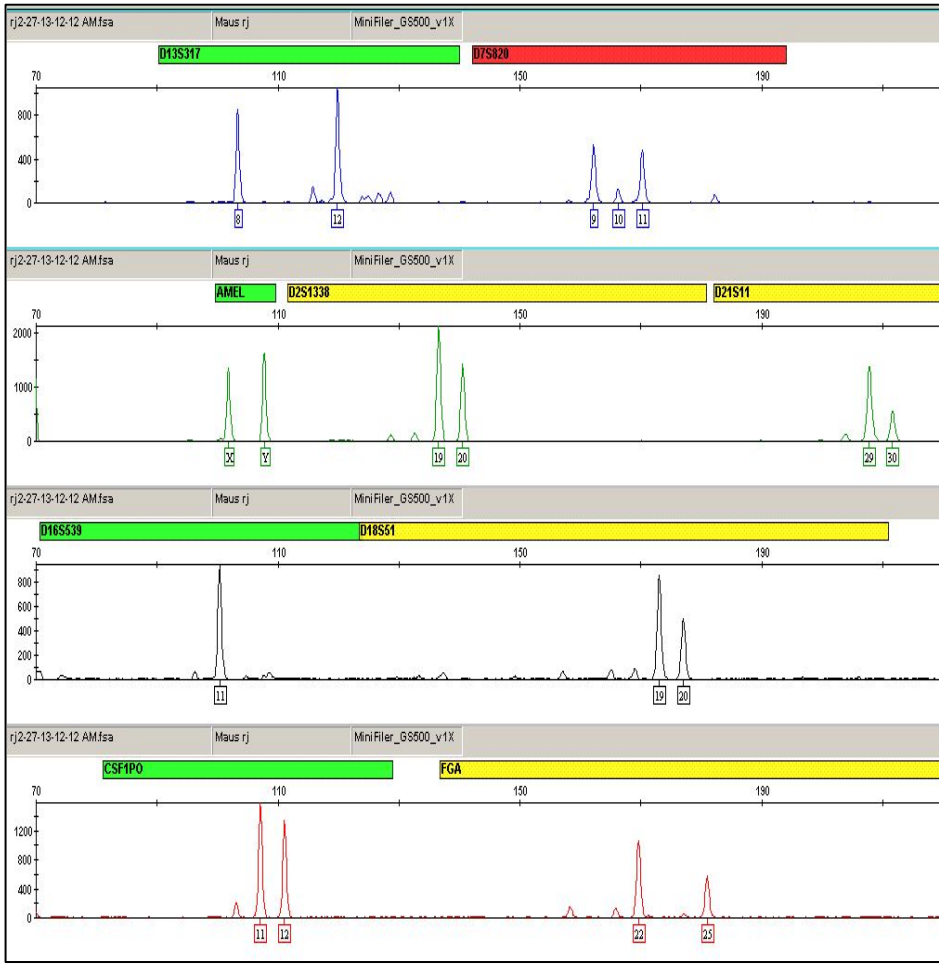
RNA results: stains I

Swab from PC mouse, Qiagen AllPrep RNA/DNA kit

Hanson 5plex



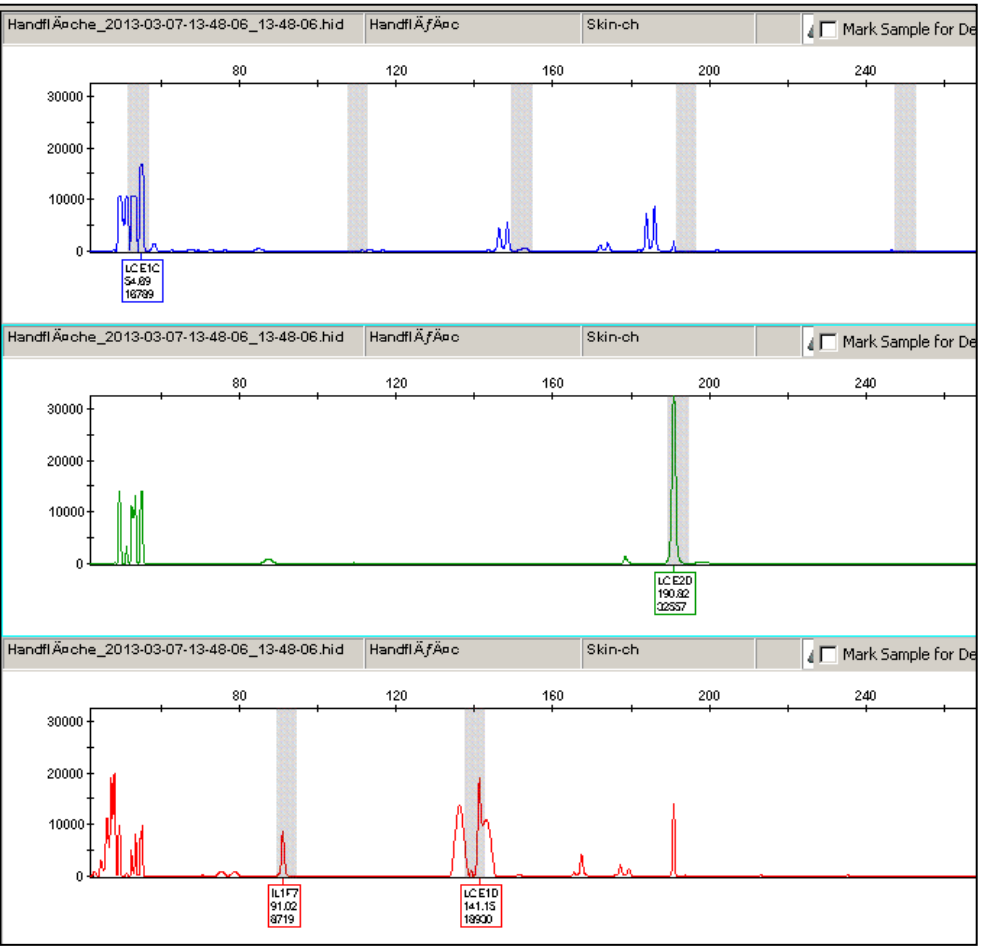
MiniFiler



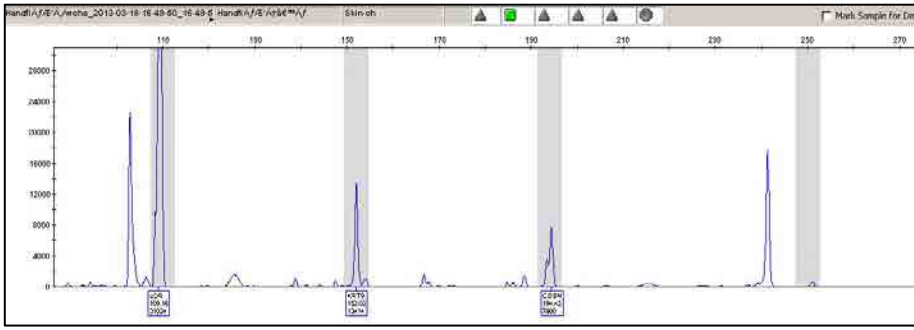
RNA results: stains I

Swab from left palm, Arcturus RNA isolation kit

Hanson 5plex



Visser 3plex

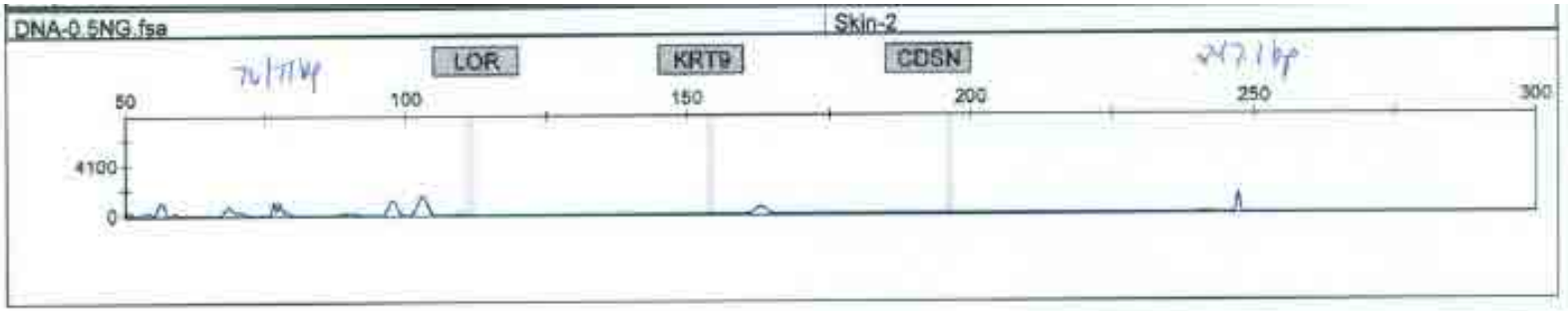


RNA results: stains II

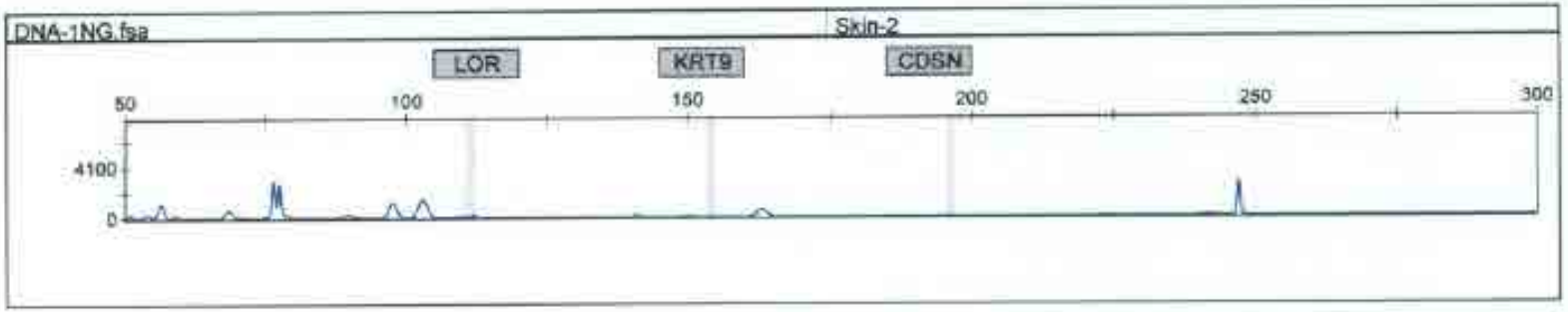
Method	Stain	5plex - Hanson markers					3plex Visser markers		
		LCE1C 56/58 bp	**IL1F7** 92 bp	**LCE1D** 142 bp	**LCE2D** 193 bp	**CCL27** 254 bp	**LOR** 109 bp	**KRT9** 152 bp	**CDSN** 193 bp
Qiagen Allprep RNA/DNA	D key rj	31030	-	2153	-	-	-	-	-
Arcturus	V key rj	20587	-	2456	-	-	-	-	-
Pinpoint	X key rj	17470	-	11251	7312	-	12807	-	-
Qiagen Allprep RNA/DNA	forefinger rj on glass	1343	-	-	-	-	-	-	-
Arcturus	middle finger rj on glass	1182	-	-	-	-	4471	-	-
Pinpoint	ring finger rj on glass	28371	-	-	-	-	4690	-	-
Qiagen Allprep RNA/DNA	swab from left palm rj pressed on paper	21114	-	2774	-	-	-	-	-
Arcturus	swab from right palm rj pressed on paper*	-	-	-	-	-	-	-	-
Qiagen Allprep RNA/DNA	swab from right palm rj	21442	-	22350	5934	-	32482	4410	1637
Arcturus	swab from left palm rj	16728	-	24167	4180	-	32418	3742	245
	pos.control	25120	4518	25772	7801	3234	30410	-	6986
	20 ng DNA (2800M)	n.d.	n.d.	n.d.	n.d.	n.d.	22661	23144	24031

RNA results: Visser 3plex - DNA

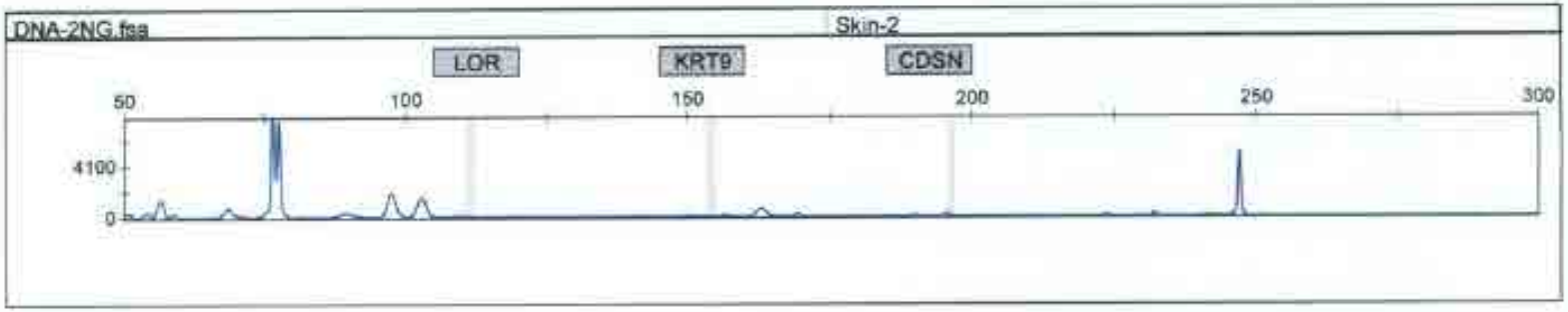
0.5ng



1ng



2ng



Suggestions: EDNAP exercise 6

Samples:
- 6-8 mock casework samples (skin, non-skin, non-human)
- 1 dilution series of skin RNA on swabs
- *optional: additional sensitivity and specificity testing using own casework samples*

Extraction:
- any RNA/DNA kit or manual method
- *optional: RNA quantitation*

Reverse transcription:
- any kit/protocol

PCR:
- RNA: skin 5plex and 3plex, additionally Housekeeping gene 3plex? provided (standardized protocol)
- *DNA (optional): any commercial STR kit*
- *optional: post PCR purification*

Thank you for your attention!

Cordula Haas / Erin Hanson / Jack Ballantyne

April 23, 2013, Bratislava

EDNAP Exercise on IrisPlex

DNA-based prediction of human eye colour

Manfred Kayser
With Lakshmi Chaitanya and Susan Walsh

and all EDNAP study collaborators

21 Participants

First name	Last name	Address 1	Address 2	Address 3	Address 4
Kaye	Ballantyne	Office of the Chief Forensic Scientist			Victoria Police Forensic Service Department
Walther	Parson	Institute of Legal Medicine			Innsbruck Medical University
Vlastimil	Stenzl	Institute of Criminalistics Prague			
Helle	Mogensen	Section of Forensic Genetics	Department of Forensic Medicine	Faculty of Health Sciences	University of Copenhagen
Antti	Sajantila	Department of Forensic Medicine	Hjelt Institute		University of Helsinki
Regine	Banemann	KT31 - Humanspuren			Bundeskriminalamt
Per	Hoff-Olsen	Department of Forensic Biology			National Institute of Public Health
Martina	Turanska	Institute of Forensic Science			Slovenská L'upca
Ricky	Ansell	Biology Unit	National Laboratory of Forensic Science		
Gunilla	Holmlund	Department of Forensic Genetics and Forensic Toxicology	National Board of Forensic Medicine		
Cordula	Haas	Institut für Rechtsmedizin Zurich			
Tita	Sijen	Department WISK	Netherlands Forensic Institute		
David	Ballard	Academic Haematology	Blizard Institute of Cell and Molecular Sciences		Barts and The London
Peter	Vallone	Biotechnology Division	National Institute of Standards and Technology		
Adrian	Linacre	South Australia Justice Chair in Forensic Science		School of Biological Science	Flinders University
Christine	Keyser-Tracqui	Intitut de Médicine Legale			Universite de Strasbourg
Peter	Schneider	Institute of Legal Medicine			University of Cologne
Francesca	Brisighelli	Instituto di Medicine legale e delle Assicurazconi			Universita Cattolica
Maria João Anjos	Porto	Forensic Genetic Service	Instituto de Medicina Legal		University of Coimbra
Chris	Phillips	Forensic Genetic Unit	Department of Legal Medicine		University of Santiago de Compostela
Wojciech	Branicki		Institute of Forensic Research		

Task 1-known phenotype info

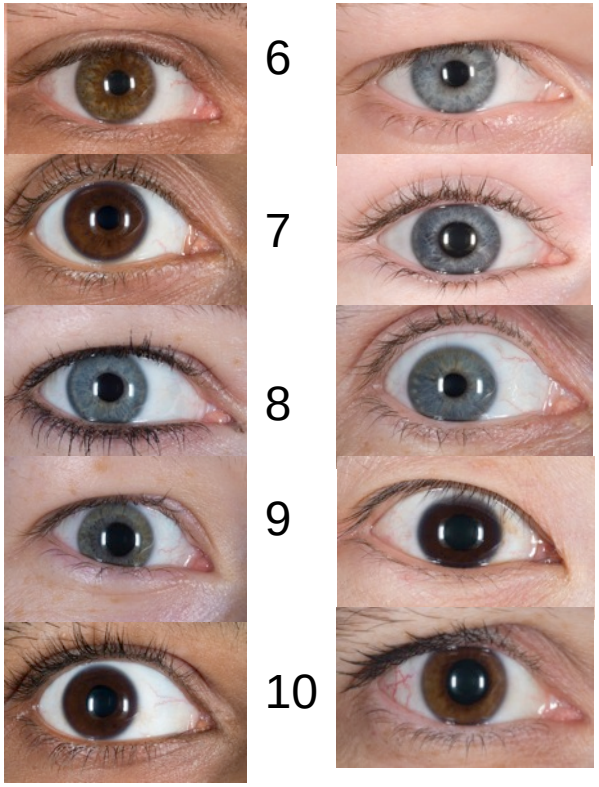
- Each group will receive samples from 10 individuals: 5 blood samples on FTA card and 5 buccal swabs on FTA card.
- A digital eye image for each of the individuals will be included.

What was done for task 1:

- Groups were asked to extract DNA from the samples and make to a concentration recorded by the lab
- They were asked to produce a IrisPlex genotype profile for each individual sample
- They were asked to state what the most likely eye color prediction outcome and its likely accuracy is based on IrisPlex.

RESULTS TASK 1

10 individuals



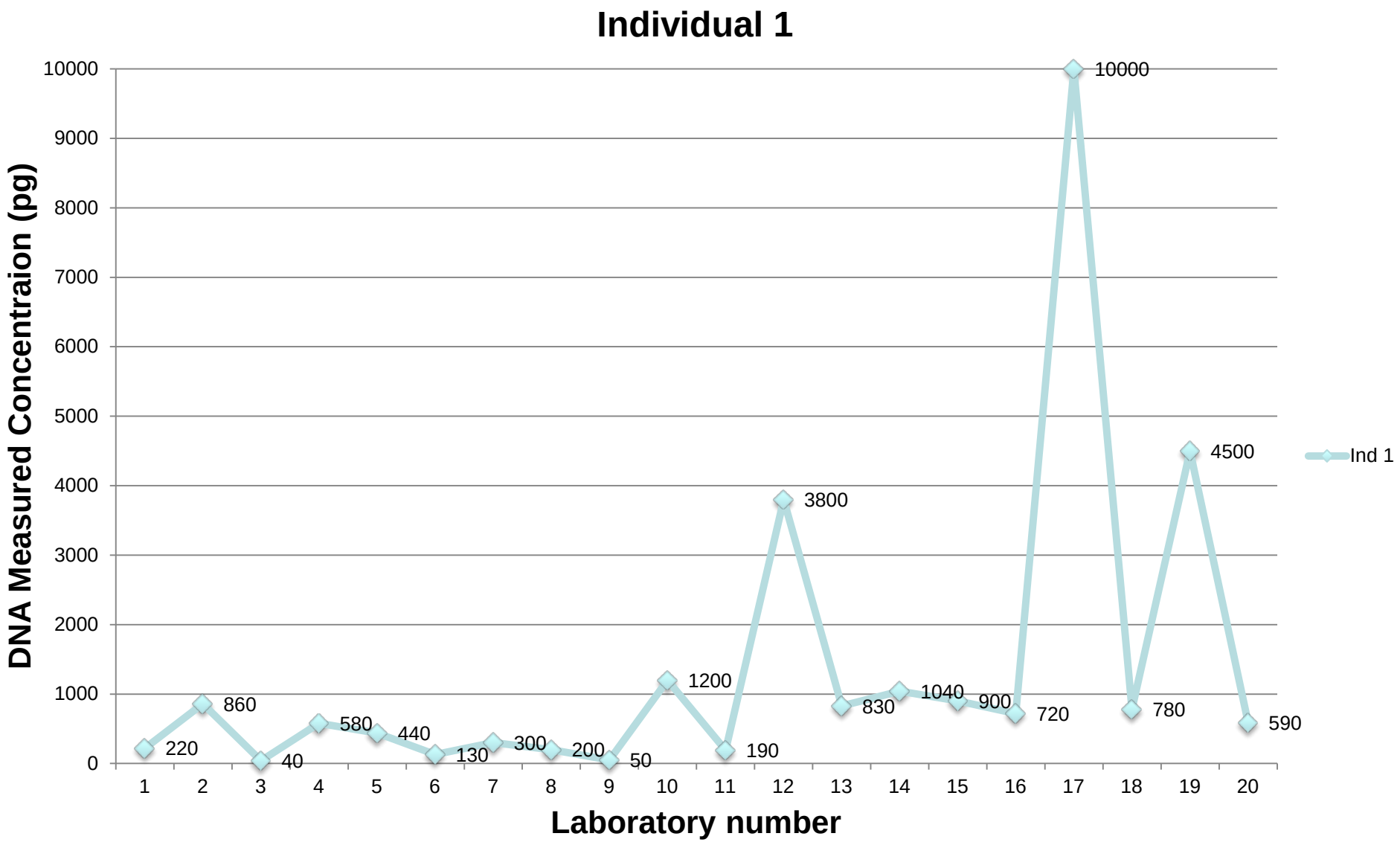
Some labs experienced
some incorrect calls, due to drop in/out,
and produced differing final
% prediction probabilities and accuracies

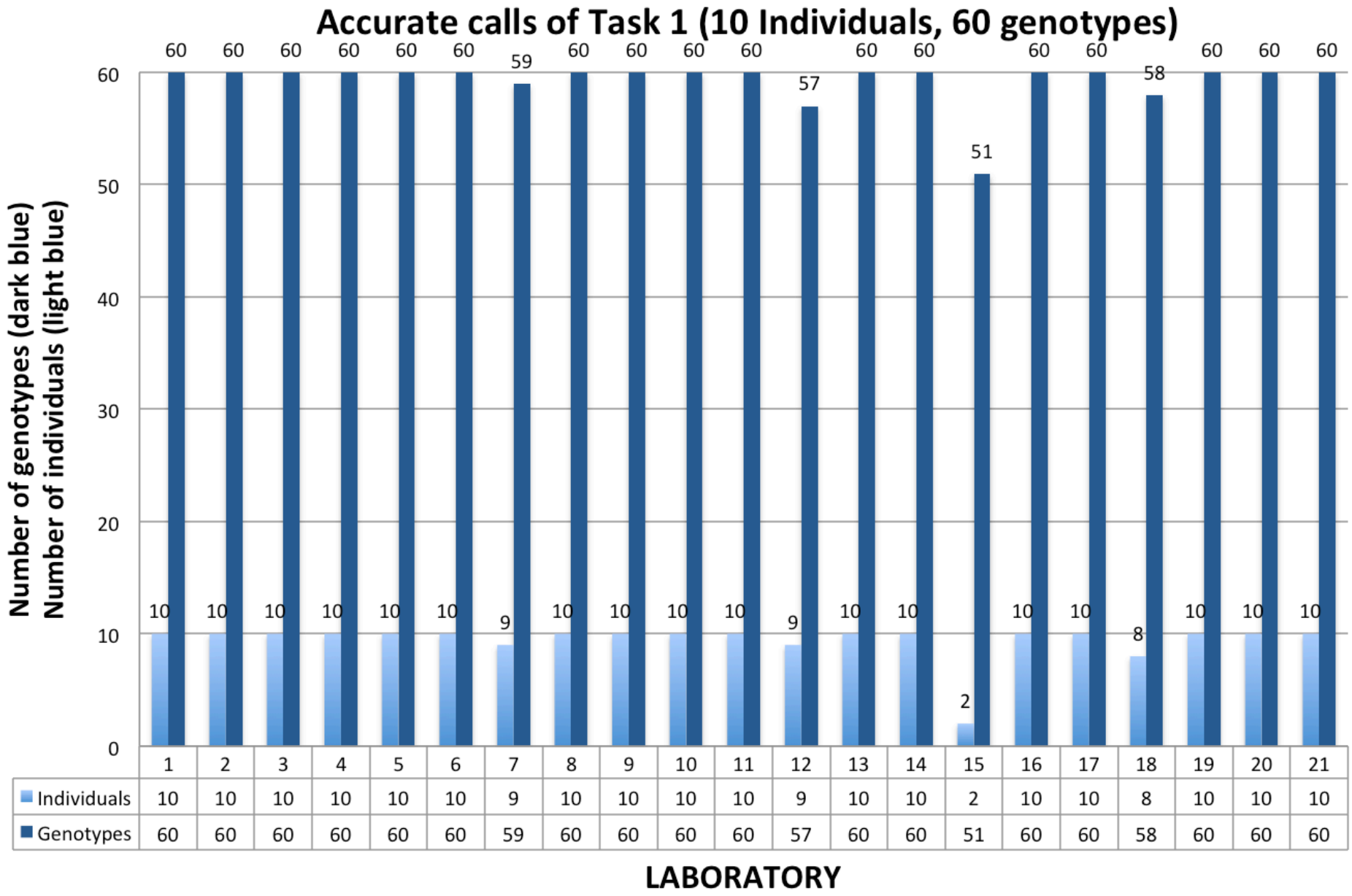
Possible explanations for drop in/out are varying DNA concentration, each lab ran the 10 individuals at varying extracted concentrations

17 out of 21 Labs did not have <u>any</u> problems with Task 1

20 out of 21 Labs predicted the correct eye color

Example of different extracted DNA concentrations between labs

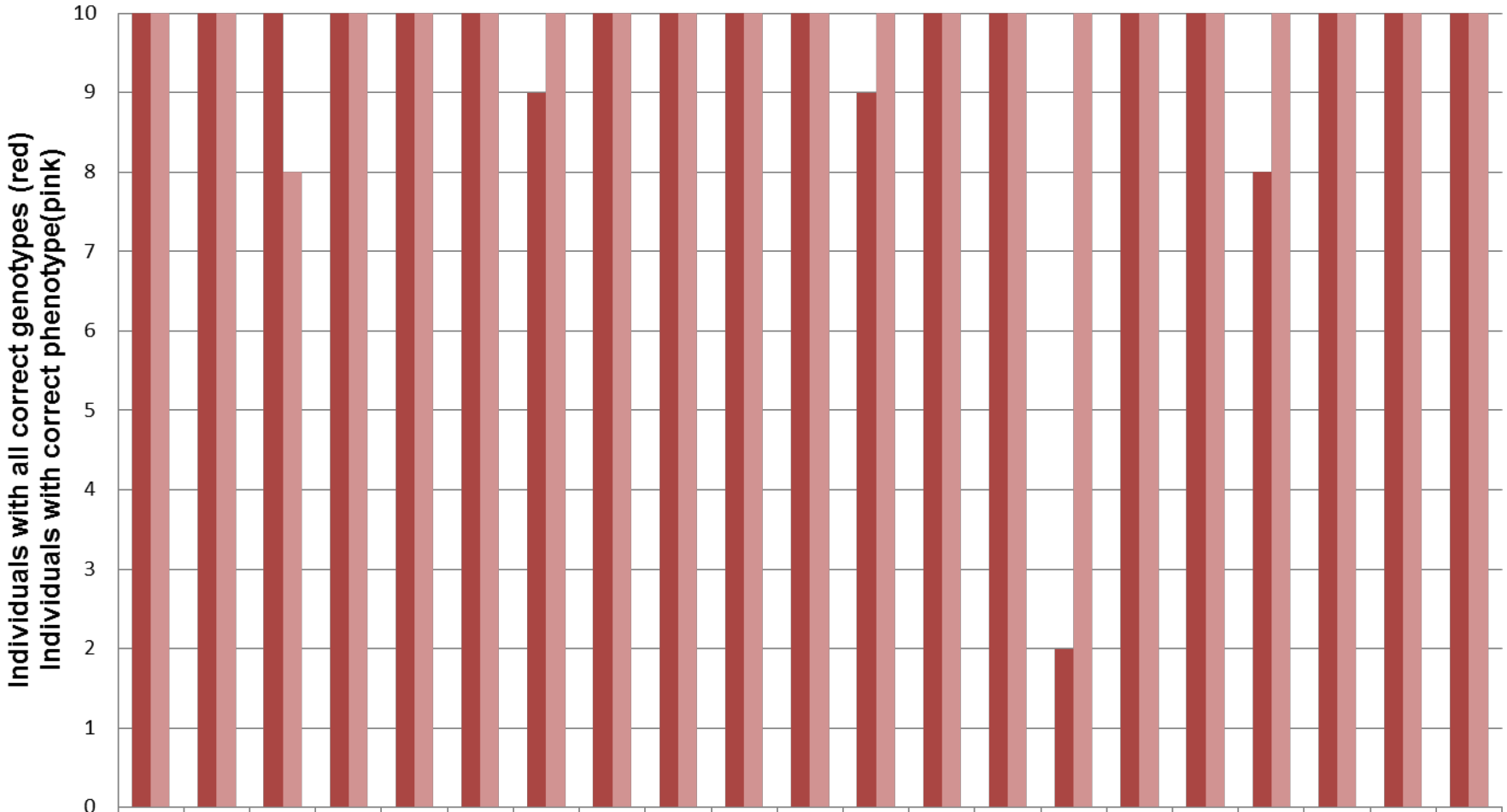




	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Individuals	10	10	10	10	10	10	9	10	10	10	10	9	10	10	2	10	10	8	10	10	10
Genotypes	60	60	60	60	60	60	59	60	60	60	60	57	60	60	51	60	60	58	60	60	60

1245 out of 1260 genotype calls correct = 98.8% accuracy

Phenotype Prediction Accuracy for Task 1



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Individuals	10	10	10	10	10	10	9	10	10	10	10	9	10	10	2	10	10	8	10	10	10
Phenotypes	10	10	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

20 out of 21 Labs predicted the eye color phenotypes correctly

Task 2 – Blind testing Casework

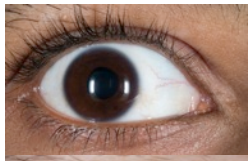
- Each group will receive 5 additional samples of already extracted DNA that have been subject to simulated casework conditions.
- No phenotype information on these samples will be available for the groups.

What was done for task 2:

- Groups are asked to produce a IrisPlex genotype profile for each individual sample
- They are asked to state what the most likely eye color prediction outcome and its likely accuracy is based on IrisPlex.

RESULTS TASK 2

5 individuals

1

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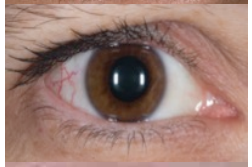
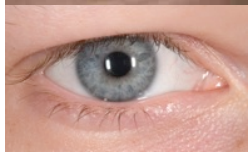

3

4

5


- 5 Simulated Casework samples
- Should have been run as is by laboratory using 1 ul sample for IrisPlex assay

Sample 1: Buccal swab
measured at 500 pg
UV treated 1 min

Sample 2: Buccal swab
measured at 100 pg
UV treated 1 min

Sample 3: Saliva on slide
measured at 250 pg

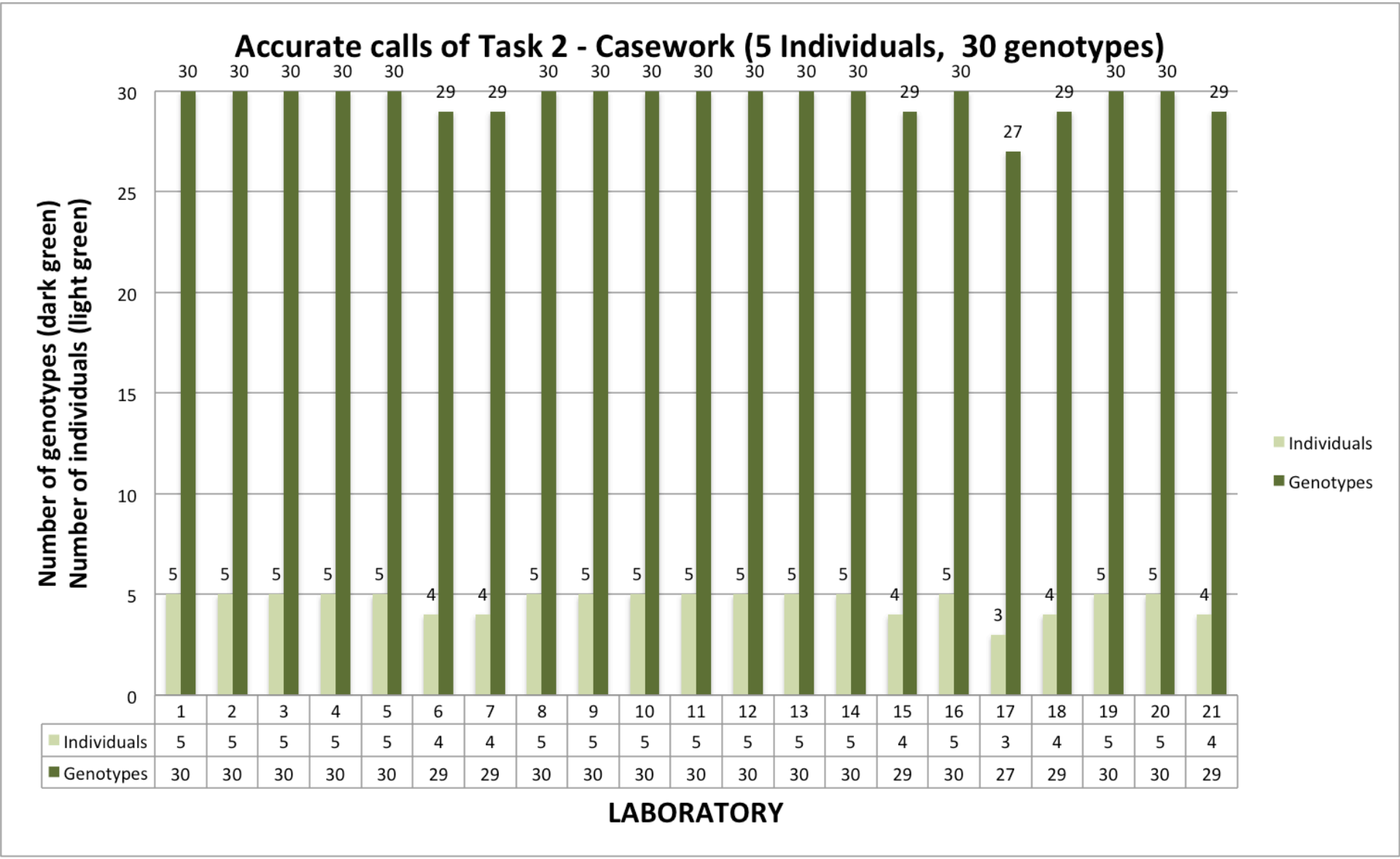
Sample 4: Blood on slide
measured at 2000 pg

Sample 5: Semen
measured at 50,000 pg

Out of 105 predictions
(5 individuals, 21 labs)
An individual was predicted wrong
in only 2 cases, due to drop out

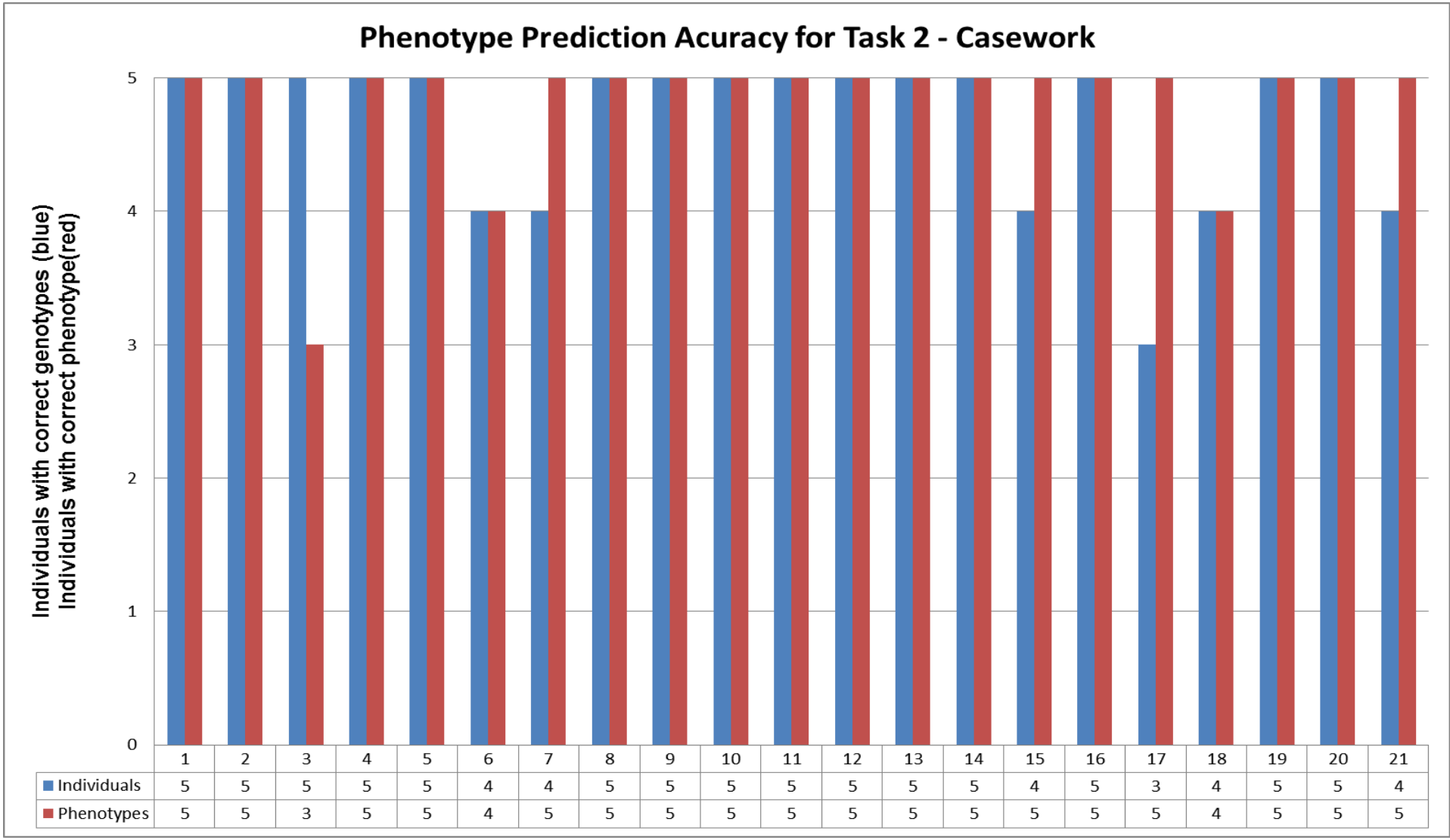
15 out of 21 labs did not have <u>any</u> problems with Task 2

20 out of 21 labs predicted the eye color phenotypes correctly



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Individuals	5	5	5	5	5	4	4	5	5	5	5	5	5	5	4	5	3	4	5	5	4
Genotypes	30	30	30	30	30	29	29	30	30	30	30	30	30	30	29	30	27	29	30	30	29

622 out of 630 genotype calls correct = 98.7% accuracy



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Individuals	5	5	5	5	5	4	4	5	5	5	5	5	5	5	4	5	3	4	5	5	4
Phenotypes	5	5	3	5	5	4	5	5	5	5	5	5	5	5	5	5	5	4	5	5	5

20 out of 21 labs predicted the eye color phenotypes correctly

Lab ID	Polymer	Genetic Analyzer	Samples with correct genotype assignments of all 6 SNPs	Samples with correct phenotype	Performed as directed	Comments
1	?	?	15	15	yes	
2	POP 6	3100	15	15	yes	
3	POP 4	3130	15	11	yes	Inconclusive phenotype was stated for 4 individuals - although the genotypes were correct
4	POP 4	3130xl	15	15	yes	run time increased to 1000s from 500s
5	?	?	15	15	yes	
6	POP 4	3500xl	14	14	yes	
7	POP 7	3130xl	13	15	yes	
8	POP 4	3130	15	15	yes	
9	POP 7	3500	15	15	yes	
10	POP 7	3500	15	15	yes	
11	POP 4	3130xl	15	15	yes	
12	POP 7	3130xl	14	15	yes	
13	POP 7	3130xl	15	15	yes	
14	POP 4	3130xl	15	15	yes	
15	?	?	6 (rs12203592 always incorrect)	15	yes	Non-concordant result for one marker throughout all the samples. Need to verify the primers they used (was it from the original Irisplex paper or the Dev Validation Paper)- perhaps strand problem? Primers were sent by other participant (not by us)
16	POP 7	3500		15	yes	
17	POP 4	3130	13	15	No	Quantified the samples and diluted them. Repeated genotyping due to wrong quantification results for some samples and used their own primer set for this in task 2.
18	POP 4	3130	12	14	Yes	
19	POP 7	3130	15	15	Yes	
20	POP-4	3130	15	15	No	Used different CE settings Used their own set of extension primers for one of the samples in Task 1 and cross checked the results of Task 2 with their own set of primers. They reported disconcordance for one sample between the two primer sets and reported the result based on their own extension primer set...
21	POP 7	3130xl	14	15	yes	

Task 3 (voluntary)

- Each group was asked to collect 5 additional DNA samples from 5 individuals in their own group of any eye color.
- It is important to note that IrisPlex is most suitable for predicting blue and brown but has difficulty in the prediction of non-blue and non-brown eye colors; however,…
- we do not ask groups to restrict their choice of individuals that they take, we just ask that groups be aware of this.

What to do for task 3

- Take a digital high-resolution photo of both eyes.
- the iris photo should be taken in natural light conditions (no fluorescent bulb light) with and without flash lens using a digital camera focusing on eyes only (no full portrait).

What to do for task 3:

- Groups are asked to produce a IrisPlex genotype profile for each individual sample
- They are asked to state what the most likely eye color prediction outcome and its likely accuracy is based on IrisPlex.

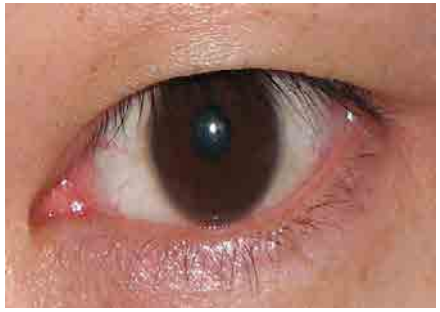
Task 3 Results

Lab ID	Number of individuals	Individuals with correct phenotype*	Comments
1	5	All	
2	6	All but 1	Colour appears brown, reported high probability for blue with IrisPlex primers but high probability for brown with USC primers (??)
3	5	All but 1	Reported inconclusive for 1 sample, for which eye colour clearly appeared brown to us
4	5	All	
5	5	All	
6	5	All	
7	-	-	
8	5	All	
9	5	All	
10	9	All	
11	5	All	
12	8	-	No images included
13	5	All	
14	5	All	
15	5	All	
16	4	All	
17	6	All	
18	5	All	
29	5	All	
20	5	All	
21	5	All	

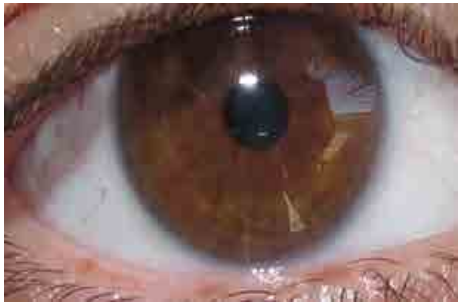
* As judged by us from image inspection

17 out of 19 labs predicted the eye color phenotypes correctly

Some examples from labs who performed Task 3



Brown with 99% accuracy



Brown with 93.9% accuracy

Intermediate with 87.5% accuracy

Blue with 94.9% accuracy

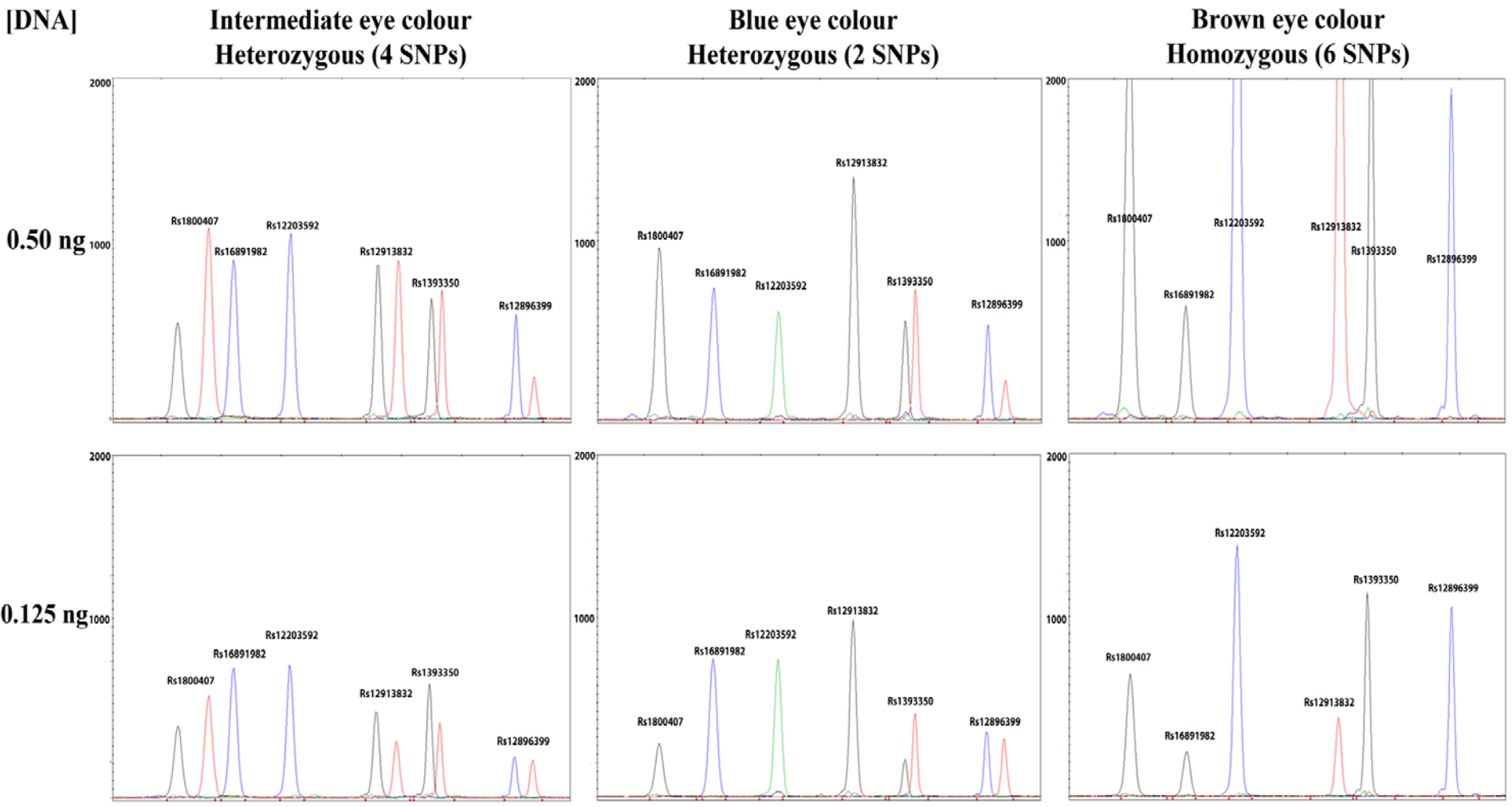
Blue with 97.4% accuracy

Questions

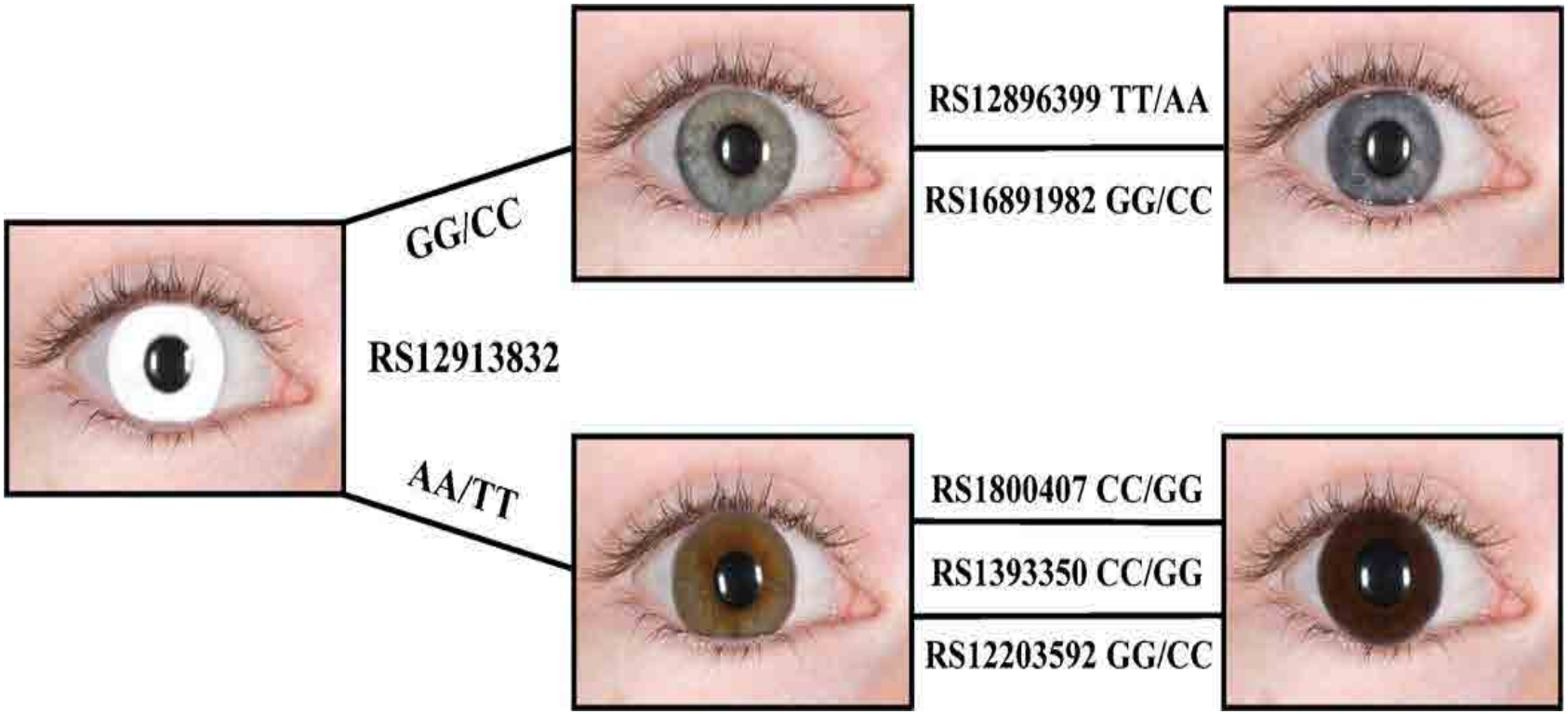
- How to deal with / report in paper results that were obtained with different primer sets?

- How to deal with / report in paper results obtained with alterations to the protocol (e.g. DNA dilutions etc)

6 SNP IrisPlex genotypes



Impact of the most influential SNP genotypes from the 6-SNP model



The most influential SNP in determining whether the eye color will be brown versus non-brown is **rs12913832** (HERC2) with its AA/TT versus GG/CC homozygote genotypes.

EDNAP Meeting, Bratislava, Slovenská republika, April 23 2013

EMPOP Update

Walther Parson
Institute of Legal Medicine
Innsbruck Medical University
Austria

EMPOP update

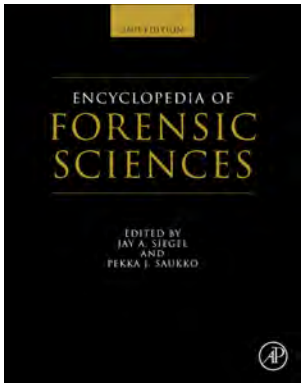
1. New publications
   - Encyclopedia of Forensic Genetics
     - Mitochondrial DNA
     - Internet Accessible Population Databases: YHRD and EMPOP
     - Future Analytical Techniques: DNA Mass Spectrometry
   - Molecular genetic investigations on Austria's patron saint Leopold III
2. Meetings
3. New mtDNA developments
4. EMPOP database

1. New publications - 1

Mitochondrial DNA

W Parson, Innsbruck Medical University, Innsbruck, Austria

This article is a revision of the previous edition article by T. Melton and G. Sensabaugh, volume 2, pp. 499–503, © 2000, Elsevier Ltd.



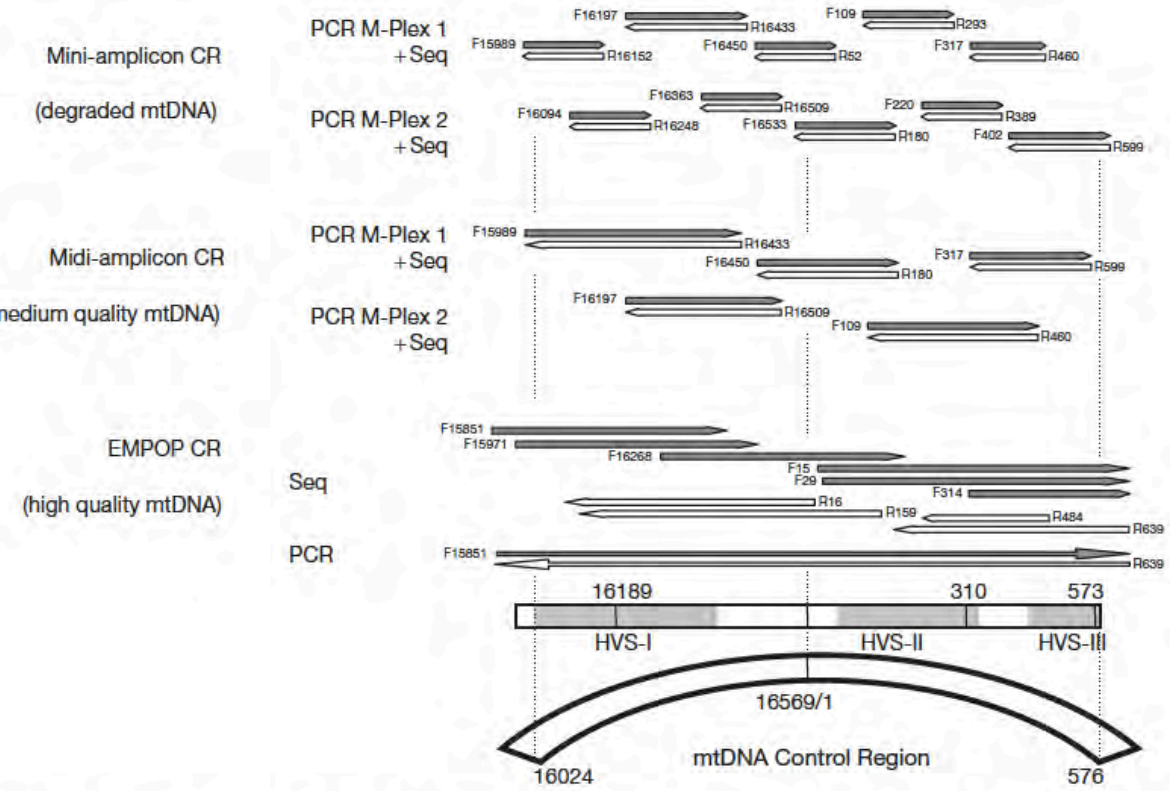


Figure 1 Scheme of the mtDNA control region with three different amplification and sequencing strategies that are applied depending to the degradation state of the available mtDNA. All three strategies lead to consenus sequences with fully double-stranded coverage. Primer designations refer to the three prime ends, primer sequences can be found in the references below. Reproduced from Eichmann C and Parson W (2007) Molecular characterization of the canine mitochondrial DNA control region for forensic applications. *International Journal of Legal Medicine* 121: 411–416; Parson W and Bandelt HJ (2007) Extended guidelines for mtDNA typing of population data in forensic science. *Forensic Science International: Genetics* 1: 13–19. Berger C and Parson W (2009) Mini-midi-mito: Adapting the amplification and sequencing strategy of mtDNA to the degradation state of crime scene samples. *Forensic Science International: Genetics* 3: 149–153.

EMPOP

1. New publications - 1

Mitochondrial DNA

W Parson, Innsbruck Medical University, Innsbruck, Austria

This article is a revision of the previous edition article by T. Melton and G. Sensabaugh, volume 2, pp. 499–503, © 2000, Elsevier Ltd.

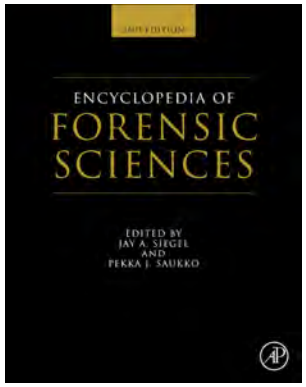
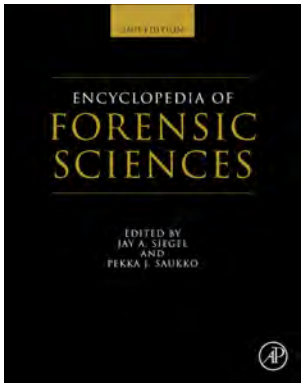


Figure 3 Spatial frequency distribution of mtDNA haplogroup U5b3 and geographical locations of populations surveyed. Reproduced from Pala et al. (2010) *American Journal of Human Genetics* 84: 1–8, with permission.

1. New publications - 2

Internet Accessible Population Databases: YHRD and EMPOP

L Roewer, Charité – Universitätsmedizin Berlin, Berlin, Germany
W Parson, Innsbruck Medical University, Innsbruck, Germany



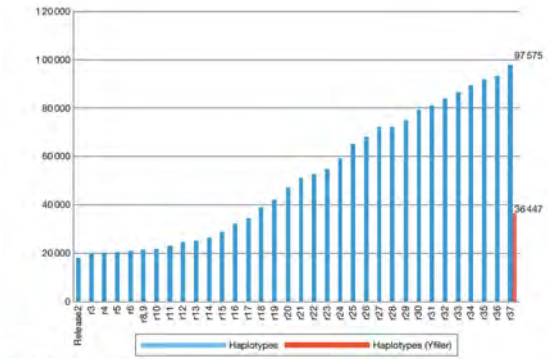


Figure 1 YHRD growth 2000–11.

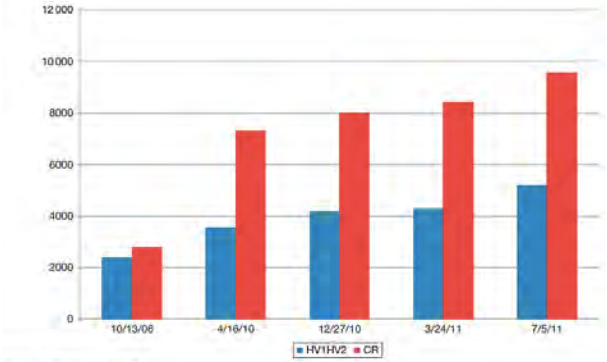
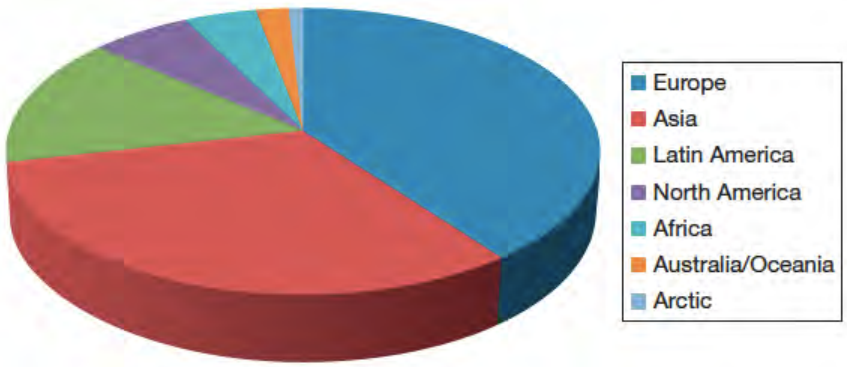
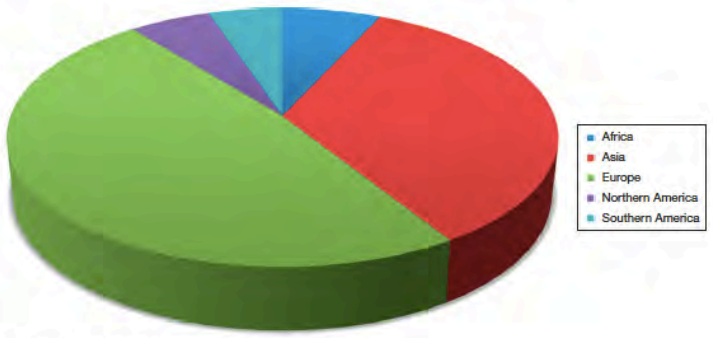


Figure 2 EMPOP growth 2006–11.

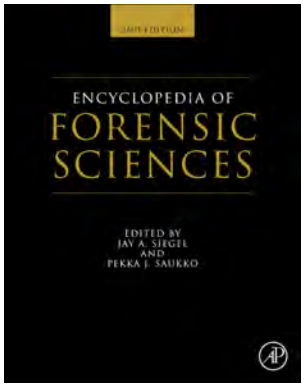




1. New publications - 2

Internet Accessible Population Databases: YHRD and EMPOP

L Roewer, Charité – Universitätsmedizin Berlin, Berlin, Germany
W Parson, Innsbruck Medical University, Innsbruck, Austria



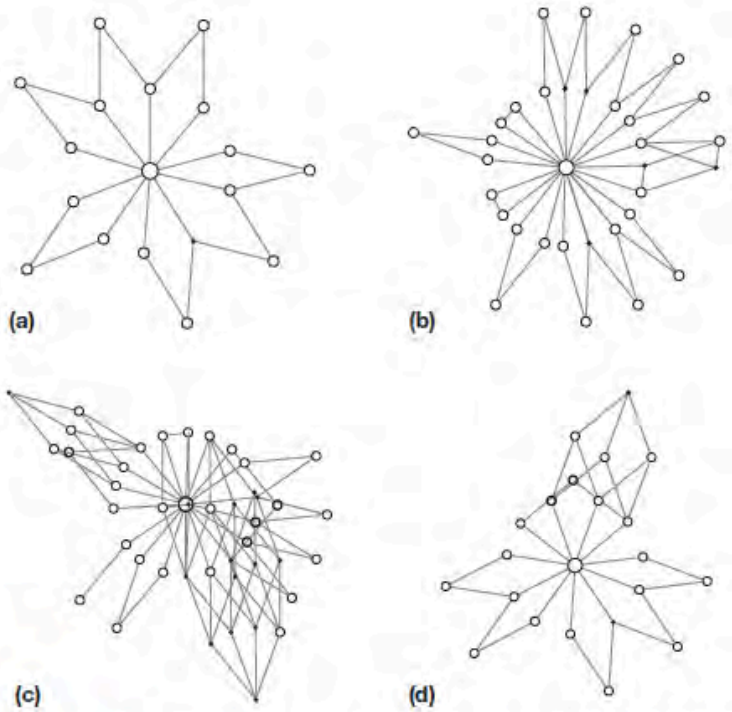
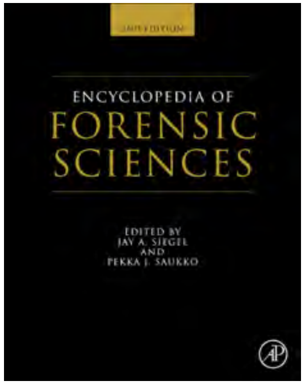


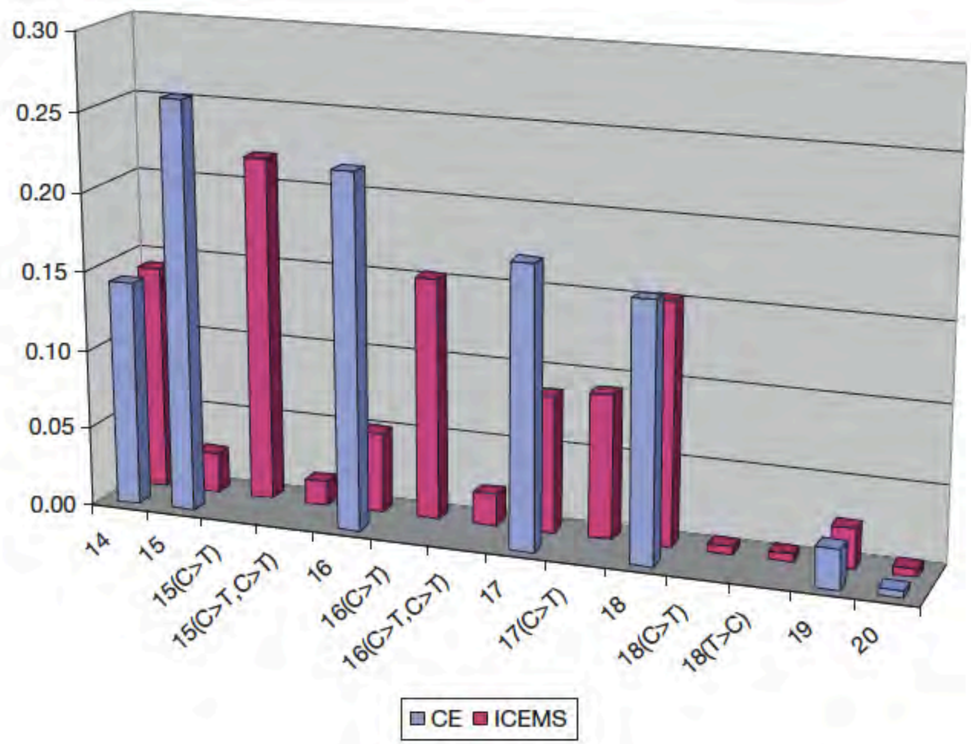
Figure 3 Depiction of four quasi-median network torsi, HVS-I (16024–16400, filtered with hotspot_EMPOPspeedyWE). (a) Etalon torso, 202 west Eurasian haplotypes of forensic quality; (b) the addition of 31 west Eurasian high-quality sequences to the etalon has only minor effects on the display of the torso; (c) the addition of 29 sequences from a Darginian data set to the etalon increases the complexity of the torso by the introduction of 13 new quasi-medians (black dots), which indicate the presence of unobserved variation. Affected polymorphisms are transitions at 16280, 16281, and 16384, and a transversion at 16391 that all turned out as phantom mutations. (d) Removal of the phantom mutations from the data in (c) results in an inconspicuous torso.

1. New publications - 3

Future Analytical Techniques: DNA Mass Spectrometry

W Parson, Innsbruck Medical University, Innsbruck, Austria
S Hofstadler, Ibis Biosciences Inc., Carlsbad, CA, USA





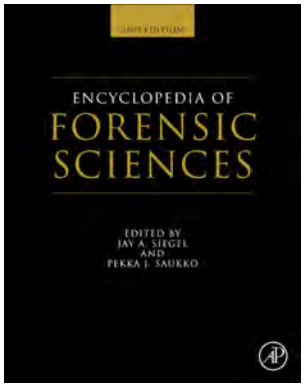
15	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGACAGATAGA	TACATG
15	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGACAGATAGA	TACATG
15(C>T)	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGATAGA	TACATG
15(C>T)	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGATAGA	TACATG
15(C>T)	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGATAGA	TACATG
15(C>T)	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGATAGA	TACATG
15(C>T)	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGATAGA	TACATG
15(C>T,C>T)	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGA TAGA	TACATG
15(C>T,C>T)	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGA TAGA	TACATG
15(C>T,C>T)	GACAGAGCAAGACCCTGTCTCA	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGA TAGA	TACATG

Figure 2 Comparison of discernible D3S1358 allele categories with CE and ICEMS based on 98 unrelated European samples. The conventional approach (CE) allows for the discrimination of seven allele classes (14 through 20), whereas the MS-based method (ICEMS) captures additional variant alleles (a total of 14 categories). Detected sequence variants for allele 15 are indicated below the graph.

1. New publications - 3

Future Analytical Techniques: DNA Mass Spectrometry

W Parson, Innsbruck Medical University, Innsbruck, Austria
S Hofstadler, Ibis Biosciences Inc., Carlsbad, CA, USA



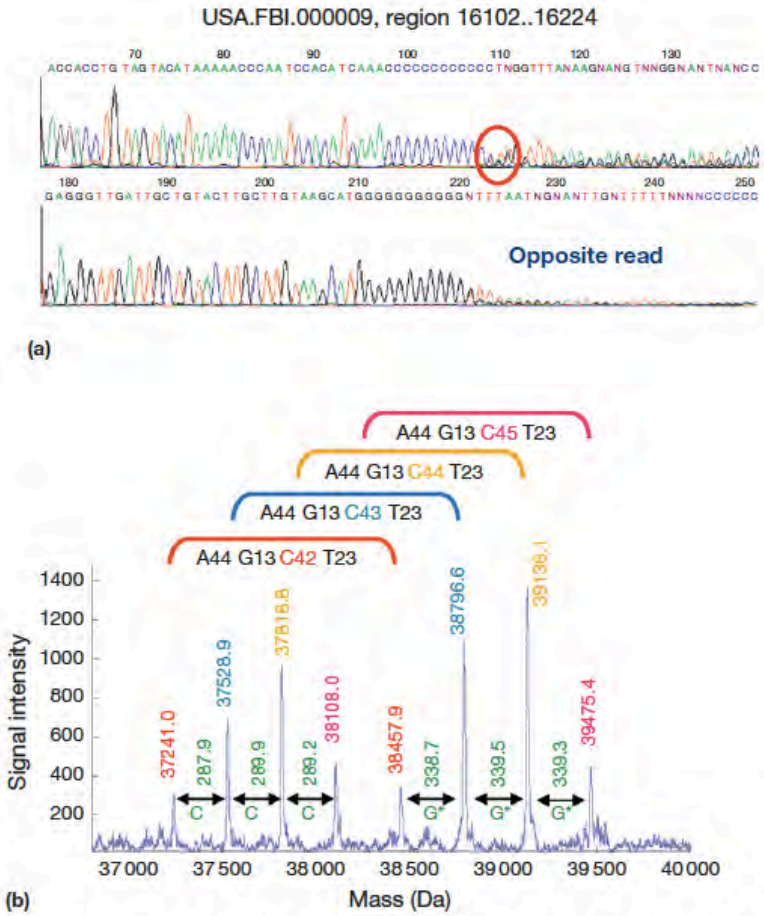


Figure 6 Effect of length heteroplasmy upon mtDNA sequencing and the mitochondrial tiling assay. Sequence analysis is severely challenged by multiple templates that differ in length. (a) Forward strand sequence electropherogram of a sequencing reaction developed by direct PCR-product sequencing from in-house saliva sample CS0033 on top. The sequence electropherogram becomes unreadable after the poly-C region because of a mixture of templates of varying length. (b) Deconvolved mass spectrum for primer pair, which amplifies coordinates 16124 . . . 16201 encompassing the HV1 poly-C stretch. There are four clearly resolved products that differ by single C residues in the poly-C tract. * ^{13}C-enriched dGTP was used in these reactions, adding ~10 Da to the mass of each G residue.

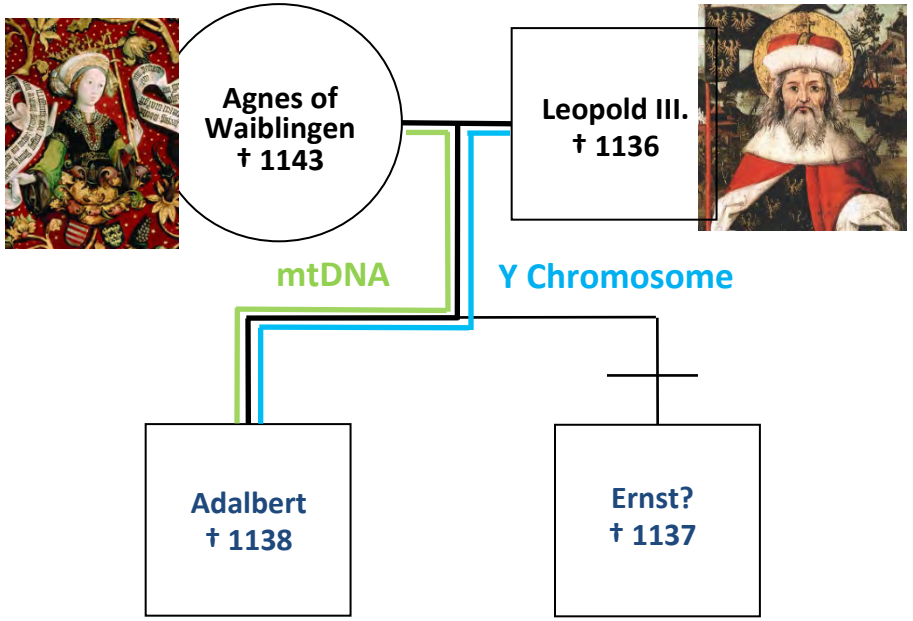
1. New publications - 4

Case report

Molecular genetic investigations on Austria's patron saint Leopold III

Christiane Maria Bauer [a], Martin Bodner [a], Harald Niederstätter [a], Daniela Niederwieser [a], Gabriela Huber [a], Petra Hatzer-Grubwieser [a], Karl Holubar [b], Walther Parson [a,c,*]

[a] Institute of Legal Medicine, Innsbruck Medical University, 6020 Innsbruck, Austria
[b] Monastery Archive, Monastery of Klosterneuburg, 3400 Klosterneuburg, Austria
[c] Penn State Eberly College of Science, University Park, PA, USA



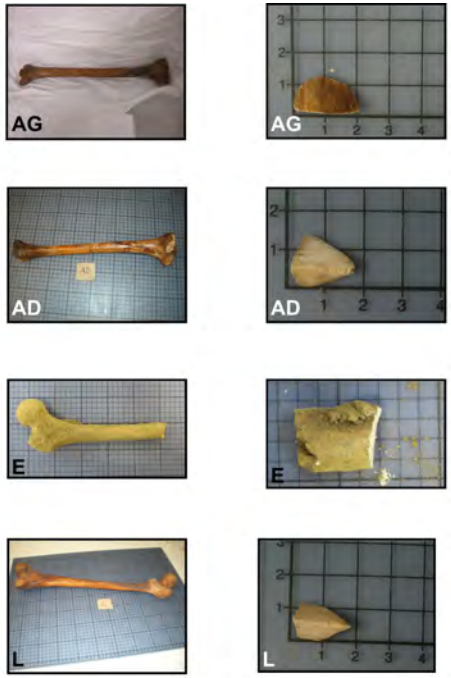


Figure S1: Femoral bone samples used for DNA analysis.
AG = Agnes, AD = Adalbert, E = Ernst, L = Leopold

2. Past meetings

Annual CODIS Conference, Oklahoma, USA (Nov 13-16, 2012)

presentation on EMPOP (QC, searching, alignment)

100th Science Meeting, Kolkata, India (Jan 03-07, 2013)

very interesting

HUGO Meeting, Singapore (Apr 12-18, 2013)

NGS mitochondrial DNA

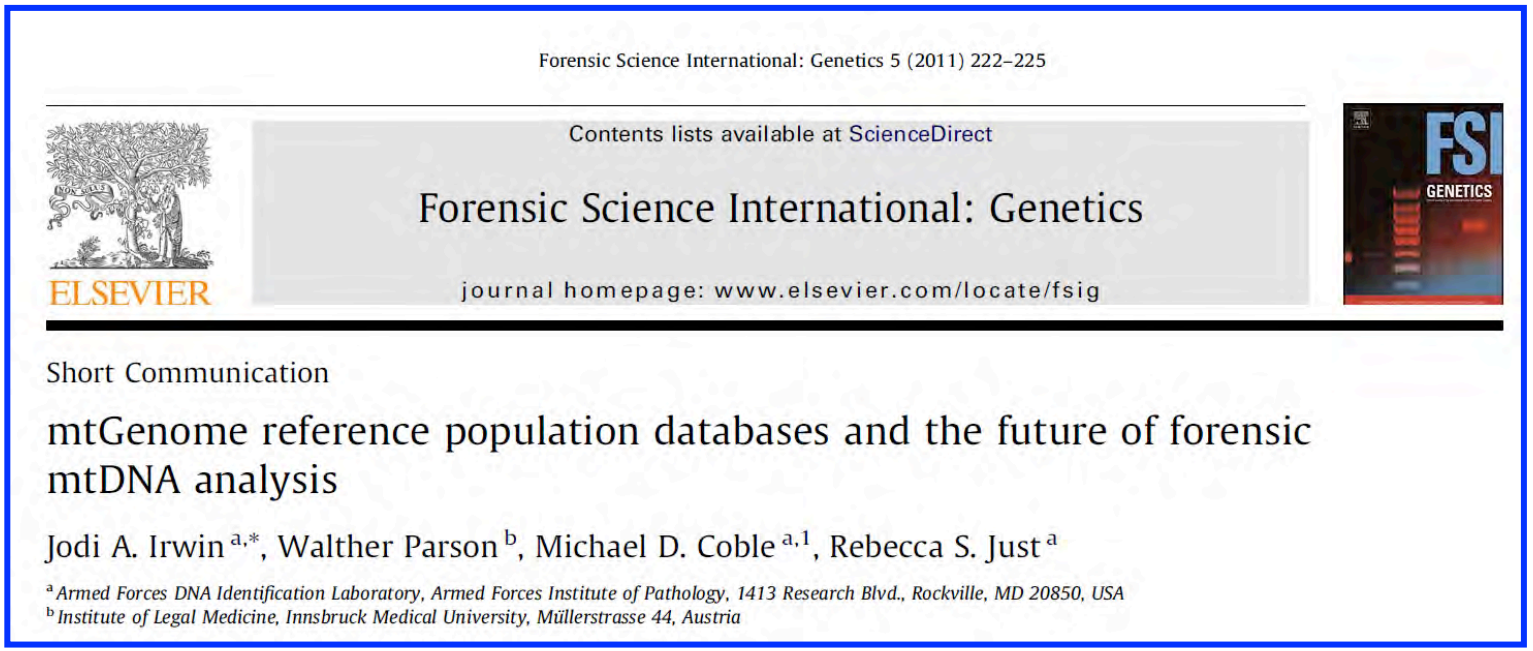
2. Upcoming meetings

Haploid marker workshop Melbourne (ISFG, Sep 07 2013)

2. Upcoming meetings

4. New developments

Evaluating full mtGenome NGS for mtDNA typing (Ion Torrent PGM, LT)



Forensic Science International: Genetics 5 (2011) 222–225

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Short Communication

mtGenome reference population databases and the future of forensic mtDNA analysis

Jodi A. Irwin [a,*], Walther Parson [b], Michael D. Coble [a,1], Rebecca S. Just [a]

[a] Armed Forces DNA Identification Laboratory, Armed Forces Institute of Pathology, 1413 Research Blvd., Rockville, MD 20850, USA
[b] Institute of Legal Medicine, Innsbruck Medical University, Müllerstrasse 44, Austria

discrimination power, phylogeny, quality control

NIJ National Institute of Justice 2011-MU-MU-K402

Maximizing mtDNA Testing Potential with the Generation of High-Quality mtGenome Reference Data

Sanger-type Sequencing of mtGenomes

BMC Genomics

BioMed Central

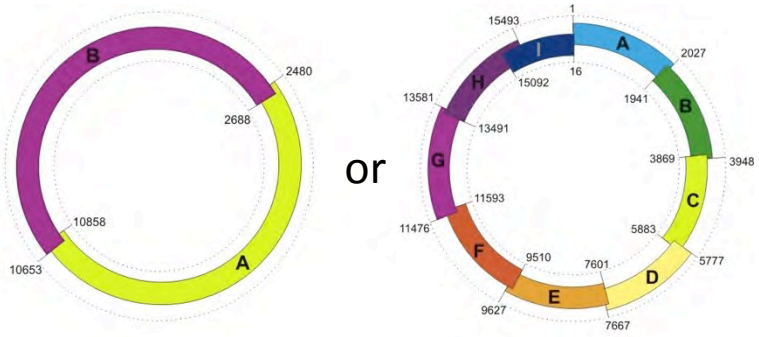
Methodology article

Open Access

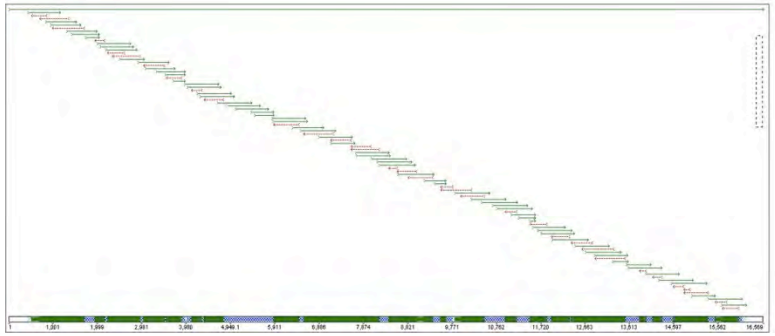
Sequencing strategy for the whole mitochondrial genome resulting in high quality sequences

Liane Fendt[1], Bettina Zimmermann[1], Martin Daniaux[2] and Walther Parson*[1]

Address: [1]Institute of Legal Medicine, Innsbruck Medical University, Müllerstrasse 44, Austria and [2]Clinical Department of Radiology, Innsbruck Medical University, Austria

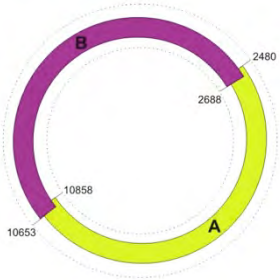


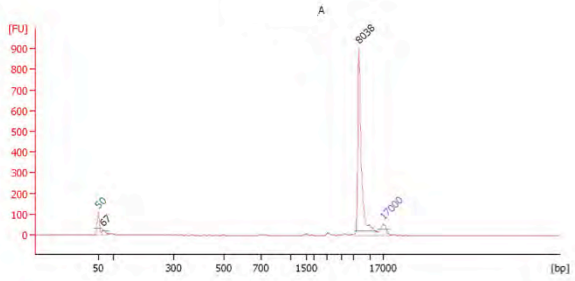
mtGenome PCR with 2 and 9 overlapping amplicons



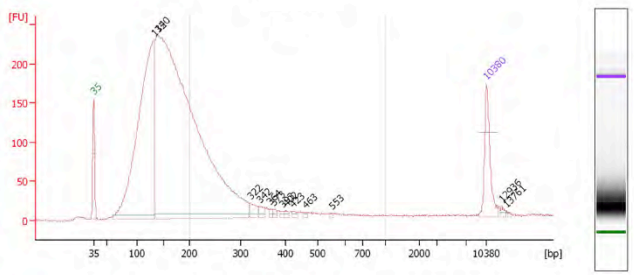
Sequencing using 106 primers

PGM Next Generation Sequencing of mtGenomes





PCR

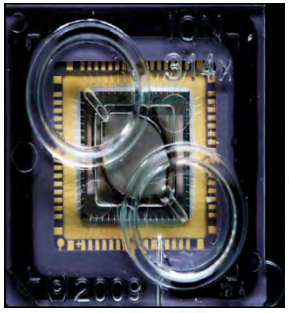


e-shearing

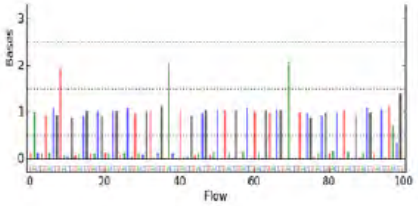
Prepare Library

Clonal Amplification

Isolate Positive Ion Sphere™ Particles



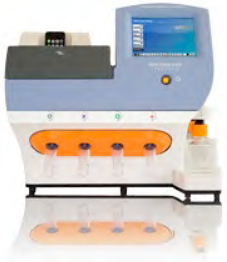
100/200 bp chemistry
316 chips



Data Analysis

OneTouch™

OneTouch™ES

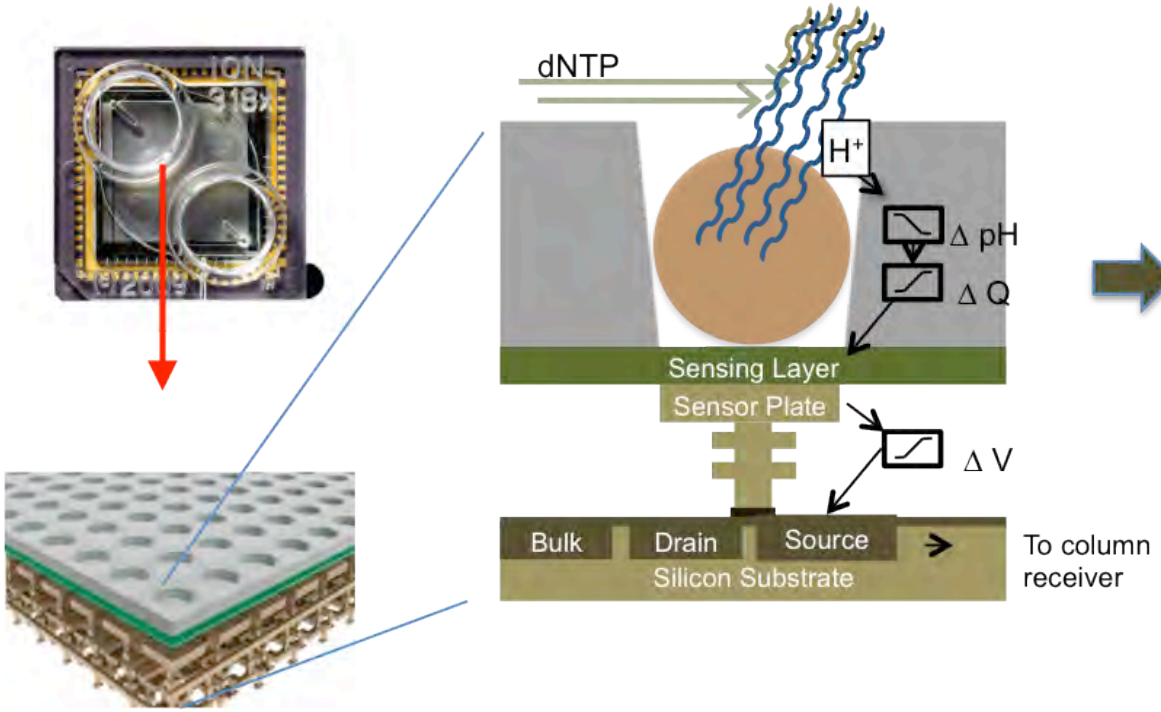


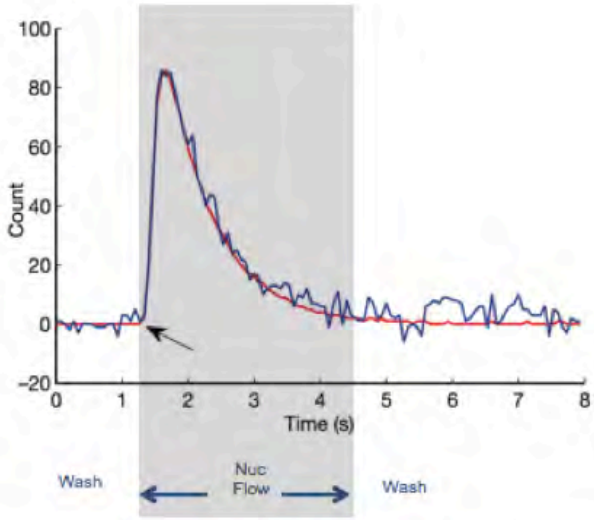
PGM™

Torrent Server & Torrent Browser

courtesy Applied Biosystems by Life technologies

Sequence Detection by pH





Rothberg J.M. *et al Nature doi:10.1038/nature10242*

NGS analysis tools used in this study

BAM (Binary Alignment Map), BAI (Binary Alignment Index)
rCRS (mtDNA Reference sequence, Andrews et al 1999)

Torrent Browser Variant Caller (Ion Torrent, Life Technologies)

- Variant Caller (Vs. 3.2.43647)
- TMAP Smith-Waterman alignment optimization (Li and Homer, 2010)

Integrative Genomics Viewer (IGV)

- Freeware to visualize alignment files (Robinson et al, 2011; Vs. 2.1.21 (2541)
- accepts BAM, BAI and other formats

NextGENe (SoftGenetics)

- modified Burrows-Wheeler transform alignment method (Vs. 2.3.1)
- paired read alignment option + visualization

Sequencher (GeneCodes)

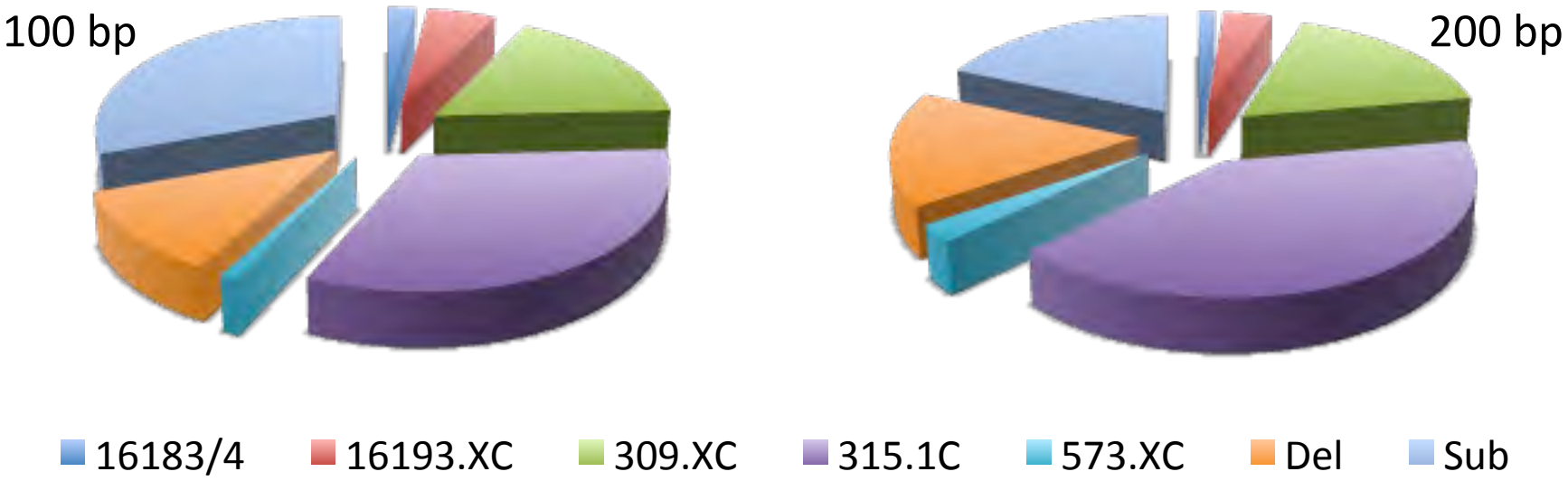
- Tablet for NGS integrated in Sequencher (5.0)
- GSNAP alignment (Wu and Nacu, 2010)

STS versus PGM variant calls

Variant caller settings: 20% variance frequency (of total coverage)

PGM seq. chem.	#	bp	differences
100 bp	31	513,651	95 (0.018%)*
200 bp	33	546,786	81 (0.015%)*

*Length heteroplasmy changes considered as single difference
*Alignment conventions 5' (PGM) vs. 3' (STS) not considered



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100 bp	31	513,651	95 (0.018%)*
200 bp	33	546,786	81 (0.015%)*

*Length heteroplasmy changes considered as single difference
*Alignment conventions 5' (PGM) vs. 3' (STS) not considered

100 bp

200 bp

16183/4 16193.XC 309.XC 315.1C 573.XC Del Sub

STS versus PGM variant calls

Variant caller settings: 20% variance frequency (of total coverage)

PGM seq. chem.	#	bp	differences
100 bp	31	513,651	95 (0.018%)*
200 bp	33	546,786	81 (0.015%)*

*Length heteroplasmy changes considered as single difference
*Alignment conventions 5' (PGM) vs. 3' (STS) not considered

PGM seq. chem.	# differences	False positives	False negatives
100 bp	95	38 (40.0%)	57 (60.0%)
200 bp	81	12 (14.8%)	69 (85.2%)

4. New developments

Evaluating full mtGenome NGS for mtDNA typing (Ion Torrent PGM, LT)

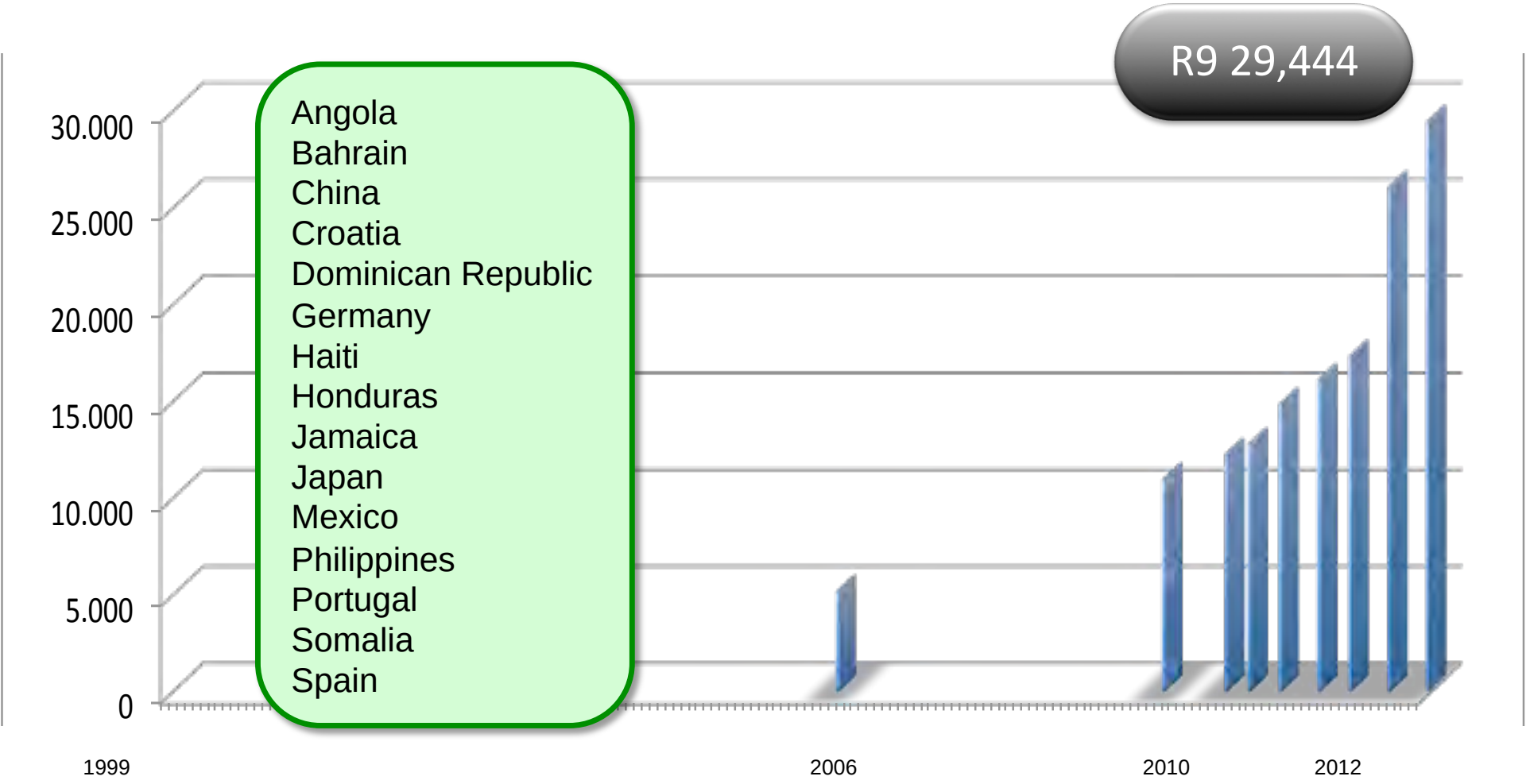
based on 33 mtGenomes (546,786 bp)

analyses performed under optimized settings for each software

33 mtGenomes (200 bp)	HVS-1	HVS-2	HVS-3	CA	DEL	SUB	total
Variant caller	4	47*	2*	0	13	15	81
NextGENe	14	2	1	2	40*	7	66

* systematic false positives

EMPOP Release 9 (14.02.2013)



Acknowledgements

EUROFORGEN Network of Excellence

FP7-SEC-2011-285487

FWF Der Wissenschaftsfonds.

Translational Research project L397
"EMPOP–an innovative human mtDNA database"

NIJ National Institute of Justice 2011-MU-MU-K402

Maximizing mtDNA Testing Potential with the Generation of High-Quality mtGenome Reference Data

applied biosystems by life technologies

Robert Lagacé
Sharon Wootton
Reina Langit
Lisa Calandro

GMI DNA EMPOP

Christina Strobl
Gabriela Huber
Bettina Zimmermann
Liane Fendt

Samples

Sibylle Marcial Gomes, Luis Souto, University of Aveiro, Portugal
Rhena Delport, University of Pretoria, South Africa

GMI DNA

EMPOP staff

NIST Update

Peter M. Vallone, Michael Coble, Becky Hill, Erica Butts, Kevin Kiesler, Margaret Kline

Applied Genetics Group

U.S. National Institute of Standards and Technology

EDNAP Meeting

April 23, 2013

Bratislava, Slovakia

NIST Human Identity Project Team

within the Applied Genetics Group

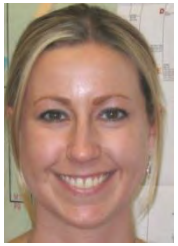
Forensic DNA Team

Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

Pete Vallone

Group Leader

Mike Coble



Becky Hill

Margaret Kline

DNA Biometrics Team

Funding from the **FBI** through NIST Information Access Division

Erica Butts

Kevin Kiesler

Data Analysis Support

Dave Duewer

As of April 1, John Butler has moved into the Office of Special Programs and is working on Forensic Science efforts across NIST

Sources of external funding

NIJ National Institute of Justice

DEPARTMENT OF JUSTICE FEDERAL BUREAU OF INVESTIGATION

U.S. DEPARTMENT OF HOMELAND SECURITY

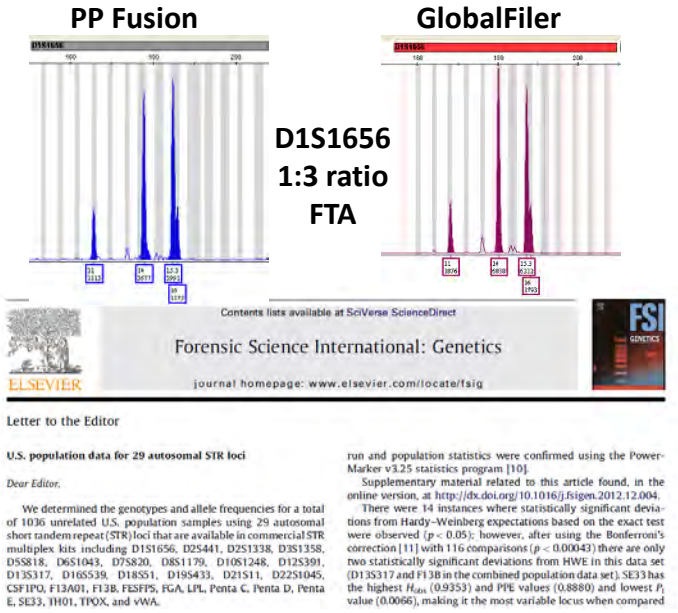
Topics

- Characterizing new STR loci
- DNA Mixture interpretation
- PLEX-ID mass spectrometer
- Next-generation sequencing
- Rapid STR typing (PCR and instruments)
- SRM 2372 (Human DNA Quantitation)

Characterizing New STR Loci

- In April 2011, the FBI announced plans to expand the core loci for the U.S. beyond the current 13 CODIS STRs to 20 total (including DYS391)
- Our group has collected U.S. population data on new loci and characterized them to aid understanding of various marker combinations
- We have recently published the genotypes, allele frequencies and population statistics from these samples at all 29 of these loci in FSI: Genetics
- Recently two commercial kits were released that include the extended core loci GlobalFiler (Life Tech) and PowerPlex Fusion (Promega)

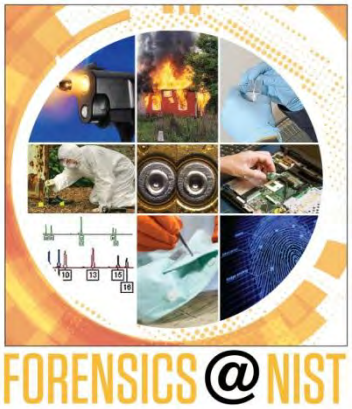
STR Kit or Core Set of Loci	Total N=1036	Caucasians (n=361)	African Am. (n=342)	Hispanics (n=236)	Asians (n=97)
CODIS 13	5.02E-16	2.97E-15	1.14E-15	1.36E-15	1.71E-14
Identifiler	6.18E-19	6.87E-18	1.04E-18	2.73E-18	5.31E-17
PowerPlex 16	2.82E-19	4.24E-18	6.09E-19	1.26E-18	2.55E-17
PowerPlex 18D	3.47E-22	9.82E-21	5.60E-22	2.54E-21	7.92E-20
ESS 12	3.04E-16	9.66E-16	9.25E-16	2.60E-15	3.42E-14
ESI 16 / ESX 16 / NGM	2.80E-20	2.20E-19	6.23E-20	4.03E-19	9.83E-18
ESI 17 / ESX 17 / NGM SElect	1.85E-22	1.74E-21	6.71E-22	3.97E-21	1.87E-19
CODIS 20	9.35E-24	7.32E-23	6.12E-23	8.43E-23	4.22E-21
GlobalFiler	7.73E-28	1.30E-26	3.20E-27	2.27E-26	1.81E-24
PowerPlex Fusion	6.58E-29	2.35E-27	1.59E-28	2.12E-27	1.42E-25
All 29 autosomal STRs	2.24E-37	7.36E-35	3.16E-37	2.93E-35	4.02E-32
29 autoSTRs + DYS391	1.07E-37	3.26E-35	1.77E-37	1.29E-35	2.81E-32

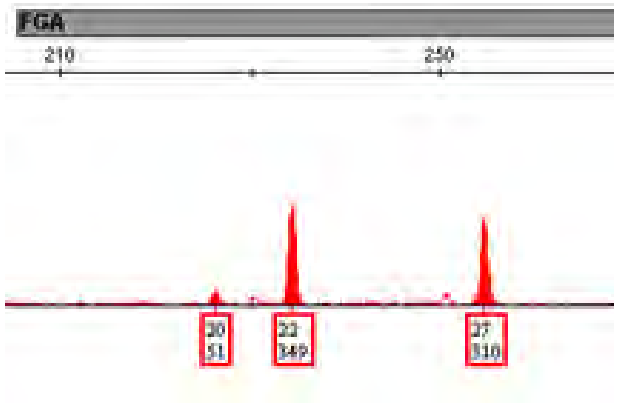


DNA Mixture Interpretation

- Currently in the planning stages for an inter-laboratory study (MIX13) with several mixture examples. This is to assess how well labs are applying the 2010 SWGDAM recommendations.
- Mike Coble recently traveled to New Zealand to receive training for STRmix. Validation studies are in progress.
- NIST sponsored an online webcast on Mixture Interpretation on April 12th. An archive of the webcast is being prepared for closed-captioning and will be available in May for up to six months at this website:

http://www.nist.gov/oles/forensics/dna-analyst-training-on-mixture-interpretation.cfm





DNA Mixture Interpretation Webcast

April 12, 2013

PLEX-ID Mass Spectrometer

- Evaluation of ESI-TOF mass spectrometer
  - Supported by the FBI
- SNP 40 Assay
  - 40 Autosomal SNPs
  - Eight 5-plex reactions
  - 194 samples typed
  - Manuscript submitted
- mtDNA 2.0 Assay
  - Eight 3-plex reactions
  - Concordance = 99.97%
    - 711 samples
    - 4 U.S. populations
  - Sensitivity ≤ 200 pg DNA
  - Mixture capability
  - No contamination observed
  - Manuscript in preparation

PLEX-ID

Discontinued
by Abbott Dec 2012

Initial report on PLEX-ID assessment 136 pages

NIST Report to the FBI:
Plex-ID Electrospray Time-of-Flight Mass Spectrometer for Mitochondrial DNA Base Composition Profiling

Experiments performed and report written by: Kevin Kiesler, M.S. (NIST)

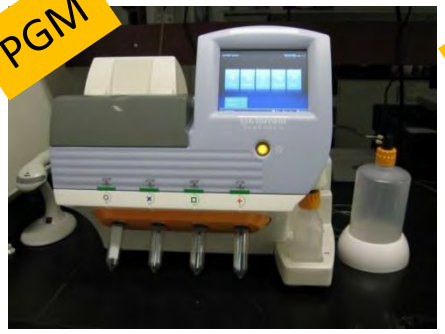
Under the direction of: Dr. Peter Vallone (NIST)

http://www.cstl.nist.gov/strbase/pub_pres/NIST-report-on-PlexID.pdf

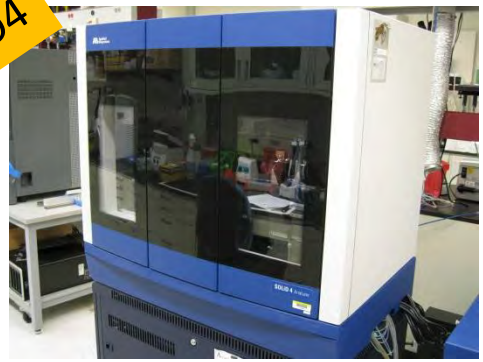
Next-Generation Sequencing

- Multiple platforms used
  - Illumina
    - HiSeq
    - MiSeq
  - Life Technologies
    - SOLiD4
    - Ion Torrent PGM
- Pilot study sequencing
  - NIST Standard Reference Materials 2392 & 2392-I
    - **For mitochondrial DNA sequencing**
  - Deep sequence coverage
    - 100x to 60,000x
    - Further Characterization
      - Heteroplasmy

PGM



SOLiD4



SRM

Results will be discussed at the Croatia meeting this June

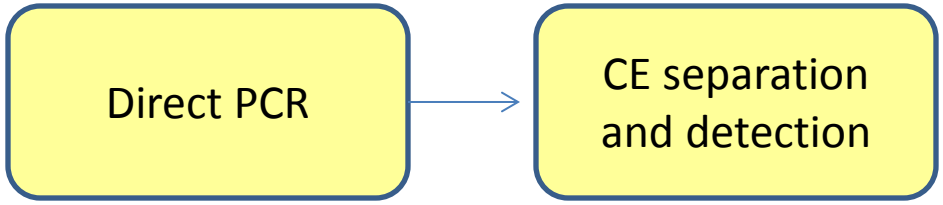
Rapid PCR and STR workflows

Example Rapid Work Flow in **Lab Setting**

single source reference samples

Lab-based fast extraction and rapid PCP protocols

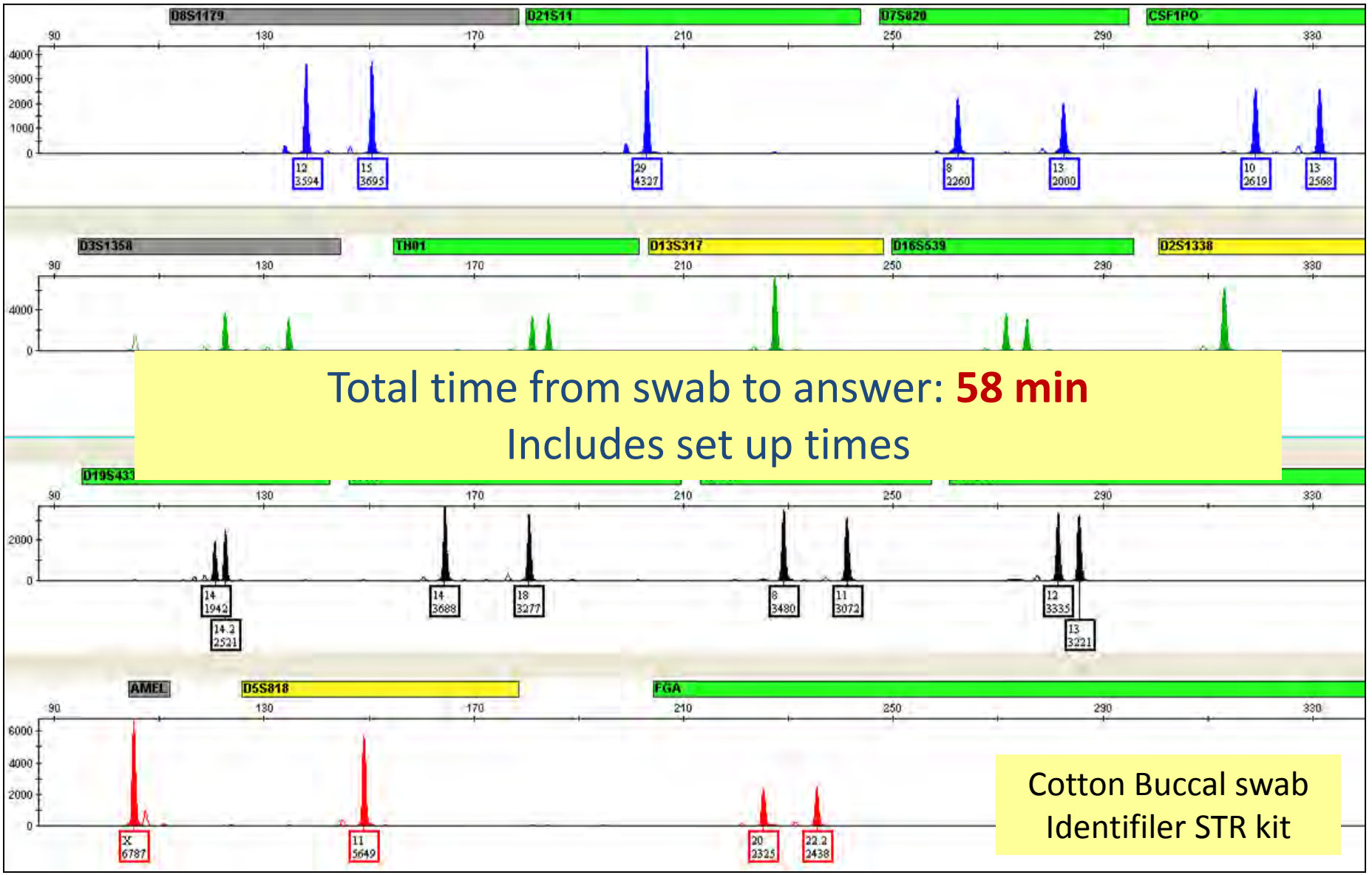
- Prep-N-Go extraction (cotton buccal)
- Rapid Identifiler (Philisa cyclers)
- Separations on an 8 capillary 3500



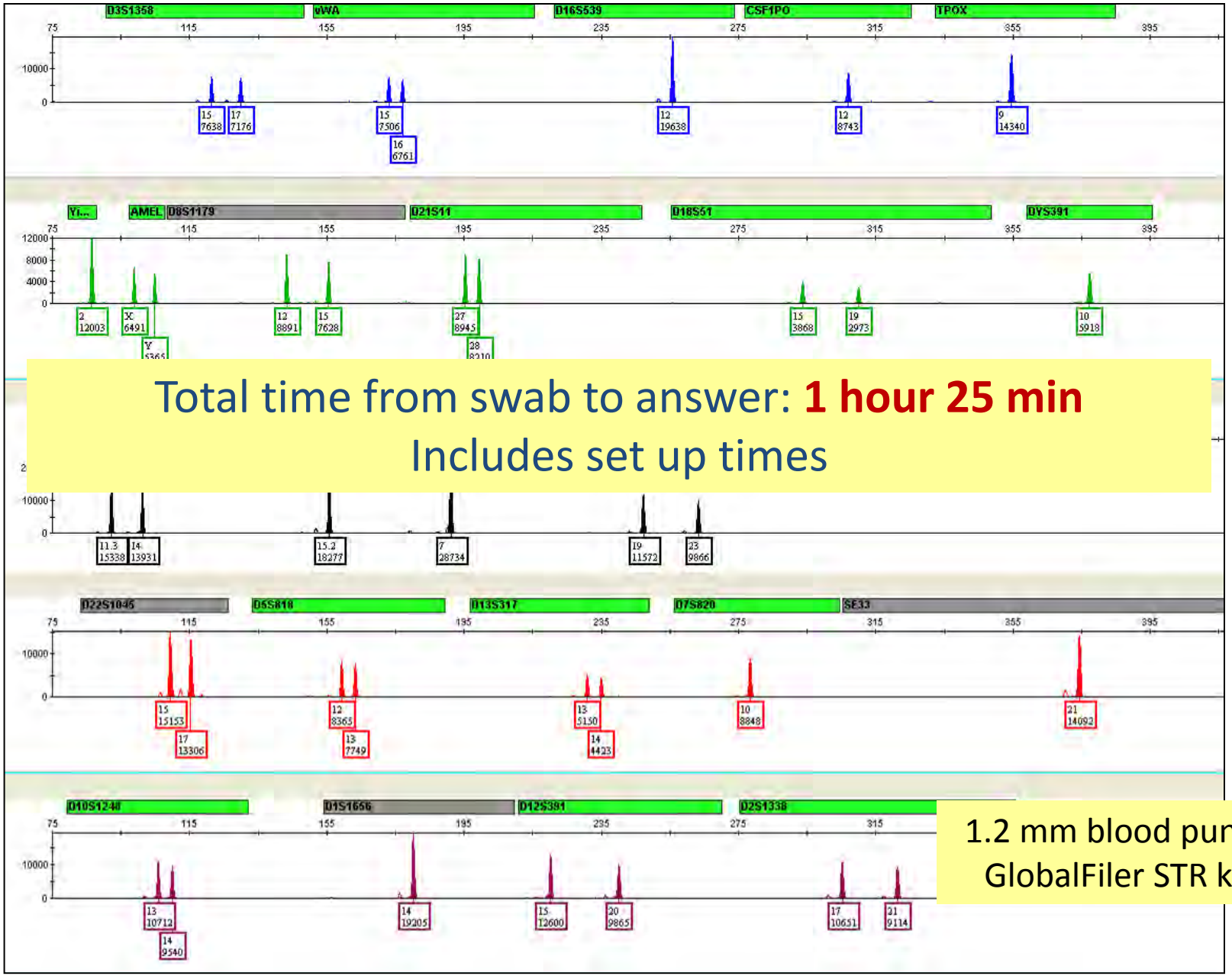
- 1.2 mm blood punch
- GlobalFiler Express (9700)
- Separations on an 8 capillary 3500

8 unique samples were typed in parallel

Prep-N-Go → Philisa (2-step) → 3500



FTA → 9700 → 3500



Total time from swab to answer: **1 hour 25 min**

Includes set up times

1.2 mm blood punch

GlobalFiler STR kit

Status of SRM 2372

- NIST SRM 2372 Human DNA Quantitation Standard – **returned to sale** (as of January 8, 2013)
- Certified based on absorbance value
  - Material in the vials is the same BUT has been re-characterized by NIST
  - Single stranded form of DNA
  - Driven to single stranded form using NaOH (0.4 mM) and recertified for absorbance
  - 1 OD at 260 nm ≈ 37 ng/µL

SRM transitioned from ds to ss DNA over time in dilute salt solution (TE^{-4})

Status of SRM 2372

Updated values

Table 1. Certified Apparent Absorbance ($A_{260} - A_{320} = D_{10}$ at 260 nm – D_{10} at 320 nm) Values for Components Treated with NaOH

Component A	Component B	Component C
0.777 ± 0.060	0.821 ± 0.095	0.804 ± 0.068

Table 2. Information Values for Untreated Components

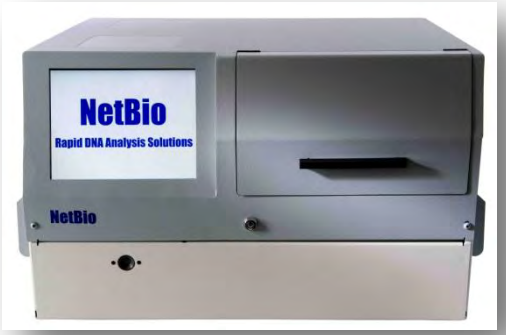
Component	Conventional ssDNA Concentration (ng/µL)	$A_{260-320}$
A	57	1.2
B	61	1.3
C	59	1.3

https://www-s.nist.gov/srmors/view_cert.cfm?srm=2372

Currently carrying out work to certify copy number using digital PCR methods

Rapid DNA Prototype Assessment

- Carrying out testing on IntegenX and NetBio R-DNA prototype STR typing instruments



- Over 250 samples (buccal cells on swabs) have been run on each platform

- In the process of assessing genotyping success and providing early feedback for improvement to the vendors

Improvements and optimization are being made to cartridge manufacturing, expert system software, chemistry, and hardware robustness

Questions/Contact

Peter.vallone@nist.gov
1-301-975-4872

Also:
Congrats to Erica Butts!!!
Levi William Butts →

- 4/9/2013
- 8 lbs 12 oz
- 22 inches long

FORENSIC BIOLOGY RESEARCH

Jack Ballantyne

National Center for Forensic Science
University of Central Florida, Orlando, FL

Ballantyne Lab Projects

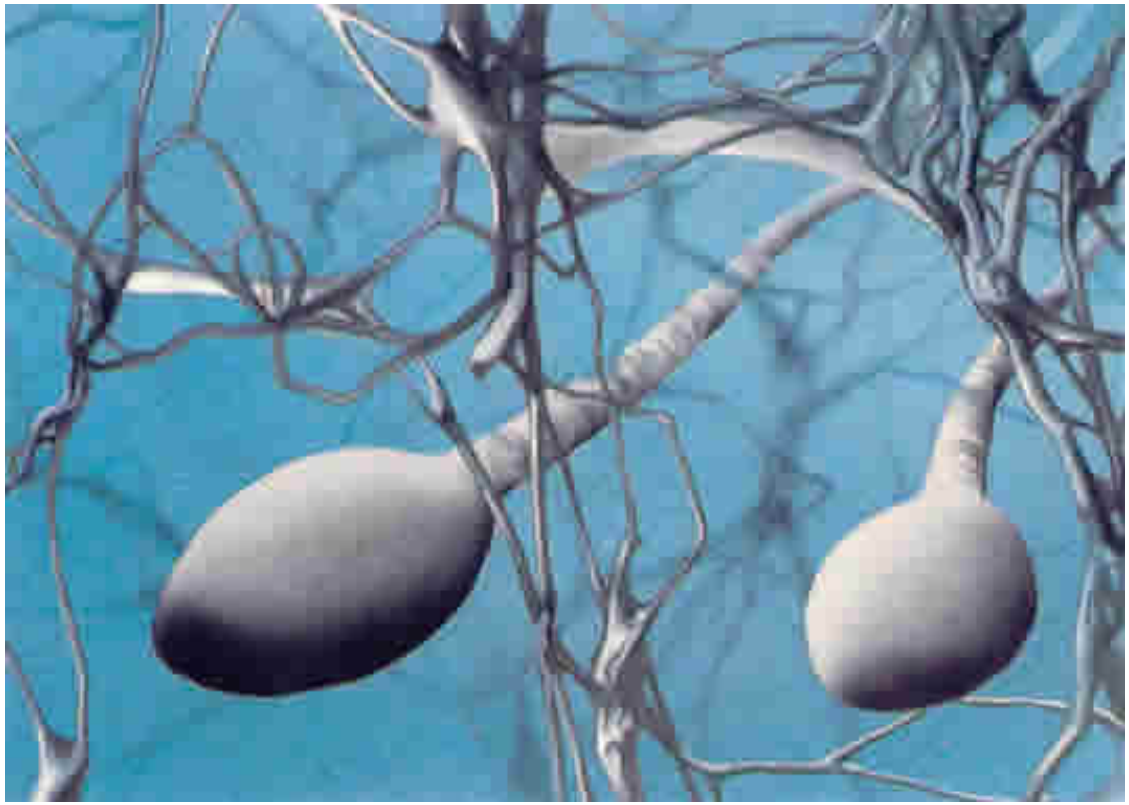
- Improve effectiveness of of bio-molecular forensic analysis (more probative information)
  - RNA profiling for body fluid identification
    - mRNA
    - miRNA
    - Co-extraction methods
  - Y chromosome
    - Next generation Y-STR markers
    - Extended Interval Post-Coital Samples
      Selective enrichment of Male DNA
  - Low copy number/single cell analysis
    - Laser capture micro-dissection (single/few cell analysis)
    - Characterization of ‘touch DNA’

Ballantyne Lab Projects

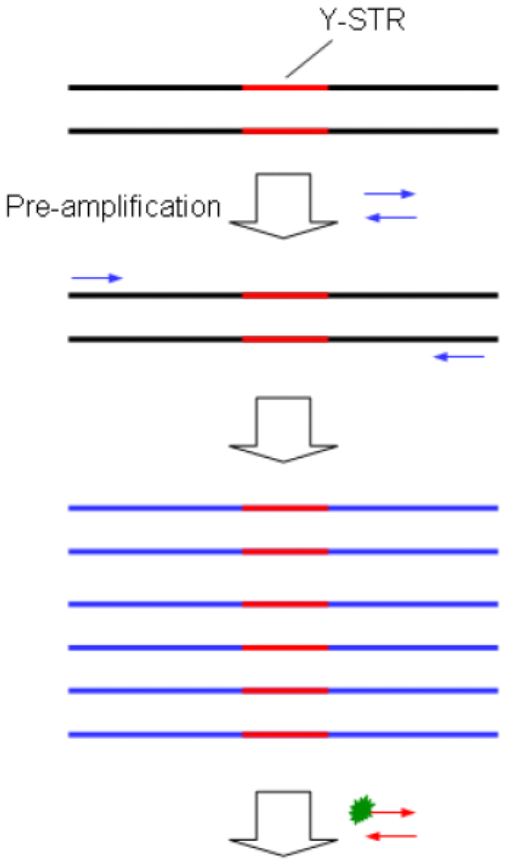
- Mixture de-convolution
- Determination of biological age of a blood stain donor
- Novel/Rapid DNA typing strategies (STRs)

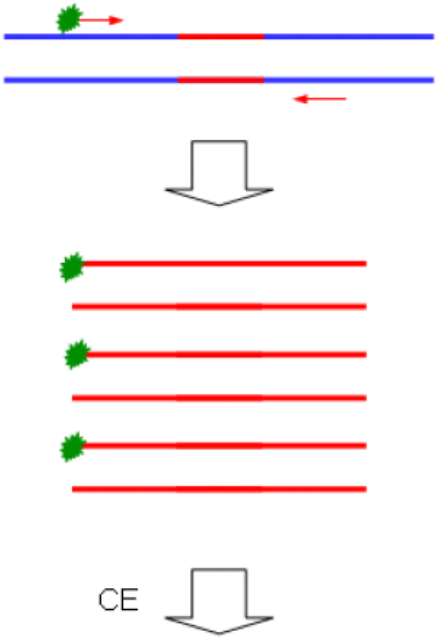
- Operational support to casework laboratories
  - National US Y-STR Database

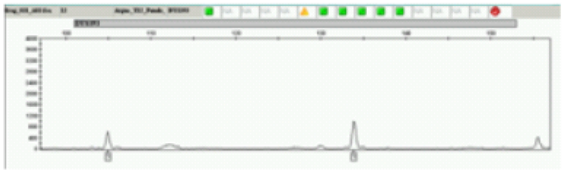
DNA Profiling of the Semen Donor in Extended Interval Post Coital Cervicovaginal Samples



Nested-PCR Y-STR Strategy







MinElute purification to
remove excess primers

Improvement in Post Coital Interval

using nested PCR pre-amplification

Post Coital Interval (Days)	Pre-Amplification ?	Allele recovery (out of 17 possible)			
		Couple 1	Couple 2	Couple 3	Couple 4
6	Yes	17	10	4	17
	No	1	0	0	0
7	Yes	17	16	3	17
	No	0	4	0	0
8	Yes	16	NT	NT	17
	No	0	NT	NT	4
9	Yes	12	NT	NT	14
	No	0	NT	NT	0
9*	Yes	16	NA	NA	NA
*menses on day 7	No	0	NA	NA	NA

NT = not tested; NA = not available

Current Work

- Collaborative study
  - National Center for Forensic Science
  - University of Tennessee, Health Science Center
- Purpose:
  - Evaluate DNA recovery in post coital samples collected 4, 7 and 9 days after intercourse
    - Use of enhanced Y-STR typing strategies to improve recovery of male DNA profiles
  - Evaluate various physiological conditions that may impact the recovery of DNA in post coital samples

Samples

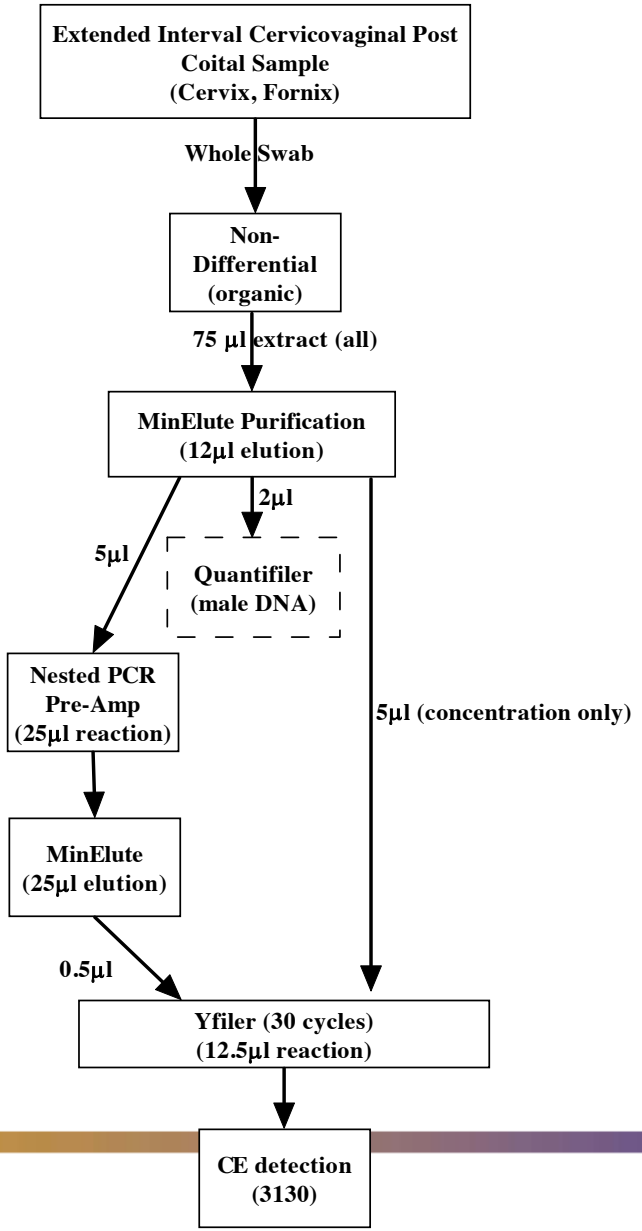
- Post coital samples collected by sexual assault nurse examiners (SANE)
  - Two locations: cervix, fornix
  - Three time intervals (after separate sexual acts)
    - 4 day, 7 day and 9 day
    - Baseline ("pre-coital") sample collected prior to one of the time intervals)
  - Currently, 72 kits available (donor couples)
    - 54 kits completed
    - 11 completed, data being entered
    - 7 kits in progress

Kits: Receipt and Log-In

- Contents:
  - Buccal swab, male
  - Buccal swab, female
  - Baseline
  - 4 day
  - 7 day
  - 9 day

- Kit inventoried to ensure all samples are included
- Collection date and time recorded
  - Time/Date of sexual act provided by UTHSC (time interval verified)

Experimental Schema



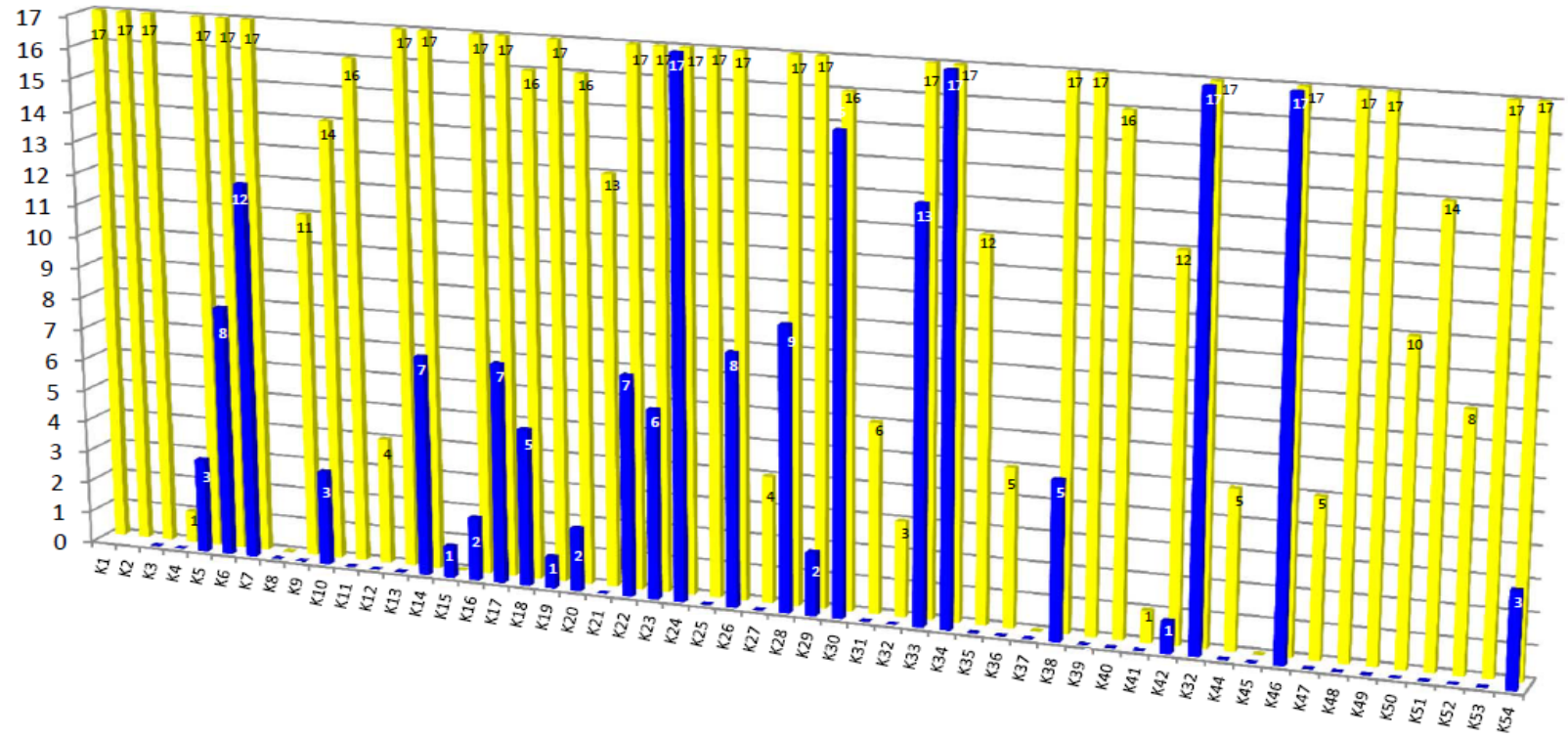
Results Summary Table

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P25		100P24		100P26		100P21		100P16	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	0	1	0	0	0	0	0	0
	Enhanced	4	0	1	1	0	0	15	15	0	0
4 day	Standard	0	3	0	0	0	0	0	0	0	7
	Enhanced	6	14	16	15	3	4	17	6	17	17
7 day	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	0	0	15	10	0	2	13	15	14	11
9 day	Standard	0	0	2	0	0	0	0	0	0	0
	Enhanced	0	5	11	15	2	0	0	0	0	1

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P18		100P29		100P20		100P30		100P28	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	2	0	0	0	0	0	1	1	0	0
	Enhanced	2	0	6	1	0	0	5	0	6	2
4 day	Standard	1	0	2	0	7	0	5	0	1	0
	Enhanced	0	0	8	17	17	17	16	0	17	17
7 day	Standard	0	0	0	0	1	0	1	0	0	0
	Enhanced	0	0	0	16	5	17	4	13	17	16
9 day	Standard	0	2	0	0	0	0	0	0	0	0
	Enhanced	0	1	0	0	3	1	8	1	8	2

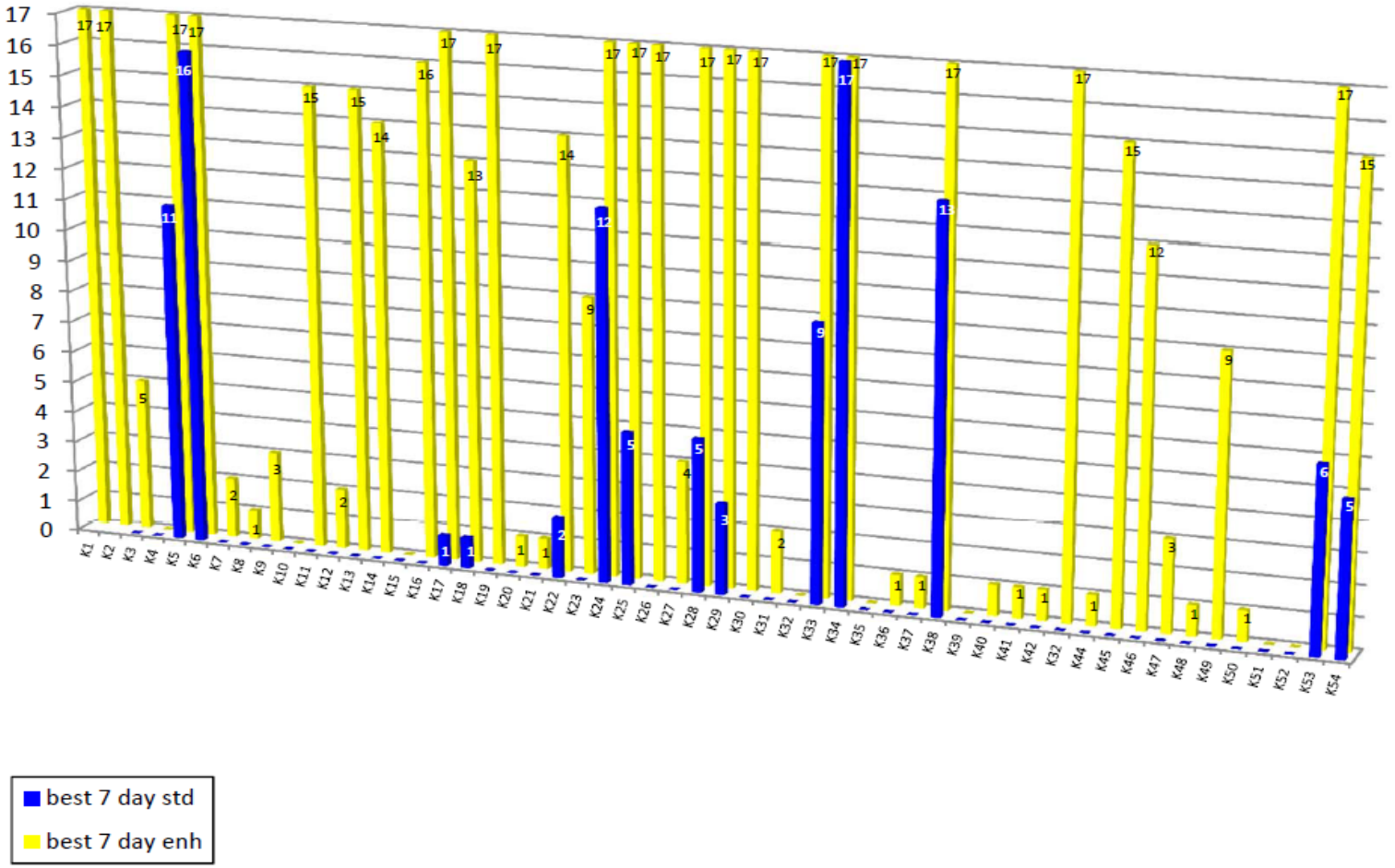
The number of alleles recovered from each swab per collection site and time interval (baseline, 4 day, 7 day and 9 days) is shown (50 RFU detection threshold). The shading represents the average RFU value of all alleles within in the profile (white – not detected; light grey 1-1000 RFUs; dark grey >1000 RFUs. NT = not tested. Baseline samples for 100P29 and 100P30 collected prior to 7 day sample.

Allele Recovery - Extended Interval Post Coital Samples(4 day)



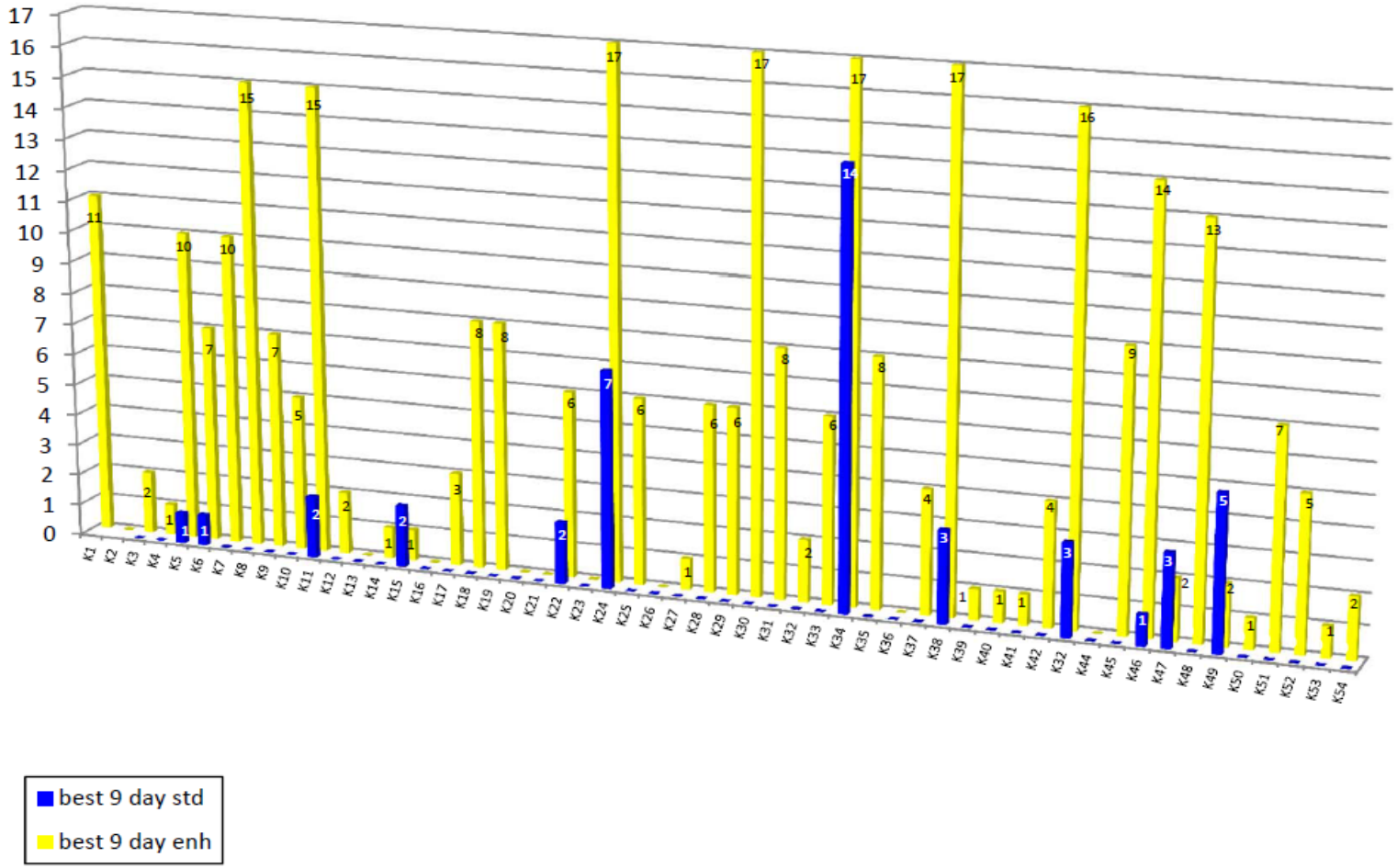
Y-axis: Number of alleles (out of 17 possible); X-axis: Kit number

Allele Recovery - Extended Interval Post Coital Samples (7 day)



Y-axis: Number of alleles (out of 17 possible); X-axis: Kit number

Allele Recovery - Extended Interval Post Coital Samples (9 day)

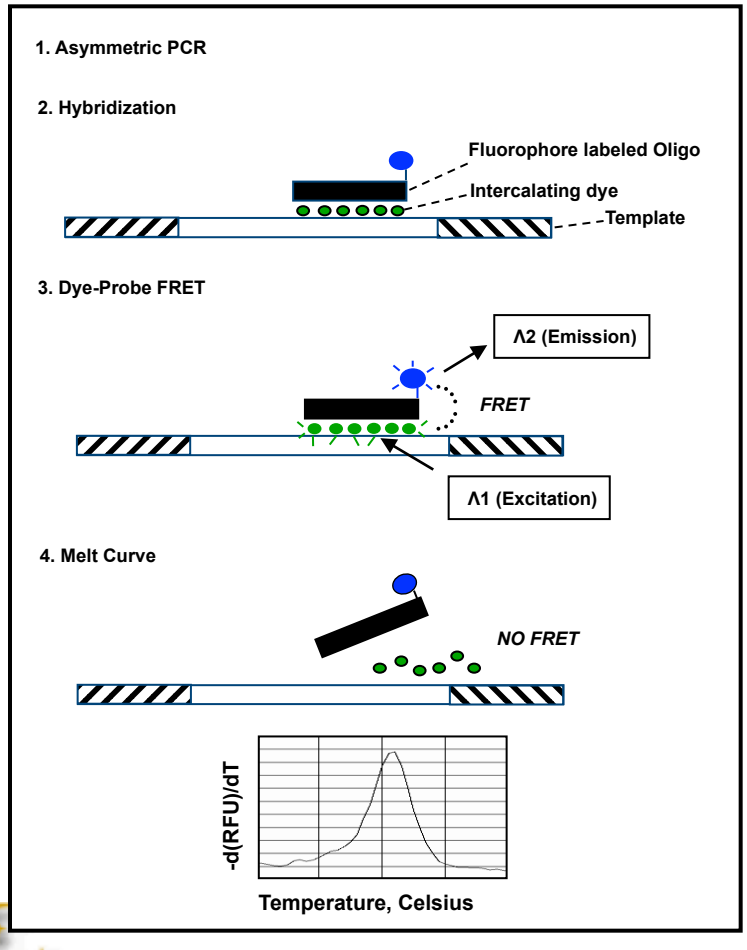


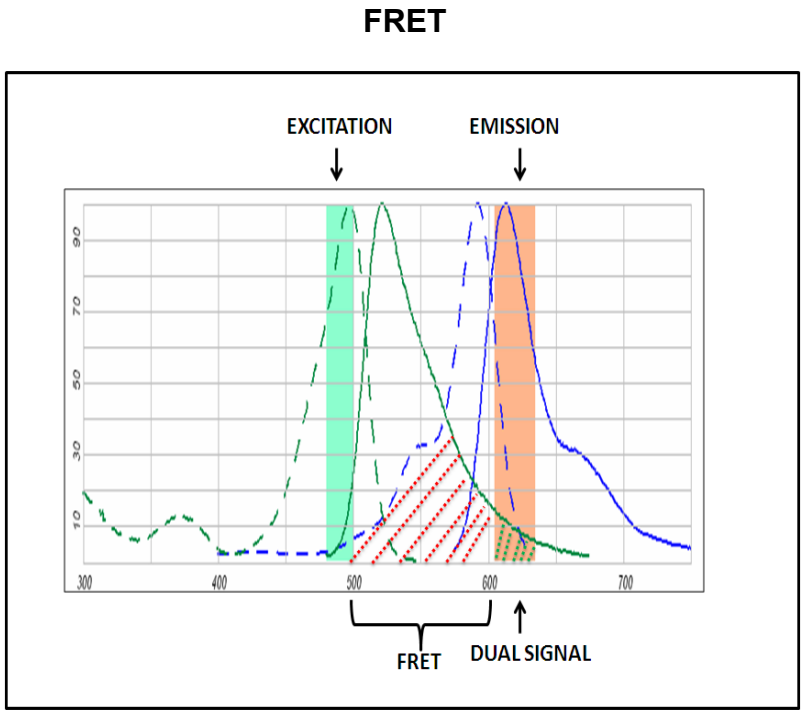
Y-axis: Number of alleles (out of 17 possible); X-axis: Kit number

Other ‘Enhanced’ Strategies

- Data clearly demonstrates improved profile recovery using Y-specific nested PCR pre-amplification
  - Cannot be readily be performed at this time due to proprietary primer mix
- Next generation Y-STR kits available
  - Promega PowerPlex Y23 (available now)
  - Life Technologies (coming soon)
  - Evaluation of PowerPlex Y23 with a sub-set of the kits

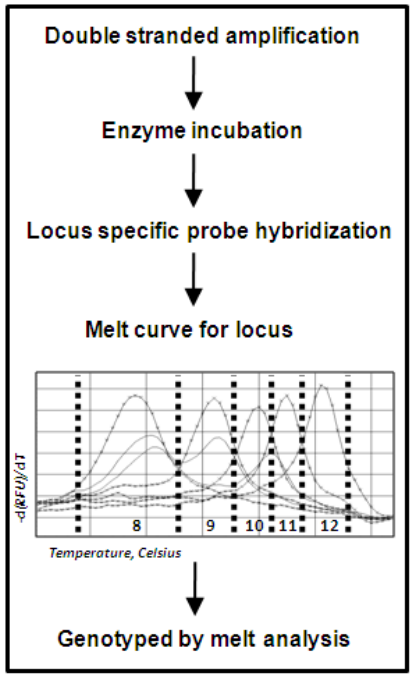
Novel DNA Typing Strategies





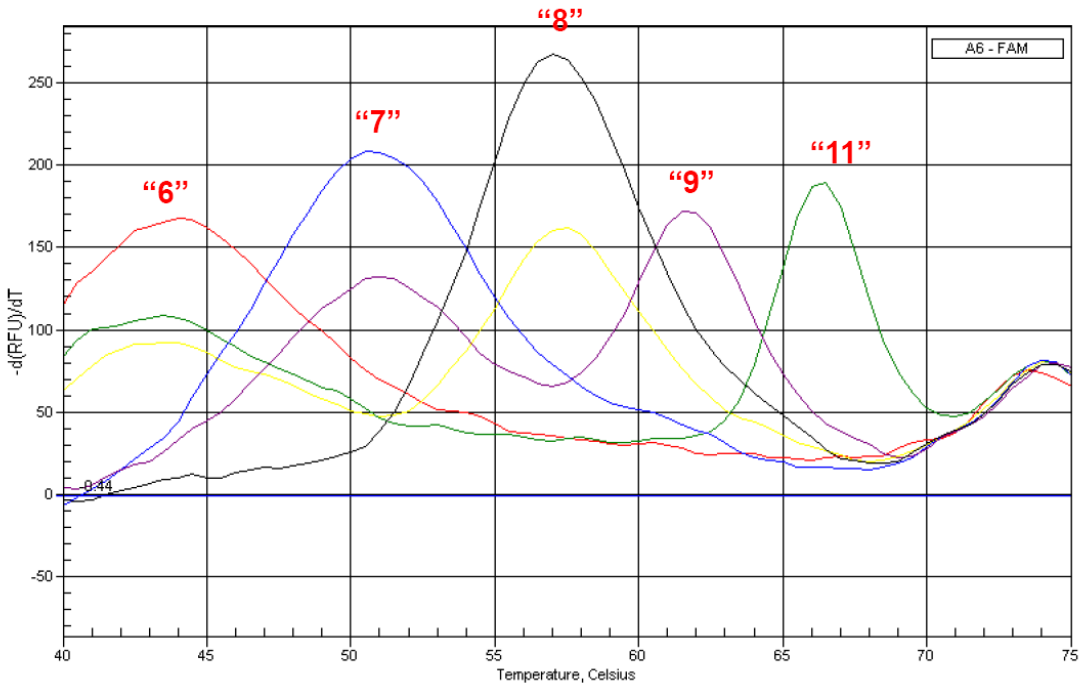
dpFRET – STR Genotyping

OPTIMIZED PROTOCOL
- one round of PCR
- asymmetric by enzymatic
- single probe set
- genotype by melt curve



TPOX

1756 (6,6); 1760 (6,8); 1736 (6,11); 1758 (7,7); 1740 (7,9); 2264 (8,8)



ParaDNA® Introduction

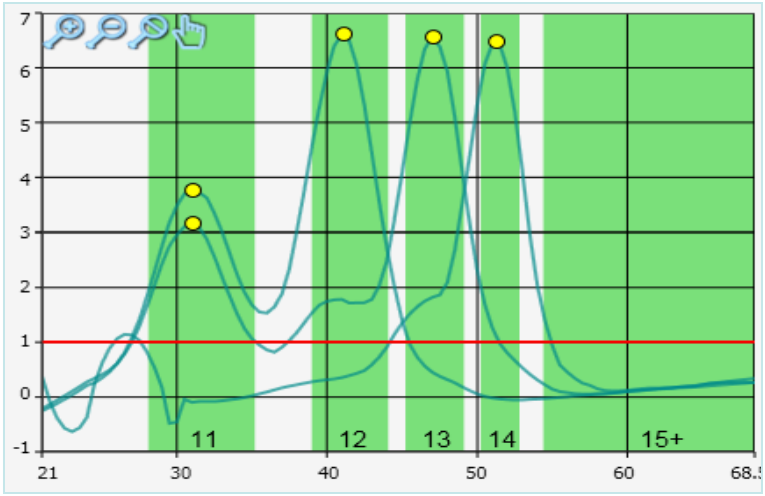
Maximize Investigative Leads

Prior to a full DNA analysis, the ParaDNA Screening System makes it quick and simple to discover:

- Which samples contain human DNA
- Which are most likely to provide a DNA result
- Provides the gender of the contributor.

ParaDNA® Technology

- Sample directly into analysis – **no extraction or quantitation required**.
- The STR positions are tagged with special fluorescent markers.
- These are then amplified by PCR process.
- Separated into different types by virtue of ***behaviour at different temperatures.***
- Results presented as peaks or series of numbers.



THO		Amelogenin		D16	
8	8	X	Y	9	12
6	9.3+	X	Y	11	14
6	8	X	Y	12	12
6	9.3+	X	Y	11	13
7	9.3+	X	X	11	11
9.3+	9.3+	X	X	12	13

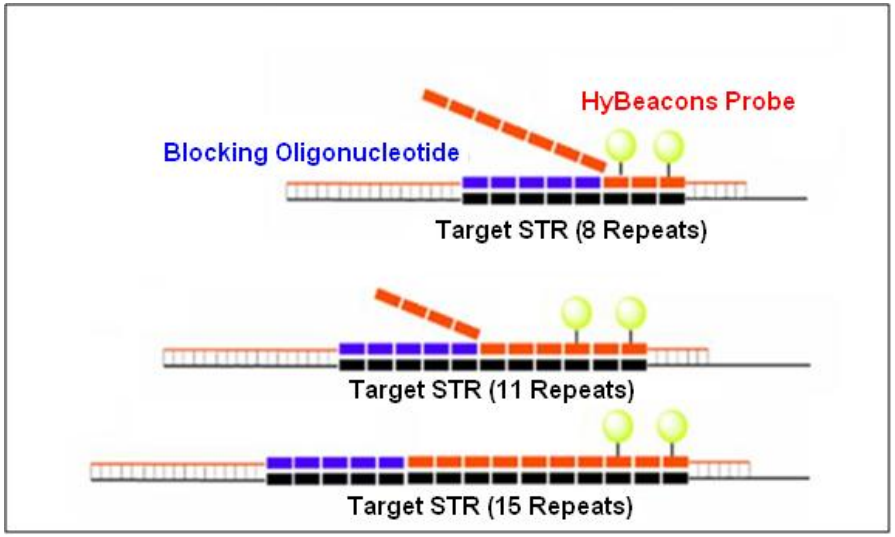
How the HyBeacon™ Assay Works

Amplification

- Sample DNA is added to the reaction tube without preparation. The reagent mix contains all the elements to perform a direct PCR and to analyse the STRs using a DNA Melt analysis.

Melt Measurement

- The longer STRs have stronger affinity to the probe.
- As the temperature is increased, the smaller STRs detach from the probe at lower temperatures.
- As the DNA become single stranded, the probes decrease in brightness.



Selection of target STRs

- Hybridization to repeating sequences
- Hybridization to sequences containing microvariants

- Select STRs with ‘well behaved’ repeat structure
- Select STRs with few (no) microvariants
- *Adsorb* and *bracket* where necessary

- Amel, Tho, D3S1358, D8S1179, D16S539, D18S51
  - **Screening unit assay in blue**
  - Field Intelligence Unit assay all STRs shown

U.S. Validation

- Validation work performed by LGC in the U.K.
  - US Validation studies
    - National Center for Forensic Science (NCFS)
    - Florida International University (FIU)
- NCFS Validation Studies
  - Concordance/Proficiency samples
  - Positive controls
  - Negative controls
  - Bloodstains
    - Concordance with PowerPlex(R) 16HS (Promega)
  - Species Specificity
  - 'Touch DNA' samples
  - Substrates
  - Environmentally compromised samples

Touch Samples

- Sampled items in laboratory and offices that had been previously touched/used/handle by various donors
  - 5 main users/donors
  - Two males/three females
  - Expected that samples might contain multiple donors
- Direct sampling of items using paraDNA collectors
  - Subsequent to sampling, the sampled area was swabbed with a sterile cotton swab pre-moistened with sterile water
    - DNA extraction performed on swab (QIAGEN DNA Investigator kit)
      - 20μl elution volume since LTDNA amounts anticipated

Touch Samples

Sample	Sampling Method	Comments
Door handle	Direct (from object itself)	male office door; smooth metal surface
Computer mouse	Direct (from object itself)	primary user - male (could be other users); used infrequently
Lab coat	Direct (from object itself)	worn by male lab member; could be worn previously by other lab members
Drink - straw	Direct (from object itself)	female user; drink taken within 1 hour of collection, but not immediately before (straw was dry)
Phone	Direct (from object itself)	in office with primarily female personnel (but could be used by others); used infrequently
Cell phone	Direct (from object itself)	primary user - female; cell phone screen with protective cover sampled
Pen	Direct (from object itself)	primary user - male; smooth surafce (not textured)
Backpack	Direct (from object itself)	primary user - male; top handle of backpack
Headphones	Direct (from object itself)	primary user - female; left headphone (in contact with ear) sampled

Sample	Sampling Method	Analyst	Date Run	ParaDNA Head #	Score (%)	Gender Call
Door handle	Direct (from object itself)	Erin	2/21/2013	1	8	male
Computer mouse	Direct (from object itself)	Erin	2/21/2013	2	46	?
Lab coat	Direct (from object itself)	Erin	2/21/2013	3	50	male
Drink - straw	Direct (from object itself)	Erin	2/21/2013	4	97	female
Phone	Direct (from object itself)	Erin	2/21/2013	1	44	?
Cell phone	Direct (from object itself)	Erin	2/21/2013	2	36	?
Pen	Direct (from object itself)	Erin	2/21/2013	3	63	?
Backpack	Direct (from object itself)	Erin	2/21/2013	4	54	male
Headphones	Direct (from object itself)	Erin	2/21/2013	1	65	female

Touch – PP16 HS

Sample	D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S17	D7820	D16S539	CSF1PO	Penta D	AMEL	vWA	D8S1179	TPOX	FGA	
Door handle	16,18	6,9	29,31.2	15	7,*	10,11,12	12	10,*	12,*,13	10	*,14	X,Y	14,18		8,11		
Computer mouse	14,15,17,18	8,9,9.3	28,29,32.2	14,17	11,19	11,12	8,11,12,13	9,10,13	8,10,12	10,12,13	10,11,12,13	X,Y	14,15,16,17,19	10,11,12,13,14	8,9,11	24,25	
Lab coat	15,16,18	7,9,9.3	28,29,30,32.2	12,13,14,17	11,19,22	11,12	11,12	10	8,10,13	12,13	9,11,12	X,Y	14,16,17,19	10,11,12,14	8,9,10,11	22,23,24,25,27	
Drink - straw	16	6,7	30,31	11,13	8,12	12,13	11,13	10,12	9,12	11,12	9,13	X	17	12,16	8,10	20,23	*correct donor genotype
Phone	16,17	7,9,9.3				10,11,12	9,11,12	10	9		9	X,Y	14,16,17,18	10,13,14	8,11		
Cell phone	16	6,7,9.3	30,31	11,12,13	8,12	12,13	11,13	10,12	9,11,12	11,12	*,13	X,Y	17,19	12,15,16	8,10,11	20,23	
Pen	14,15,16,17,18	9,9.3	28,29	14,17	11,19	11,12	11,12,13	8,10,13	8,10,11	13	11,12	X,Y	14,16,19	10,11,12	8,9,10,11	24,25	
Backpack	15,18	9,9.3	28,29	14,17	11,19	11,12	11,12	10	8,10	13	11,12	X,Y	14,19	11,12	9,11	24,25	*correct donor genotype
Headphones	16,18	7,9	28,33.2	15	*,13	11,12	9,12	10	12,13	*,12	9,12	X	17,18	13,14	8,11	20,24	*correct donor genotype

- Single source profiles for drink straw, backpack and headphones
  - Correct genotypes of “main” user/donor
- Mixtures obtained from most other items
  - Multiple users possible so not unexpected
  - Major profile observed for each of these samples
    - Main user/donor profile was the major profile observed in each case

Future Work

- Completion of Screening System validation
  - Touch DNA samples
    - Direct vs Indirect Sampling
  - Sensitivity
- Validation of Intelligence System
  - All studies performed in screening system validation
  - Mixture study

VICTORIA POLICE FORENSIC SERVICES DEPARTMENT

VPFSD

Update of BSAG activities to EDNAP and ENFSI 2013 Bratislava, Slovakia

John Scheffer

Victoria Police Forensic Services Department

Australia

VPFSD

Areas of focus

- New core loci for Australian DNA Database
- Validation of new kits
- Development of new DNA interpretation/statistics software
- Contextual bias
- When is a DNA profile identity?
- Rapid DNA instruments
- Environmental monitoring
- Australian standards
- ISFG

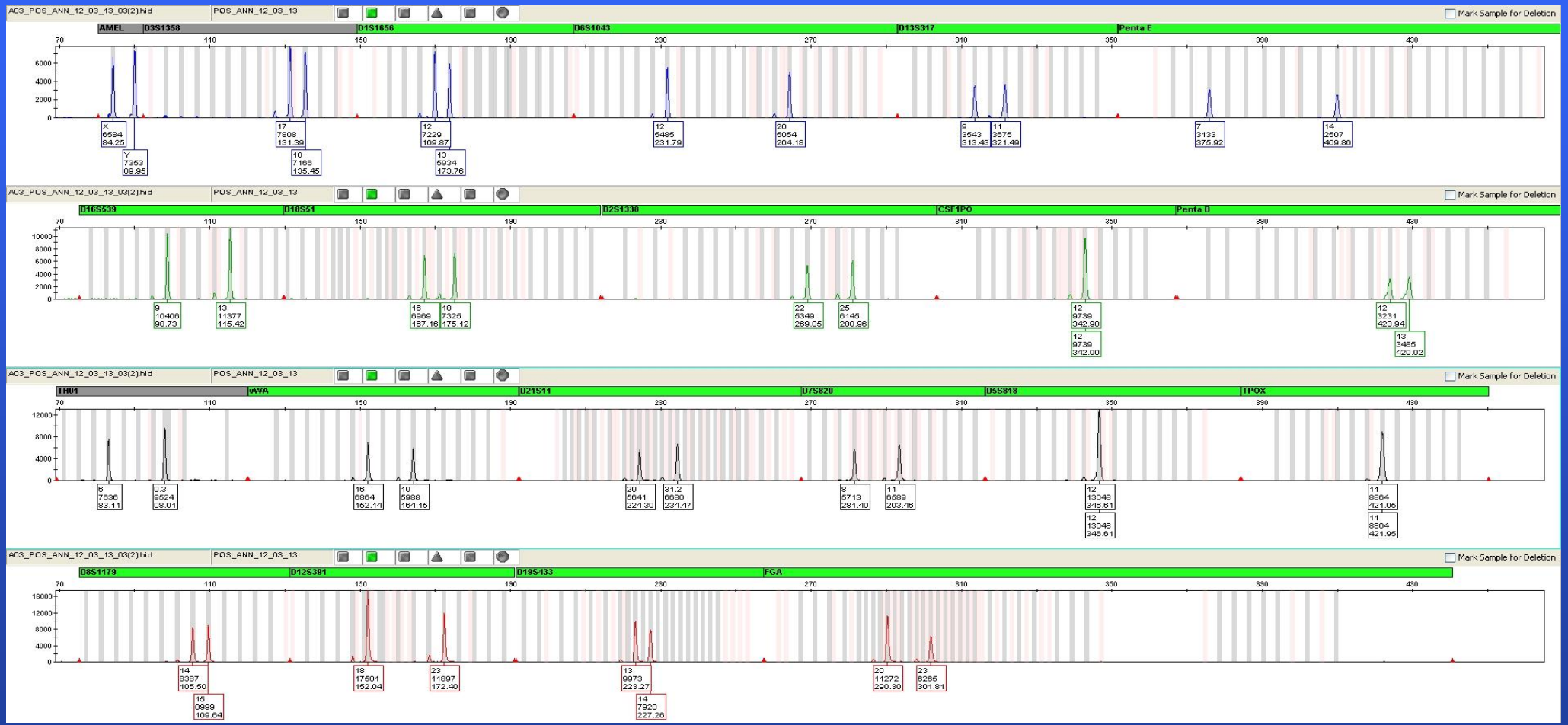
DNA core loci

- Now set at 18 loci
  - Amelogenin, D3S1358, D8S1179, D18S51, D21S11, FGA, vWA, D5S818, D7S820, D13S317, TH01, D16S539, D2S1338, D19S433, CSF1P0, TPOX, D1S1656, D12S391
- Not just a database solution but casework solution
- Roll out has commenced
  - Some observed delays due to the interpretation of additional loci
- NCIDD has been updated to accommodate all the new kits
- Communication with stakeholders in ongoing

The kit

- PowerPlex 21 is the only currently available kit that includes all the loci
- Fusion and Globalfiler to follow
- Provides for the greatest overlap for current Profiler Plus kit
- Provides significant overlap with European kits and core loci
- Concordance with previous kit

PowerPlex 21 (21 DNA sites)



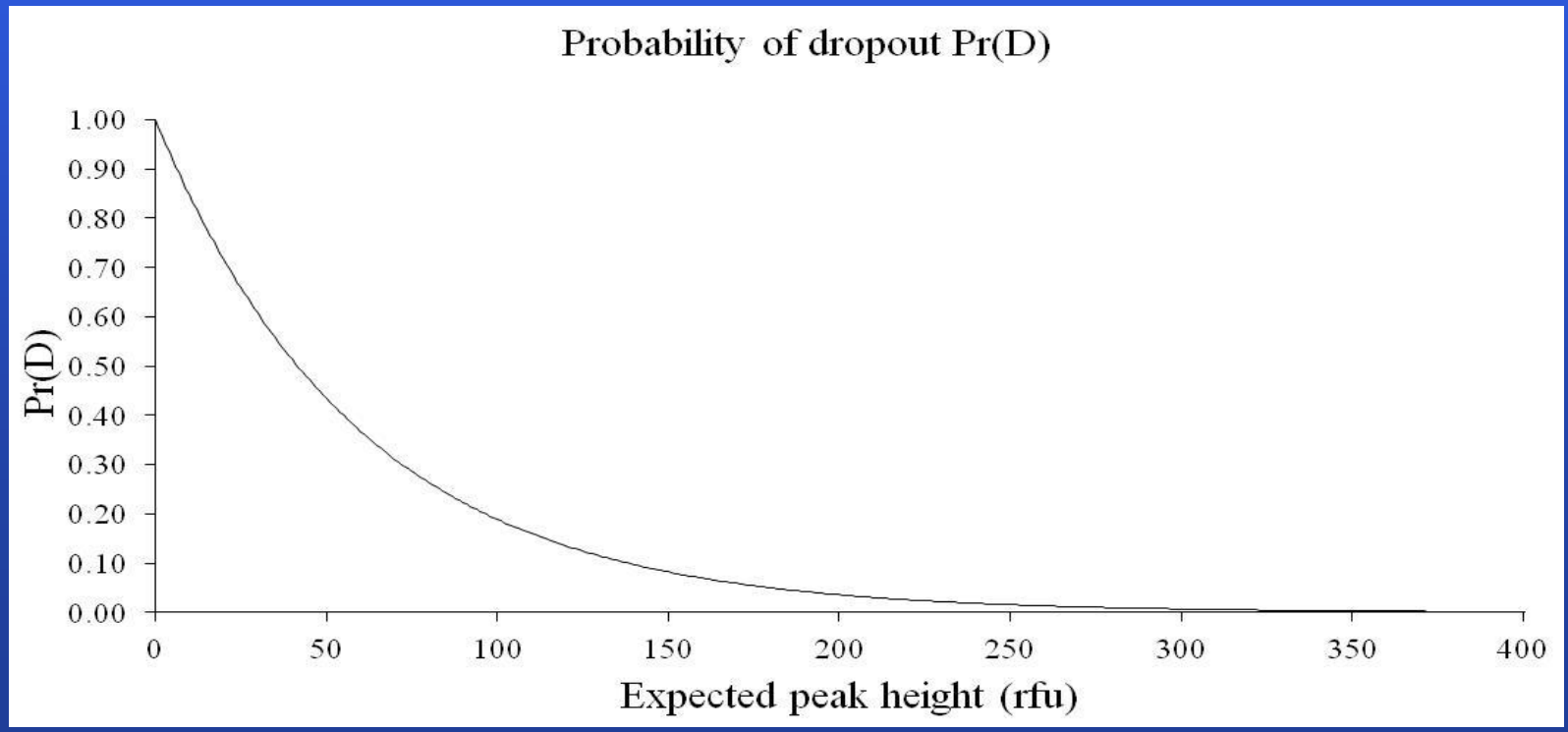
VPFSD

Expert System for DNA Interpretation

- STRmix
  - Continuous model
  - Change from cut-off binary model
  - Avoids ‘falling off the cliff’ issue
  - Takes into account peak heights to allow for interpretation of mixed profiles
  - Some combinations of genotypes, while being possible explanations of a profile, are less likely than other combinations

Change to DNA interpretation methodology – Move from SPURS to STRmix

- P+ used SPURs program – binary or "cut-off" based analysis
- PP21 profiles will be interpreted using STRmix - probability base analysis
  - StatSWG recommendation to BSAG was that a move towards a continuous probabilistic model for DNA interpretation was seen as the way forward.



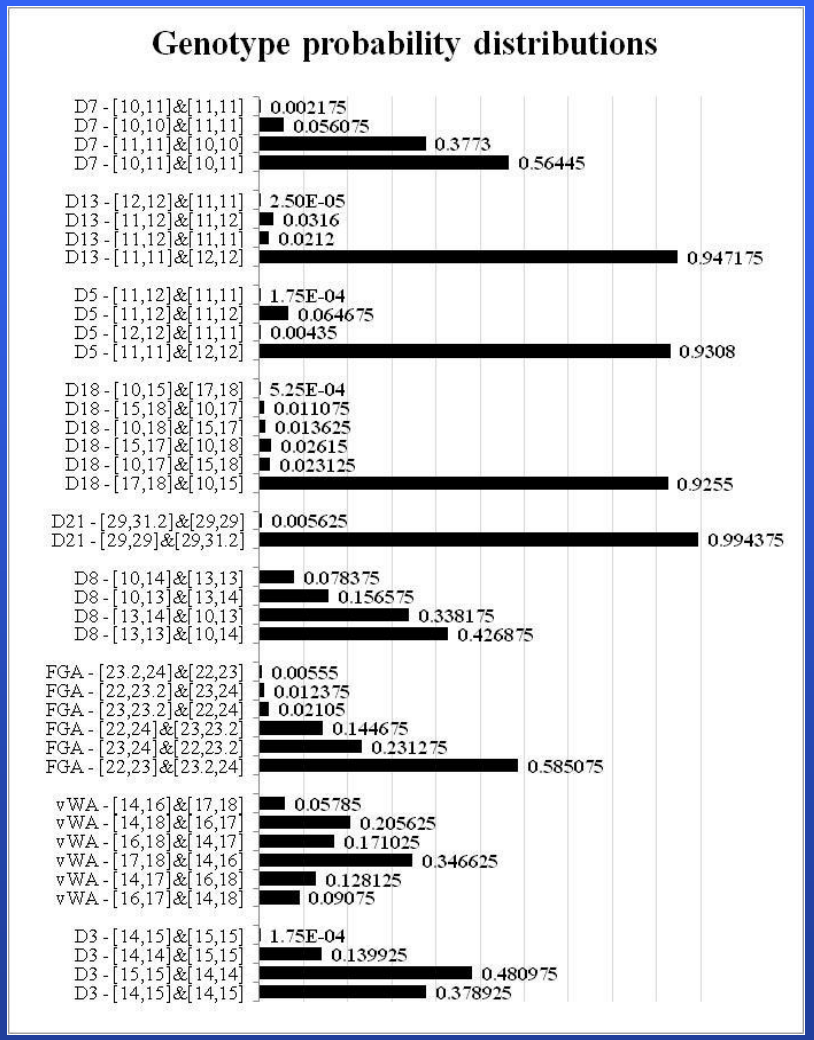
VPFSD

STRmix

- Can be used to:
  - Provide a statistical weighting (LR) for the comparison of a reference sample to an evidential sample
  - To deconvolute a mixture
    - Determine a major
    - Search a Genotype Probability Distribution (no clear major) against a database

STRmix

- An advanced probabilistic approach to dropout and peak imbalance and an algebraic solution (particularly good for dealing with mixtures)
- STRmix looks at a complicated DNA profile and tries every possible explanation for that profile
- It determines what the best explanation for the results are, using many thousands of tests to determine the most likely explanation.



VPFSD

- Does not model overstutter, artifacts or overloaded samples. This means a human is required to read and interpret Genemapper files prior to input
- The output gives the probabilities of the evidence under each hypothesis considered and the Likelihood Ratio for each locus, as well as the profile LR.
- Calculates LRs for single-source and 2- and 3-person mixtures. (3-person mixtures take at least several minutes to be analysed, and complex ones may take over an hour or even overnight.)
- It provides genotype probability distributions, to enable the operator to see whether the most- supported genotypes make sense in terms of the profile.
- It utilises the Balding and Nichols (1994) formulae for the theta subpopulation correction, and the Highest Posterior Density method to give probability intervals.

- Collaborative developmental effort between ESR, New Zealand (John Buckleton, Jo Bright) and Forensic Science South Australia (Duncan Taylor) and overseen by ANZPAA/NIFS
- STRmix has been fully validated and either in press or accepted for publication
  - J.-A. Bright, et al., Developing allelic and stutter peak height models for a continuous method of DNA interpretation, Forensic Sci. Int. Genet. (2013), http://dx.doi.org/10.1016/j.fsigen.2012.11.013

Contextual Bias

- Issue has been around for many years.
- NAS report
  - Fingerprints
  - A 2006 study by a London-based scientist, Itiel E. Dror, asked experts to analyze fingerprints that, unbeknownst to them, they had analyzed earlier in their careers. This time, however, examiners were given biasing statements, such as that a suspect had confessed or that a suspect was locked up at the time of the offense. In 16.6 percent of cases, examiners reversed earlier judgments.
- Could this happen in our DNA labs?
- When is the information about the suspect's profile used in comparison to the crime sample?
- Should the profiles be compared without the knowledge of one being from the suspect?
- Are we sometimes guilty of subconsciously fitting the suspect to the crime profile when there are no obvious exclusions? E.g. drop in, drop out etc.
  - Is this more likely when we are exposed to serious crime and the investigators/prosecutors input?

- Continuing to explore the topic
- Expert seminars have been conducted
  - Itiel Dror conducted two workshops for Australia/NZ senior caseworkers in late 2012
  - Bryan Found is in the process of conducting similar workshops across Australia/NZ and in South Africa
  - Workshops are also being conducted within the individual labs and with stakeholders
  - Workshop conducted at ANZFSS by NFI in September 2012
  - Need to discuss and challenge our processes before serious challenges are forthcoming

When is a DNA profile Identity?

- Likely to be raised in the court room
  - Particularly in adversarial jurisdictions
- Now generate a minimum profile LR of a Septillion (1x 10^{18}) for single source
- Default profile is 1x10^{11} or 100 billion
- Apart from where relatives may be involved is this now considered identity in the eyes of the public?
- Should this now be the subject of a DNA Commission?
- What about Bayes theorem?

Rapid DNA instruments

- GE Healthcare
- IntegenX
- Intrepid
- Largely waiting to see
  - Can these be used for casework?
  - Can they use PP21?
  - Are they designed only for reference samples and use in police stations?
  - What about training for police? Accreditation?
- Further demos likely at ISFG in September.

Environmental monitoring

- In wake of Jama wrongful conviction
  - Contamination prior to lab
- Expanding environmental monitoring and contamination minimisation beyond the lab
- New kits are likely to identify additional peaks due to increased sensitivity
- Are our current cleaning procedures adequate?
  - R&D project

Australian Standards

- New Australian standards
  - DNA consumables AS 5481
- The four core Standards will be:
  - Forensic Analysis Part 1: Recognition, recording, recovery, transport and storage of material (about to be published)
  - Forensic Analysis Part 2: Analysis and examination of material (about to be published)
  - Forensic Analysis Part 3: Interpretation (final stages)
  - Forensic Analysis Part 4: Reporting (in development)
- Adoption is currently under discussion by jurisdictional quality managers who are seeking a consensus approach

melbourne

25th World Congress of the International Society for Forensic Genetics

2 – 6 September 2013

ISFG 2013

Current status

- Number of abstracts
  - As of 15 April 392 abstracts submitted
    - 86 oral presentations
    - 192 poster presentations
    - 114 as either oral or poster
- Total number of authors: 277
- Distribution by country
  - 42 countries
    - 12% Australia
    - 88% International
      - 25 Europe/ middle east; 9 Asian/sub-continent; 6 North, South and central America

- Workshops
  - 10 on offer
  - Currently 70 registrations
- To be reviewed and approved at ISFG board meeting next week
- Full program should be available soon after
- Take advantage of the early bird registration on 2 June
  - Currently 69

R&D

- Formation of the Office of Chief Forensic Scientist
  - Responsible for VPFSD R&D program
  - Currently 28 main projects underway for 2013
    - 2 proposed
    - Topics broadly include
      - DNA transfer
      - Y-STRs
      - mRNA
      - Population studies
      - SNPs
      - DNA contamination and cleaning procedures
      - Predictive DNA

melbourne

25th World Congress of the International Society for Forensic Genetics

2 – 6 September 2013

ISFG 2013

The European Forensic Genetics Network of Excellence – advancing research, training and cooperation across Europe

Peter M. Schneider

Institute of Legal Medicine

University of Cologne (Germany)

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Work packages

- **WP1**
  - Project Management: Coordination and communication office
- **WP2**
  - Integrating research and networking: towards the creation of an European Virtual Center of Research in Forensic Genetics
- **WP3**
  - Exemplar reserach projects
- **WP4**
  - Ethical and legal aspects, and the societal dimension of forensic genetics
- **WP5**
  - Education, Training and Career Development

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WP2 – Integrating research and networking

- **Task 1: To establish a directory of forensic genetic research institutions across Europe**
  - **Objective:** Identification of the key members of the scientific community actively carrying out research in forensic genetics across the Europe
  - **Important developments:**
    - 06-08/12: Establishment of National Contact Points (NCPs)
    - 09/12: NCP meeting
    - 09/12: Questionnaire preparation
    - 10-12/12: Questionnaire distribution (NCPs, partners, known labs, ENFSI / EDNAP / GEDNAP mailing lists)
    - 01/13: First results

Questionnaire results: Geography

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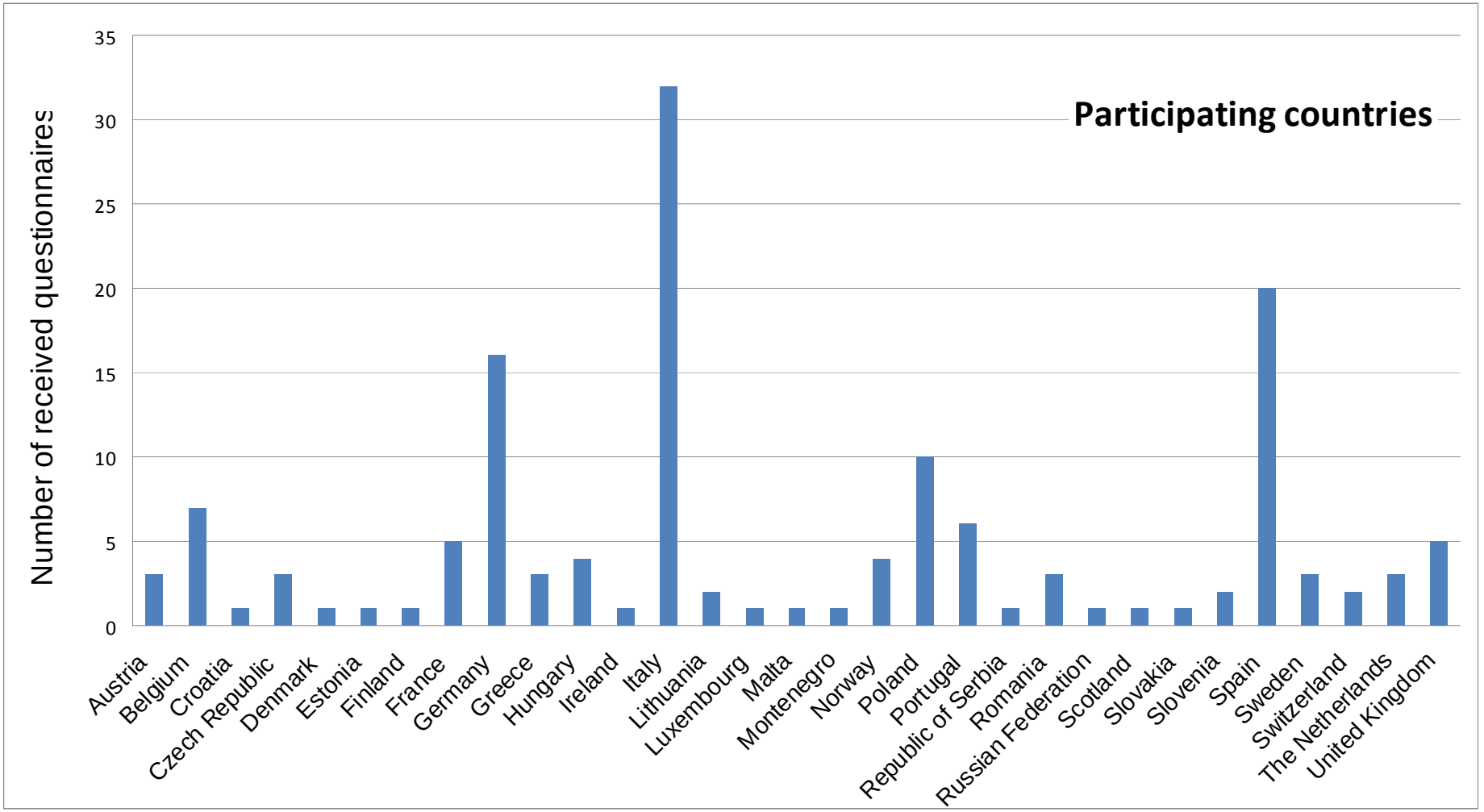
Results: 145 European DNA Laboratories



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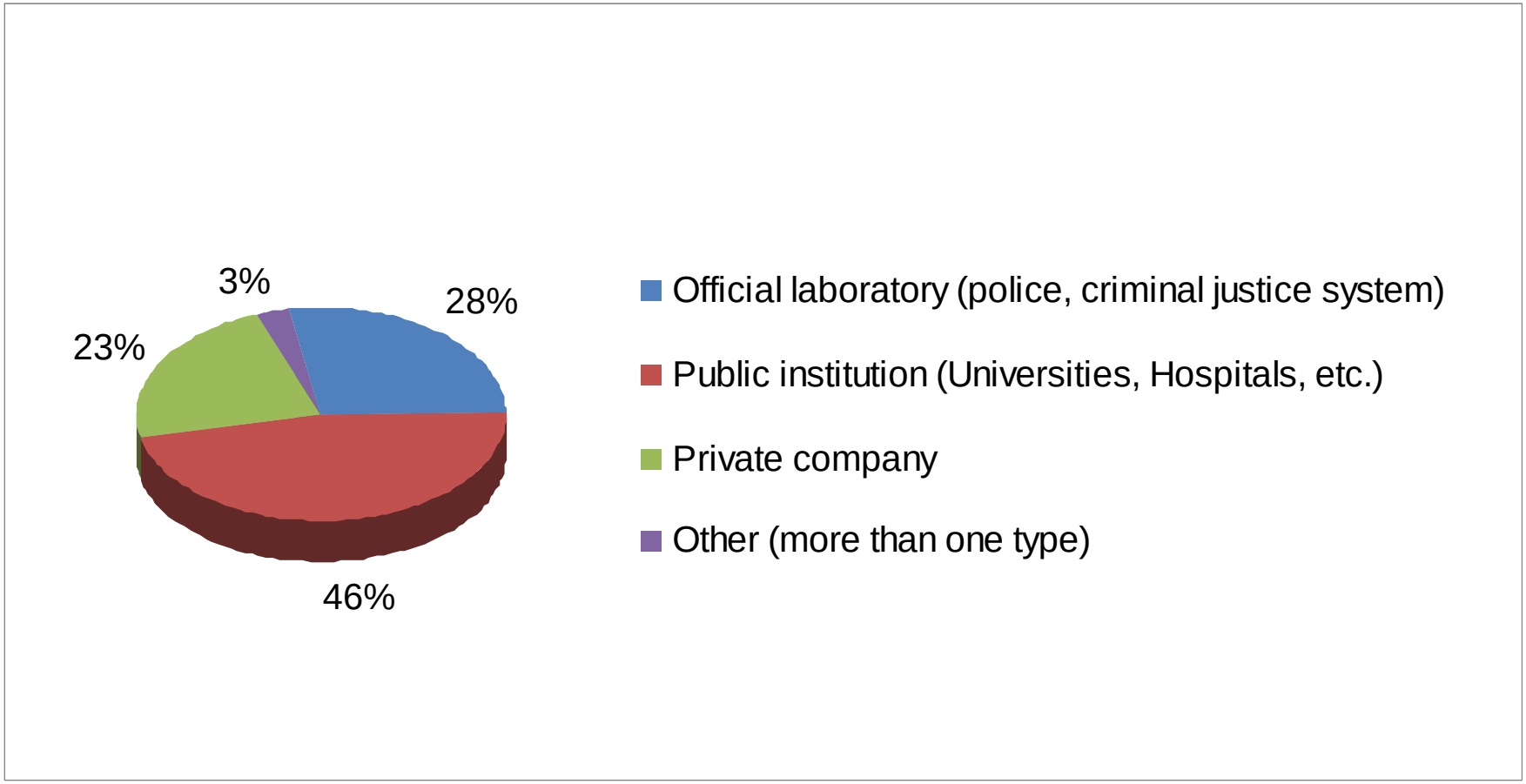
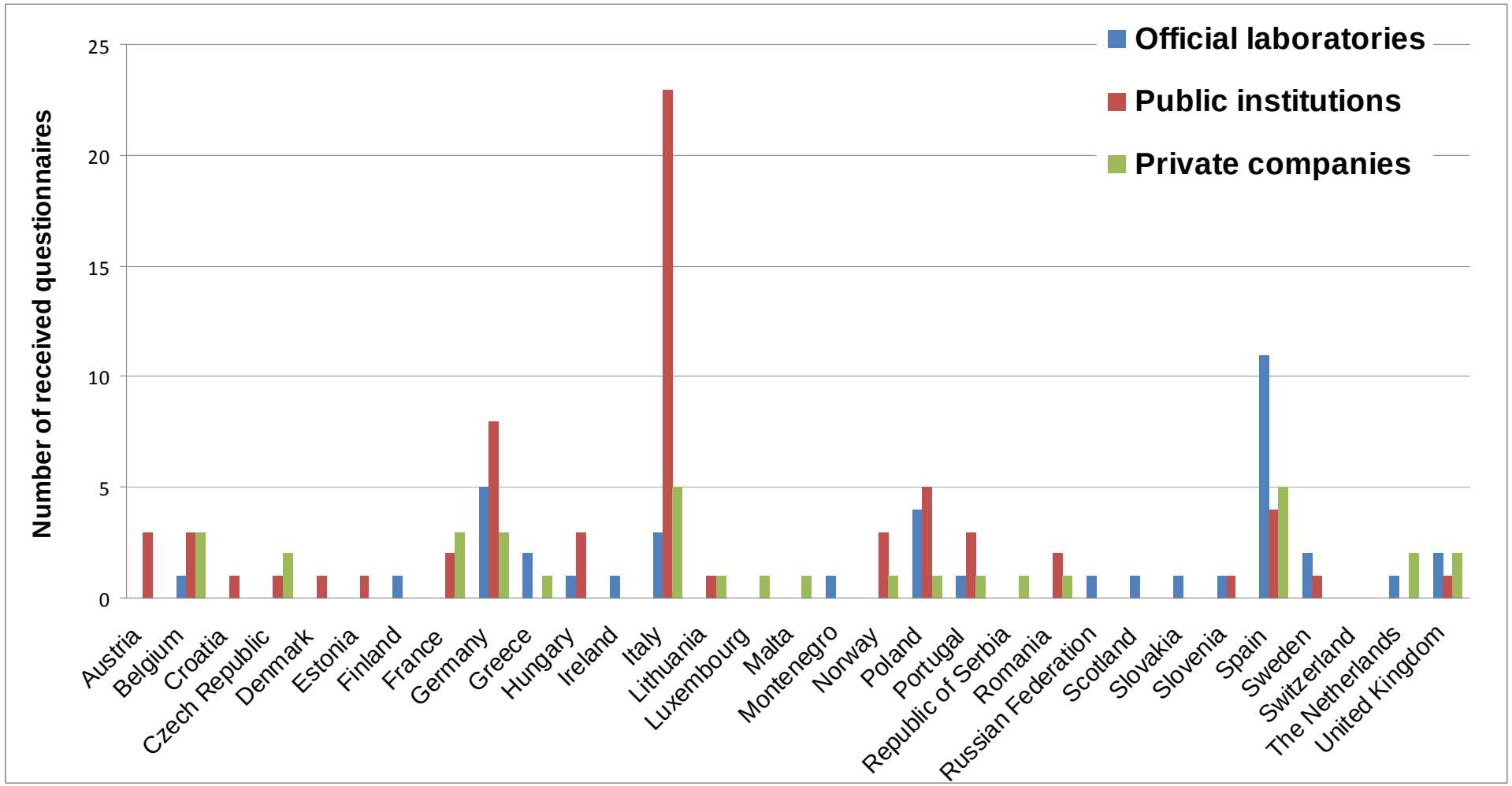
Laboratories grouped by Sector



Type of Laboratory by Country



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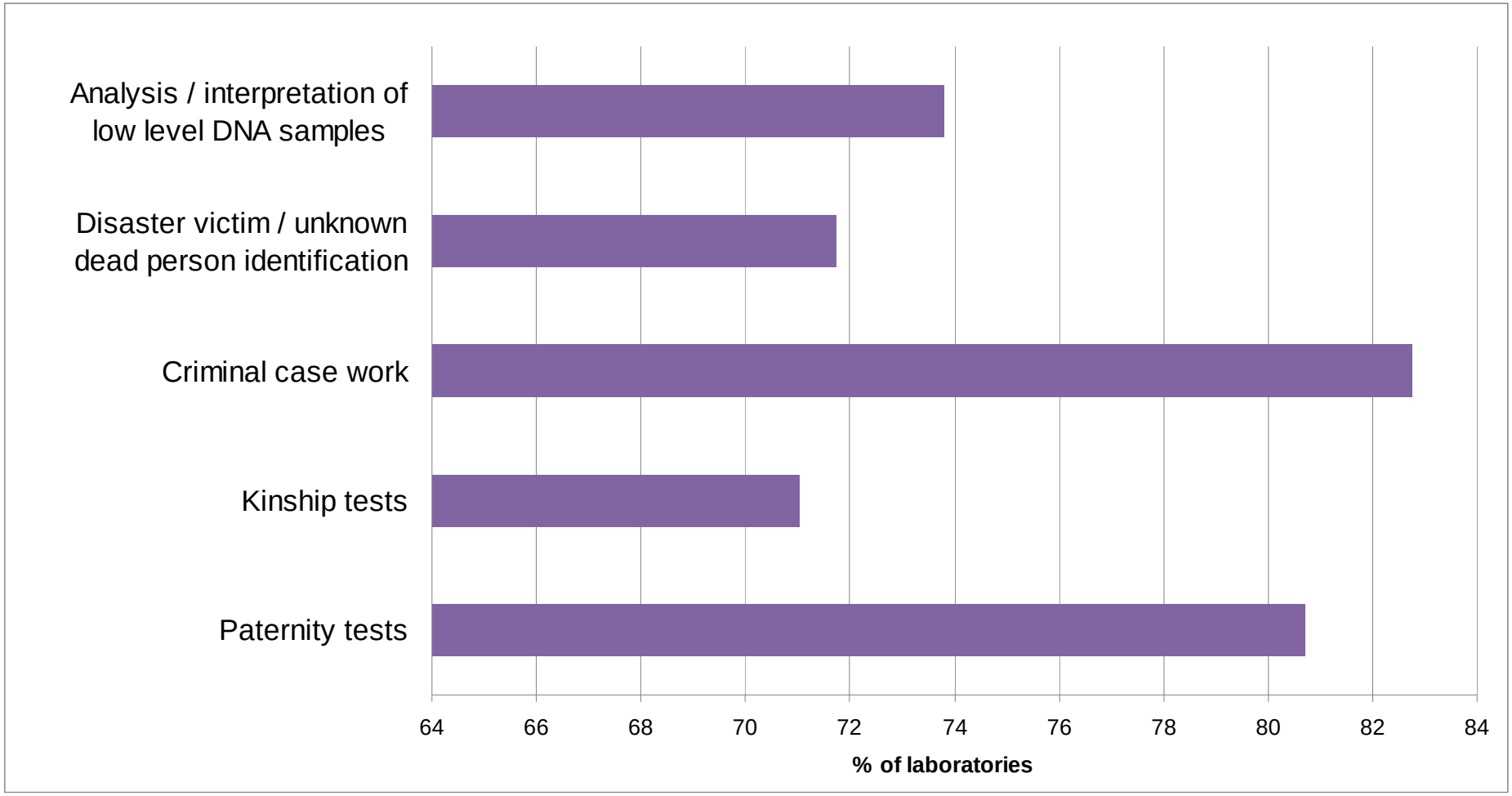
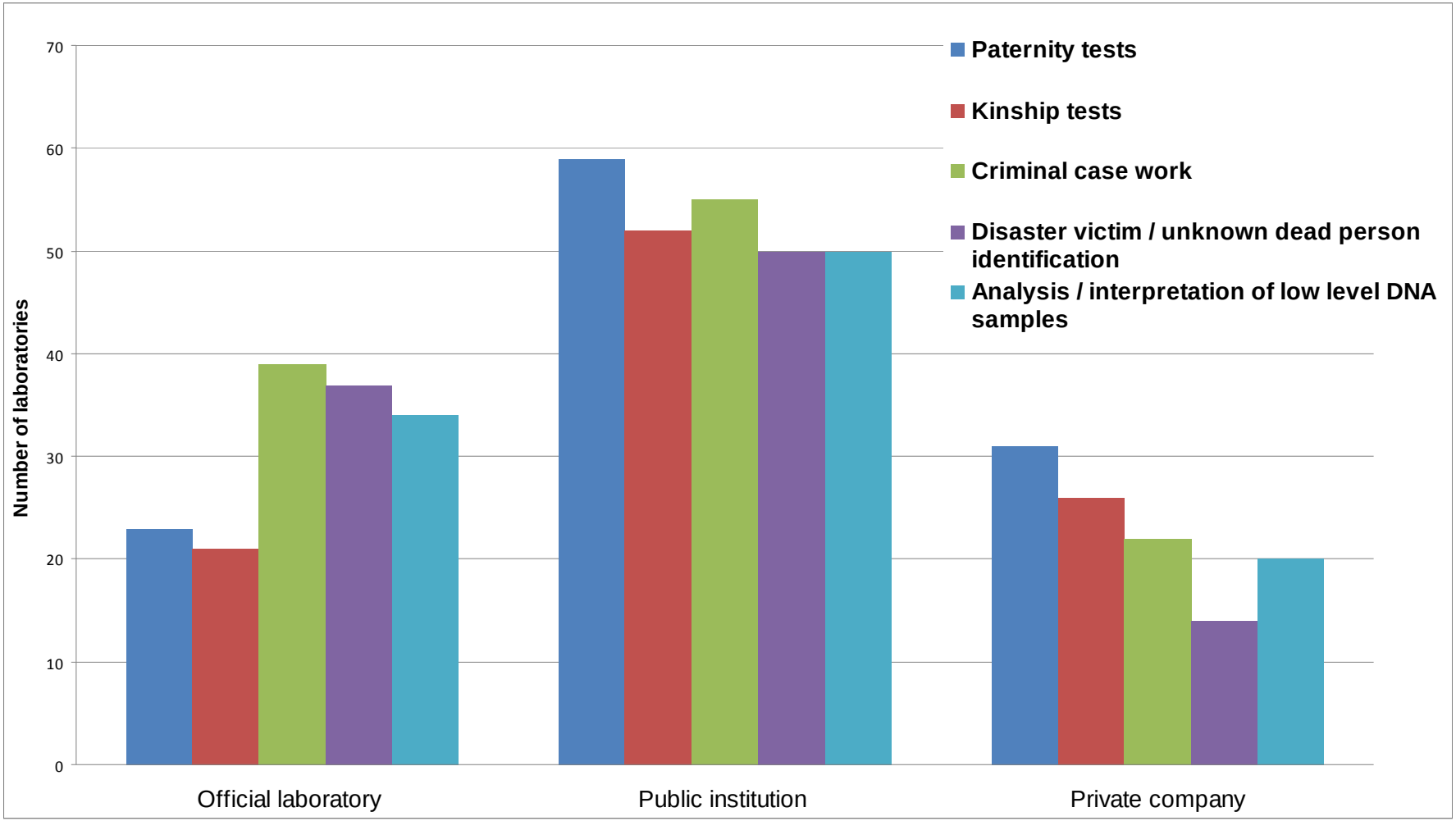
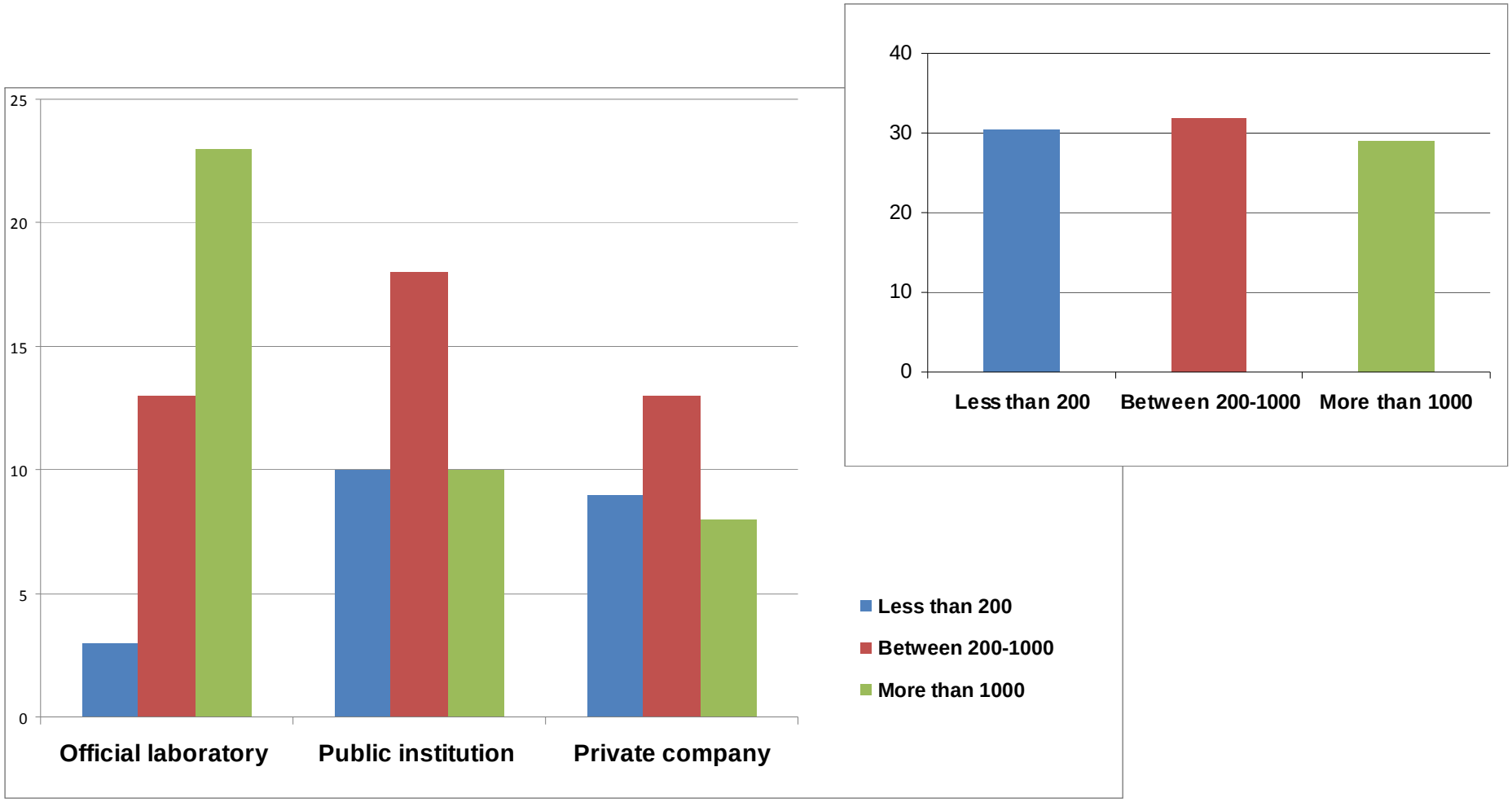
Practical casework



Laboratories and Types of Analyses



Number of Cases / Lab



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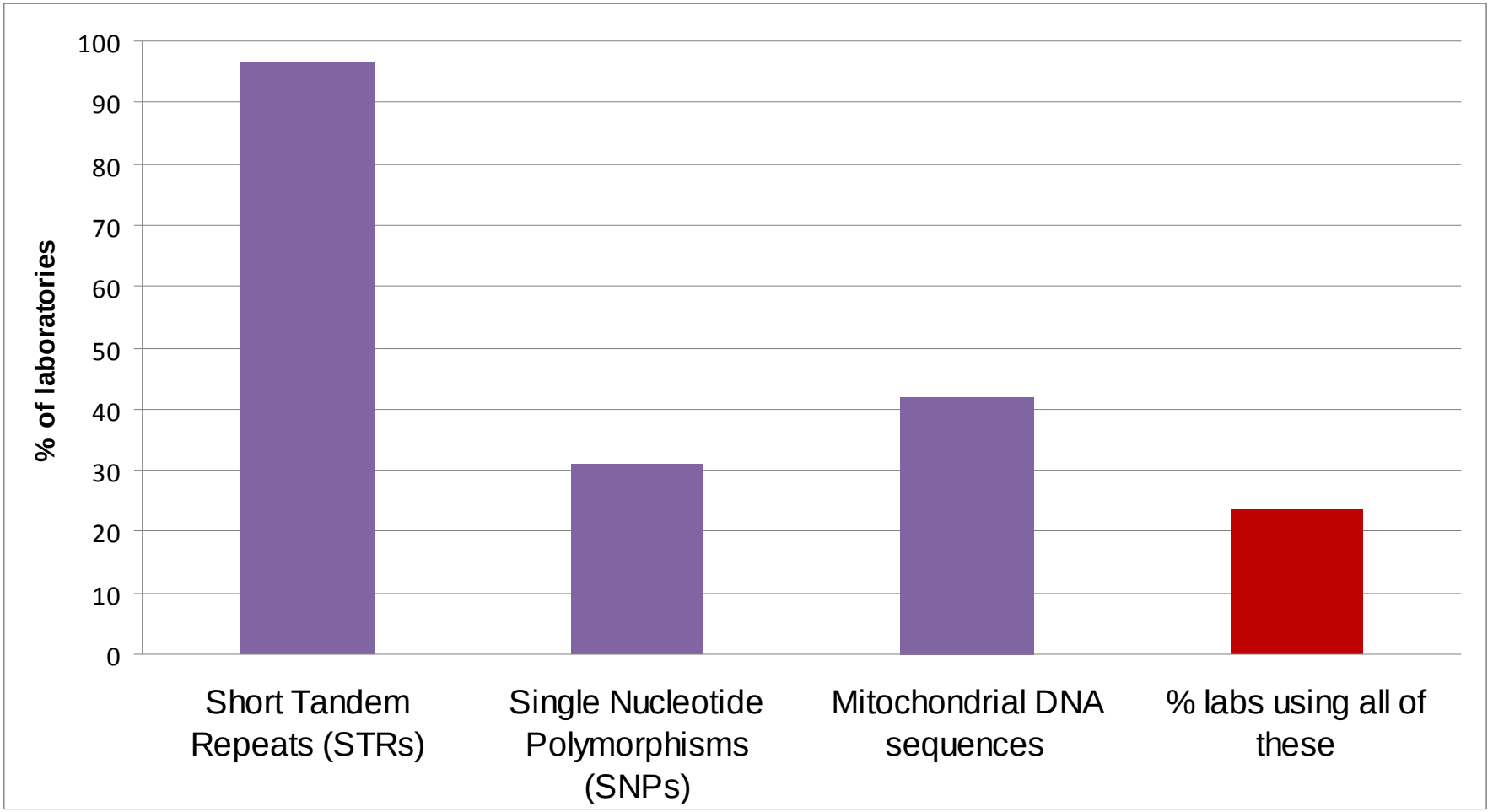
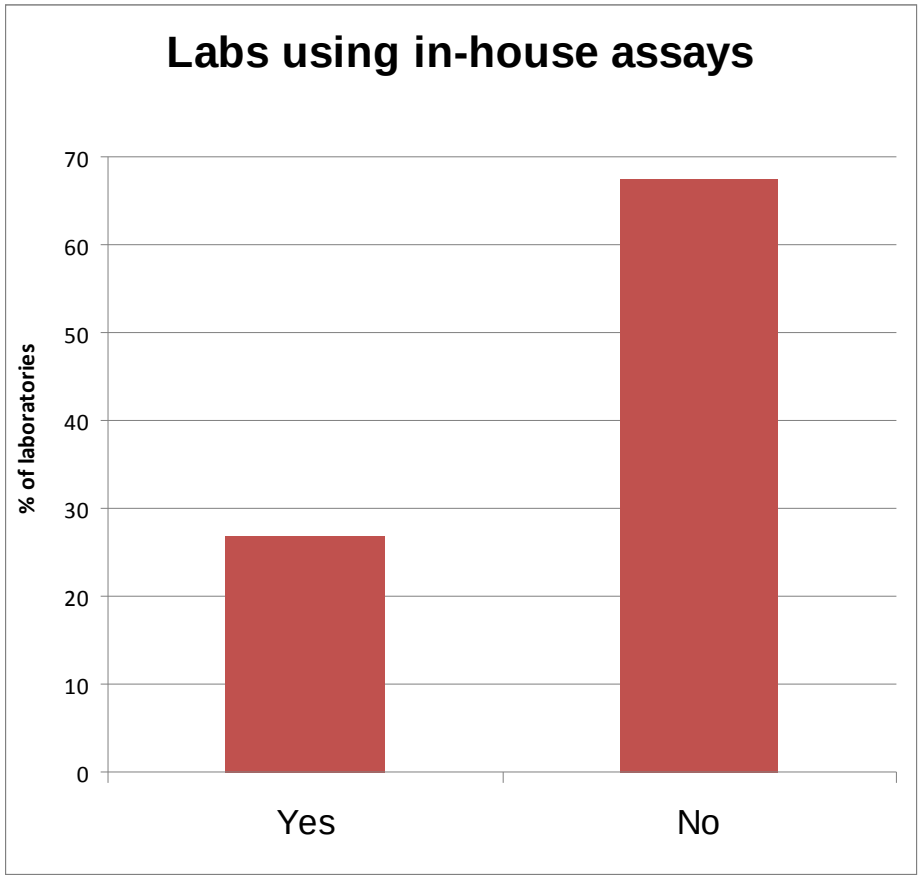
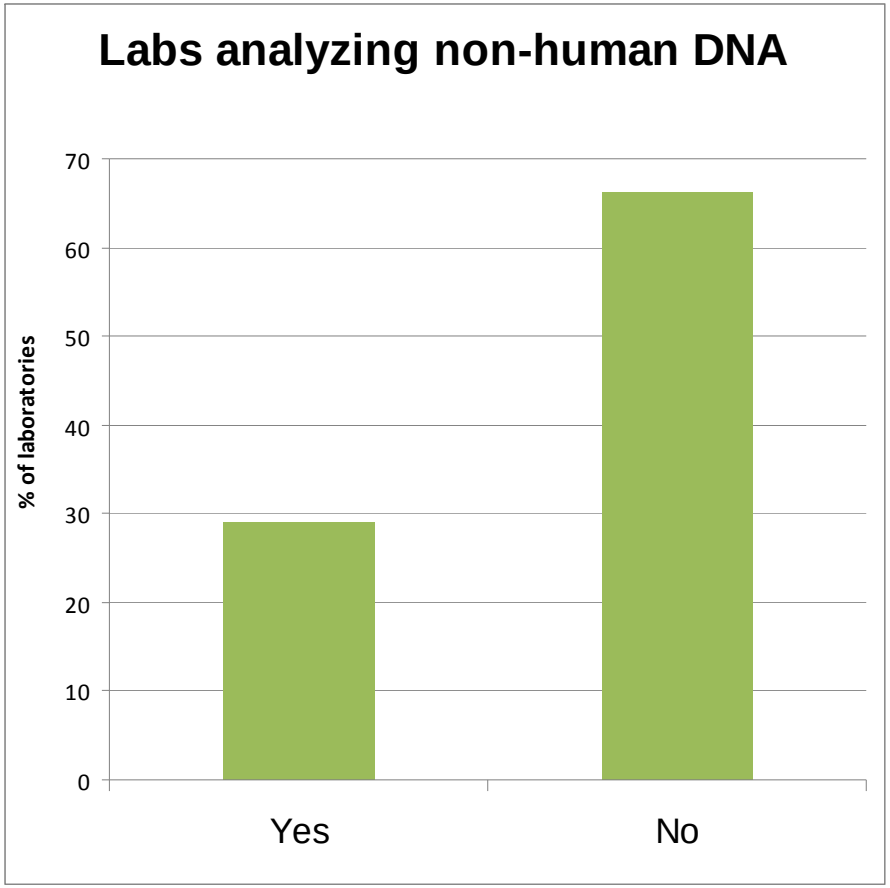
DNA Marker Types used



Other Assays





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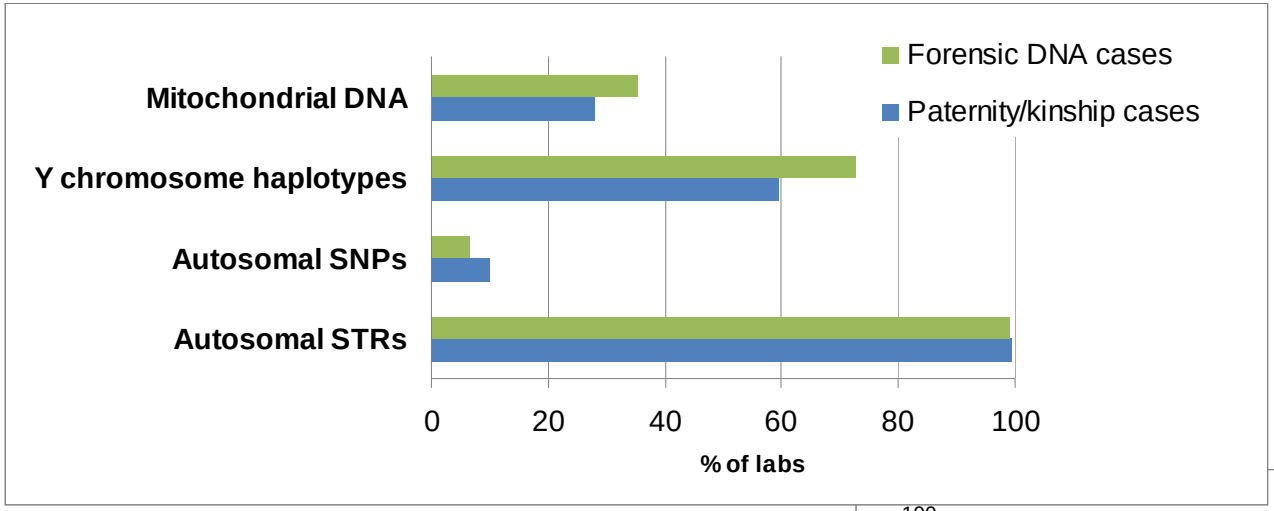
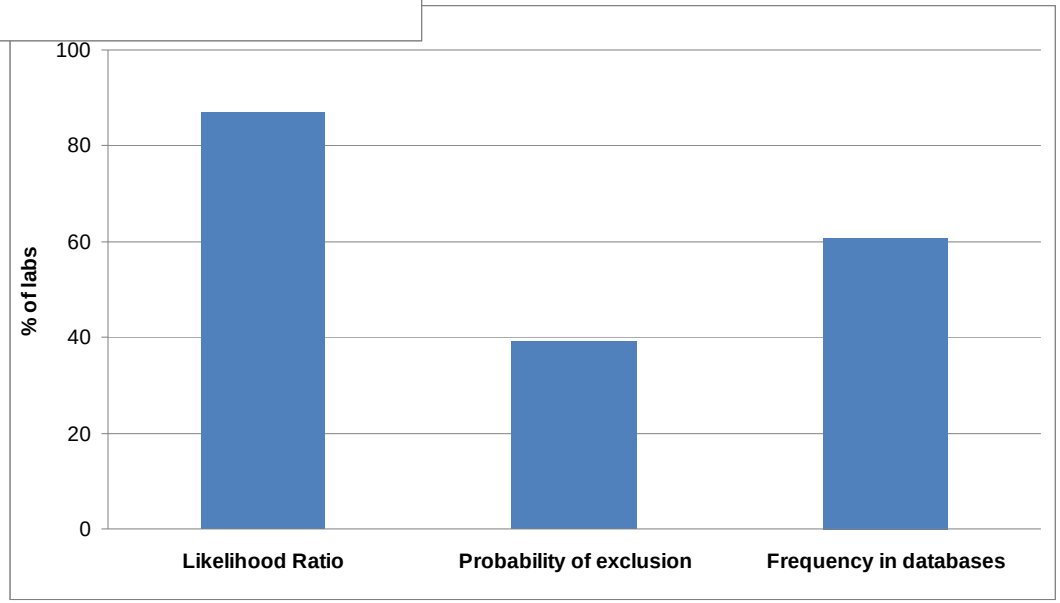
Interpretation Methods





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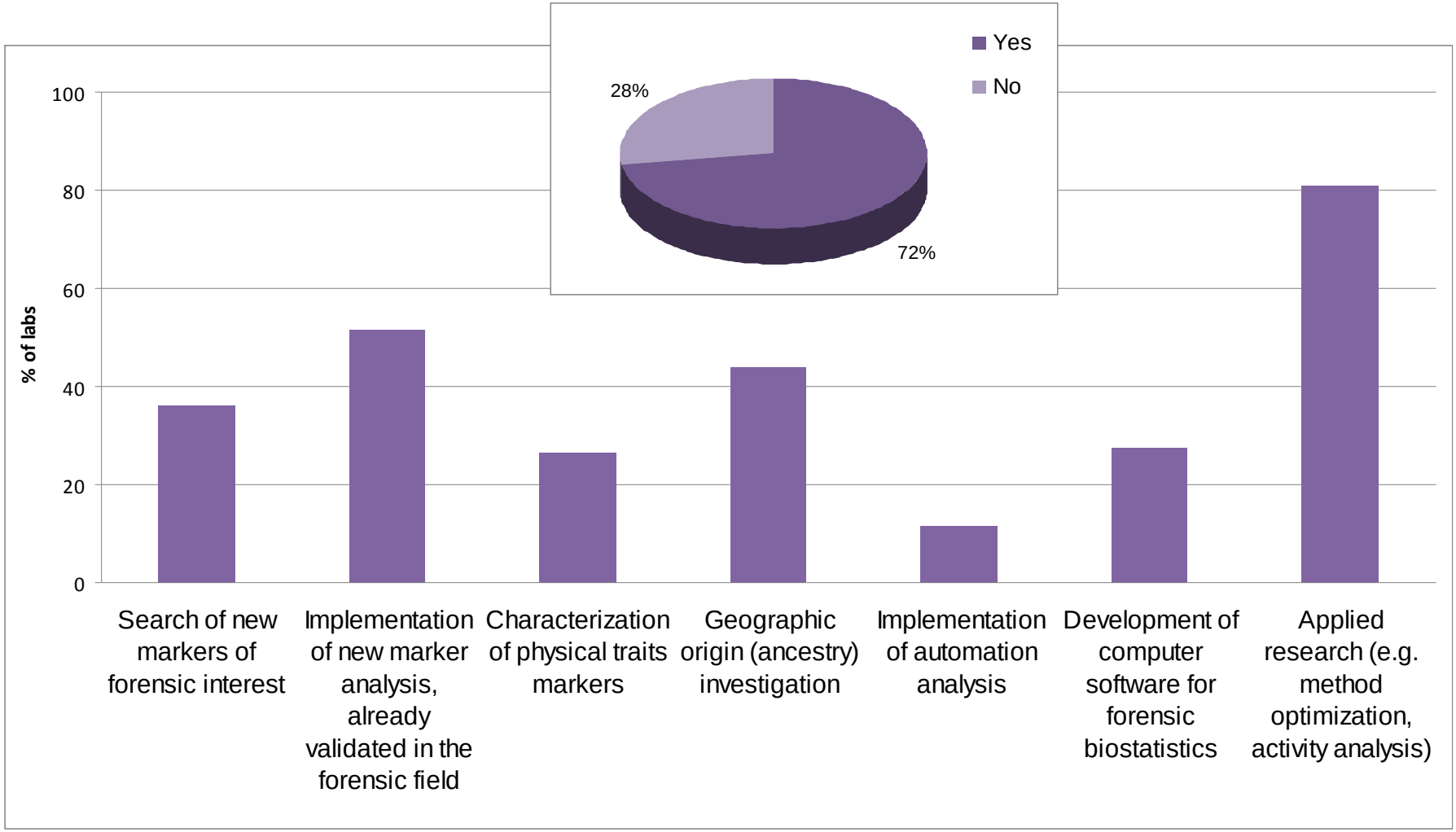
Research Activities



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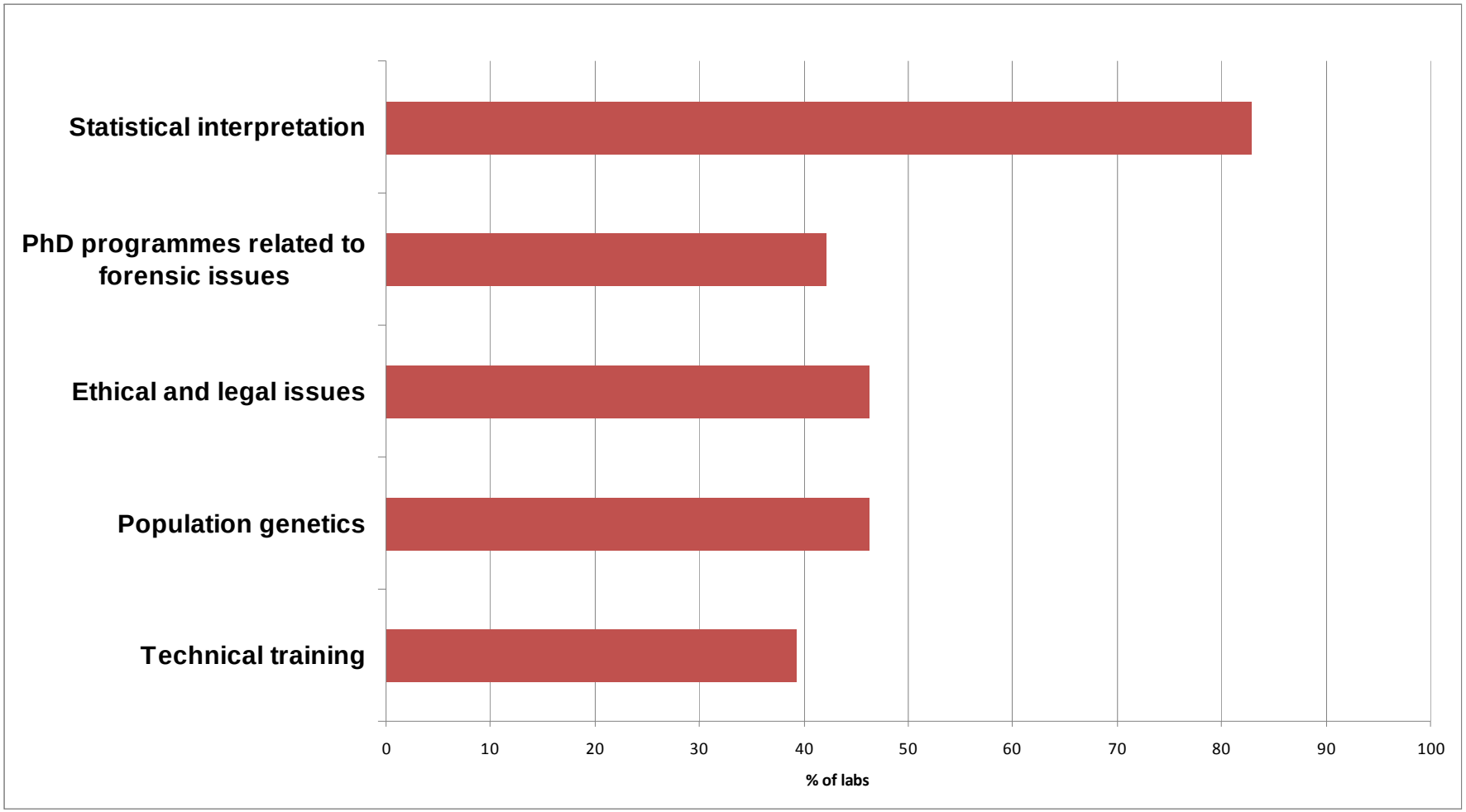
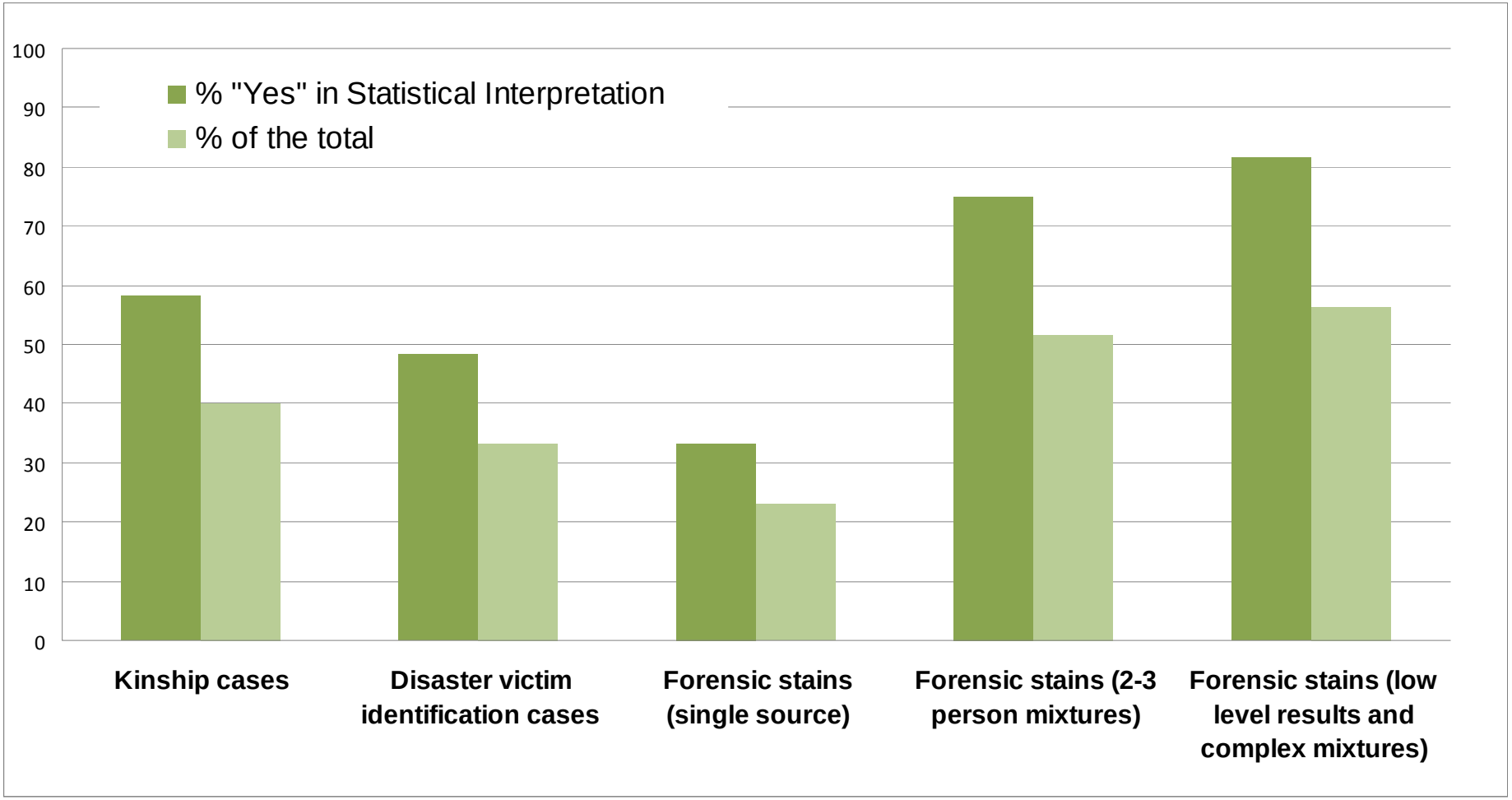
Educational Needs



Specific Training for Statistical Interpretation



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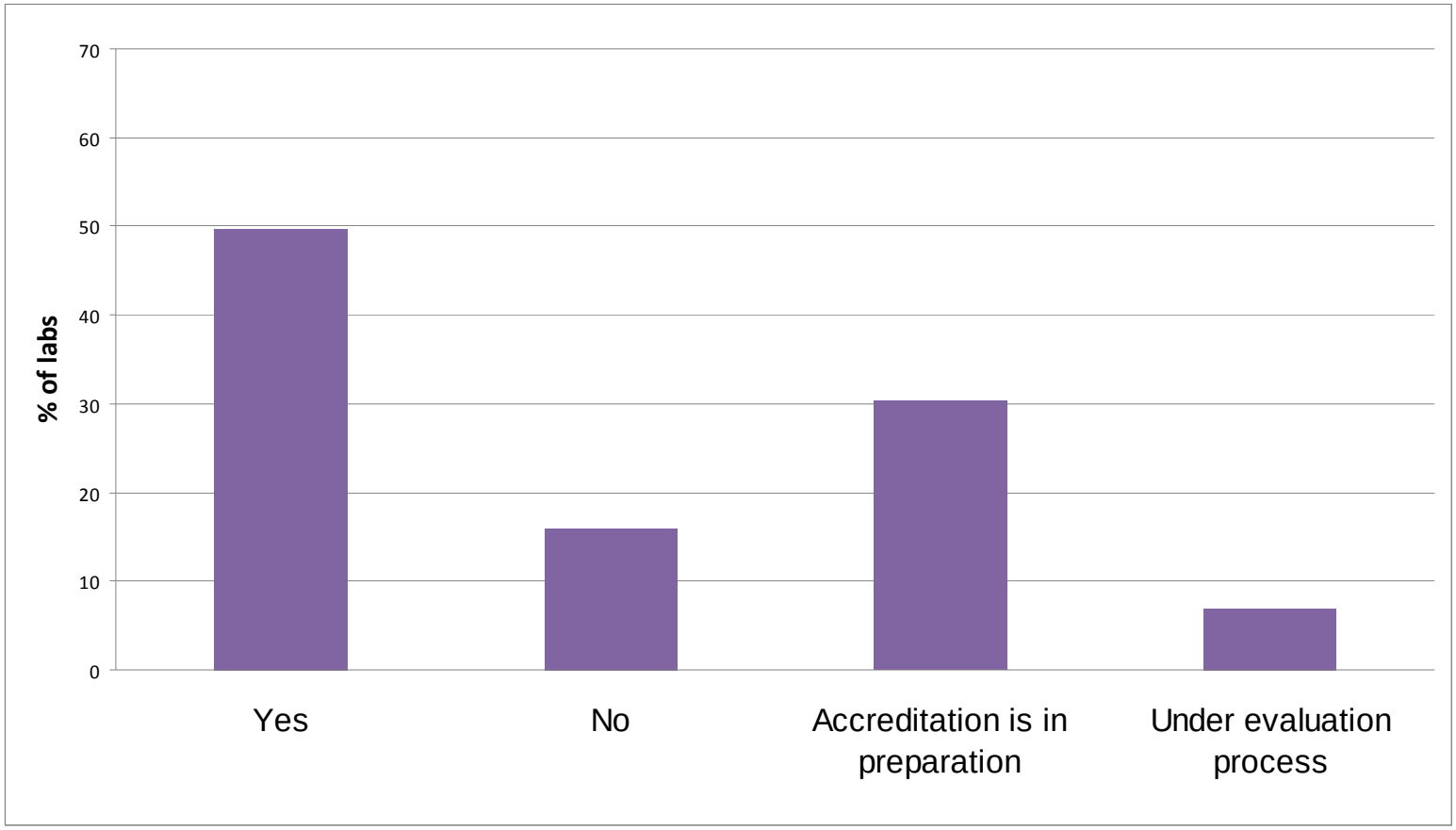
Accreditation Status



Questionnaire: further activities

- **More data are being collected**
- **All European forensic DNA laboratories are invited to participate**
- **Participants will be displayed on map and with address**
- **Data will be published online, as well as in a summary publication:**

  "Directory of Forensic Genetic Research Laboratories in Europe"

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http://www.euroforgen.eu/eu

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Network of Excellence

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SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE - The research leading to these results receives funding from the Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 285487.

Introduction

Dear Colleague,

Thank you for your willingness to complete the EUROFORGEN-NoE questionnaire!

You have heard or read about the project and you know about our aim to build a European-wide network structure for forensic genetic research.

To come closer to this aim we are looking forward to your feedback. It only takes about 10 minutes to complete and at the end of the questionnaire there is some open space for further comments. Please feel free to elaborate on your answers, for instance if you find some of the questions hard to answer briefly.

Please provide us with your contact details to display the different correspondents on the consortium website and to enable us to inform you about further network initiatives. By filling in the questionnaire you have agreed to the use of the data for EUROFORGEN-NoE internal research purposes.

When submitting the questionnaire, you will receive an automatically generated e-mail, containing the questions and your answers.

In case you have any remarks or questions, please **contact** us. Thank you in advance for your collaboration and all the best:

Angel Carracedo and Peter Schneider

→ **To the questionnaire**

Quicklinks

→ The European scene in forensic genetics

Geographical display and contact data

→ workpackages

Consortium

EUROFORGEN: Next activities

- **CEPOL course Madrid, June 4-7, 2013**
  - "Mixtures, complex DNA profiles, and familial testing: interpretation workshop schedule"
  - Open for members of police laboratories
  - www.cepol.europa.eu
- **25th ISFG World Congress, Melbourne, Sept. 2-7, 2013**
  - Support of pre-congress educational workshops
  - www.isfg2013.org
- **Kick-off workshop "Train the Trainers"**
  - Copenhagen, Oct. 2013
  - For colleagues willing to act as trainers at national level
  - Workshop series on different topics

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