EUROPEAN DNA PROFILING GROUP (EDNAP) MEETING

Athens, Greece

20 November 2013

Host: Maria Vouropoulou.
Chairman: Niels Morling.

A list of participants is attached.

Welcome
Director Penelope Manniattis, PhD, welcomed members to Athens.

Presentations of the organising laboratory

Forensic genetics in Greece (pdf attached)	Penelope Miniati
Maria's Case - A DNA Analysis With Global Impact (pdf)	Aristea Metheniti
Preparing Bones for DNA Analysis - A Home Made System (pdf)	Maria Vouropoulou

Update on exercises

mRNA Cordula Haas

The results of mRNA exercises 4 and 5 was recently published in FSIGEN 2014; 8: 203–12.
Cordula Haas presented the results of the mRNA exercise 6 on the identification of skin (pdf). Briefly, results were received from 20 laboratories that tested 8 mock casework samples and dilutions of skin mRNA with 8 skin specific mRNA markers. Cordula Haas will prepare a draft of a manuscript of the results.
Cordula Haas will explore the possibility of testing RNA quantification systems and will present a plan at the next EDNAP meeting for a collaborative exercise.

The IrisPlex exercise on genetic prediction of eye colour Niels Morling

Manfred Kayser was unable to participate. Attached please find the highlights of the results (pdf). A manuscript will be circulated very soon.

Updates from other groups

EMPOP Walther Parson

Walther Parson gave an update on the developments in EMPOP. Since the last meeting, three EMPOP/mtDNA related papers were published in Forensic Science International Genetics. EMPOP developments were presented at international conferences. EMPOP trainings took place in San Sebastian, Spain and Rio de Janeiro, Brazil. The SWGDAM group (USA) invited WP to discuss mtDNA alignment and quality control of mtDNA haplotypes. Updated SWGDAM guidelines on mtDNA analysis were published in July 2013 including EMPOP alignment principles. Walther Parson demonstrated a new concept for estimating mtDNA haplogroups using dedicated software (EMMA). EMPOP releases 10 and 11 have been issued including 34,617 haplotypes (pdf).

Interpol Ingo Bastisch

Ingo Bastich gave a short summary of the DNA activities of Interpol (pdf).

NIST Niels Morling

The new leader of NIST, Peter Vallone, sent his best wishes and a pdf with information on the latest NIST activities (pdf).

Euroforgen - NoE Peter Schneider

Peter Schneider gave a general update concerning the EUROFORGEN Network of Excellence project (pdf).

Euroforgen – NoE mRNA collaborative exercise Peter Schneider

The European Forensic Genetics Network of Excellence (EUROFORGEN-NoE) carried out a collaborative exercise on mRNA profiling and RNA as well as combined RNA/DNA data interpretation to assess the usefulness of mRNA markers for forensic stain type inference. The nine participating laboratories used a 20-marker multiplex that identifies blood, saliva, semen, vaginal mucosa, menstrual secretion and skin. In the exercise, specimens of increasing complexity were assessed that ranged from the analysis of reference PCR products, cDNAs of various amounts of single source RNAs with indicated body fluid/tissue type, single source and mixed cDNAs with unspecified composition to challenging mock casework stains. A scoring system was tested for reliable inference of cell types in a specimen. This scoring system builds on fourfold replicate RNA analyses and calculating a ratio based the number of observed and possible peaks for all markers of a given body fluid/tissue type (Lindenbergh et al., Forensic Sci. Int. Genet. 7; 2012; 159-166). The exercise results support the usefulness of this scoring system. When interpreting the results for the stains, participating laboratories were asked to integrate the DNA and RNA results and associate donor and cell type when possible. A large variation for the integrated interpretations of the DNA and RNA data was obtained including correct interpretations. The overall results indicate that clear guidelines for data interpretation and awareness regarding potential pitfalls in associating donors and body fluids/tissues are needed for the implementation in forensic casework.

EDNAP web site update Peter Schneider

Members are encouraged to visit the website (www.isfg.org/EDNAP).

Future activities Niels Morling

Chris Phillips offered to organise a collaborative exercise on ancestry informative markers (AIMs). The method is based on (1) SNaPshot, single base extension and capillary electrophoresis (CE) of 34 SNPs and (2) PCR and CE of 46 insertion-deletion (Indels) polymorphisms. Critical reagents will be distributed from Santiago de Compostela in January 2014, most likely the second week. The reagents will include DNA extracts acting as typical samples from five continental regions plus a mixed-source DNA sample prepared by KCL, London, comprising two different donors with different ancestries. Laboratory protocols and a file of 80 reference genotypes for the standard 9947a control DNA will be provided.
Participants are asked to assign ancestry to the five ethnic samples using the Snipper online classifier and to identify the ancestry of the contributors of the mixed DNA sample.
The results are expected to be submitted to Santiago de Compostela no later than 22 March 2014 so that we can discuss the results at the next EDNAP meeting (cf. below).

Nomenclature of DNA sequences of STRs Niels Morling

The challenge concerning nomenclature of STRs with precise DNA sequence information was discussed.

Any other business Niels Morling

There was no other business.

Next meeting Niels Morling

The next EDNAP meeting will take place on 22 April 2014 in Tibilisi, Georgia in connection with CODIS and ENFSI meetings.

Closing of the meeting Niels Morling

The meeting closed with the sincere thanks to Penelope Miniati, Maria Vouropoulou and their colleagues at the laboratory in Athens.

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

- List of participants
- Presentations
  - Penelope Miniati: Forensic genetics in Greece (pdf)
  - Aristea Metheniti: Maria's Case - A DNA Analysis With Global Impact (pdf)
  - Maria Vouropoulou: Preparing Bones for DNA Analysis - A Home Made System (pdf)
  - Cordula Haas: Results of the skin cell mRNA exercise (pdf)
  - Manfred Kayser: Presentation of IrisPlex results (pdf)
  - Walther Parson: EMPOP report (pdf)
  - Peter Vallone: NIST report (pdf)
  - Peter Schneider: EUROFORGEN-NoE report (pdf).

Prof. Dr. Walther Parson
Institute of Legal Medicine
Innsbruck Medical University
Müllerstrasse 44
A-6020 Innsbruck
Austria
Tel: +43 512 9003 70640
Fax: +43 512 9003 73640
E-mail: walther.parson@i-med.ac.at

Dr. Fabrice Noël
National Institute of Forensic Science
98-100 Chaussée de Vilvorde
B-1120 Bruxelles
Belgium
Tel: +32 2243 4604
Fax: +32 2240 0501
E-mail: fabrice.noel@just.fgov.be

Dr. Helle Smidt Mogensen
Section of Forensic Genetics
Department of Forensic Medicine
Faculty of Health Sciences
University of Copenhagen
Frederik V's Vej 11
DK-2100 Copenhagen
Denmark
Tel: +45 3532 6212
Fax: +45 3532 6270
E-mail: helle.smidt@sund.ku.dk

Professor, dr.med. Niels Morling
Section of Forensic Genetics
Department of Forensic Medicine
Faculty of Health Sciences
University of Copenhagen
Frederik V´s Vej 11
DK-2100 Copenhagen
Denmark
Tel: +45 3532 6115
Fax: +45 3532 6270
E-mail: niels.morling@sund.ku.dk

Dr. Auli Bengs
Department of Biology
Forensic Laboratory
National Bureau of Investigation
Jokiniemenkuja 4, PO BOX 285
FIN-01310 Vantaa
Finland
Tel: +358 71878 6377
Fax: +358 71878 6303
E-mail: auli.bengs@poliisi.fi

Dr. Regine Banemann
KT31
Bundeskriminalamt
Thaerstrasse 11
D-65193 Wiesbaden
Germany
Tel: +49 61155 16053
Fax: +49 611 5545 089
E-mail: regine.banemann@bka.bund.de

Dr. Ingo Bastisch
KT31
Bundeskriminalamt
Thaerstrasse 11
D-65193 Wiesbaden
Germany
Tel: +49 61155 16030
Fax: +49 611 5545 089
E-mail: ingo.bastisch@bka.bund.de

Prof.Dr. Peter M. Schneider
Institute of Legal Medicine
University of Cologne
Melatenguertel 60-62
D-50823 Cologne
Germany
Tel: +49 221 4788 8345
Fax: +49 221 4788 8370
E-mail: peter.schneider@uk-koeln.de

Dr. Penelope Miniati
Dept. Biological Material Analysis
Division of Criminology
Hellenic Police
Antigonis 2-6 & L.Anthinon
GR-104 42 Athens
Greece
Tel: +30 210 748 6791
Fax: +30 210 748 6791
E-mail: dna@astynomia.gr

Dr. Ioulia Skitsa
Athens Legal Medicine Department
10 Anapajseos Street

11636 Athens
Greece
Tel: +302109219909
Fax:
E-mail: ninaskit@otenet.gr

Dr. Maria Vouropoulou
Dept. Biological Material Analysis
Division of Criminology
Hellenic Police
Antigonis 2-6 & L.Anthinon
GR-104 42 Athens
Greece
Tel: +30 210 510 3407
Fax: +30 210 510 3408
E-mail: dna@astynomia.gr

Dr. Maureen Smyth
Forensic Science Laboratory
Garda Siochana
Phoenix Park
Dublin 8
Ireland
Tel: +353 1666 2905
Fax: +353 1666 2929
E-mail: msmyth@fsl.gov.ie

Dr. Francesca Brisighelli
Instituto di Medicina legale e delle
Assicurazconi
Universita Cattolica
Largo Francesco Vito 1
I-00168 Roma
Italy
Tel: +39 6 3550 7031
Fax: +39 6 3550 7033
E-mail: francesca.brisighelli@rm.unicatt.it

Dr. Maria João Anjos Porto
Forensic Genetic Service
Instituto de Medicina Legal
University of Coimbra
Largo da Sé Nova
P-3000-213 Coimbra
Portugal
Tel: +351 239 854230
Fax: +351 239 826132
E-mail: mariajoao.porto@dcinml.mj.pt

Dr. Livia Zatkalikova
Institute of Forensic Science
Slovenská L'upca
Priboj 560
976 13
Slovak Republic
Tel: +421 961 60 6333
Fax: +421 961 60 6309
E-mail: livia.zatkalikova@minv.sk

Dr. Chris Phillips
Forensic Genetic Unit
Department of Legal Medicine
University of Santiago de Compostela
San Francisco, s/n
E-15705 Santiago de Compostela
Spain
Tel: +34 98158 2327
Fax: +34 98158 0336
E-mail: c.phillips@mac.com

Dr. Ricky Ansell
National Laboratory of Forensic Science
S-58194 Linköping
Sweden
Tel: +46 1056 28119
Fax: +46 1014 5715
E-mail: ricky.ansell@skl.polisen.se

Dr. Cordula Haas
Institut für Rechtsmedizin Zurich
Winterthurerstr. 190
CH-8057 Zurich
Switzerland
Tel: +41 44 635 5656
Fax: +41 44 635 6858
E-mail: cordula.haas@irm.uzh.ch

Dr. Denise Syndercombe Court
Academic Haematology
Blizard Institute of Cell and Molecular Sciences
Barts and The London
4 Newark Street, Whitechapel
E1 2AT London
UK
Tel: +44 20 78822276
Fax: +44 20 7882 2182
E-mail: Denise.syndercombe-court@kcl.ac.uk

HELLENIC POLICE
FORENSIC SCIENCE DIVISION

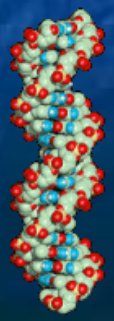
Subdivision of Biological & Biochemical Examination & Analysis - DNA

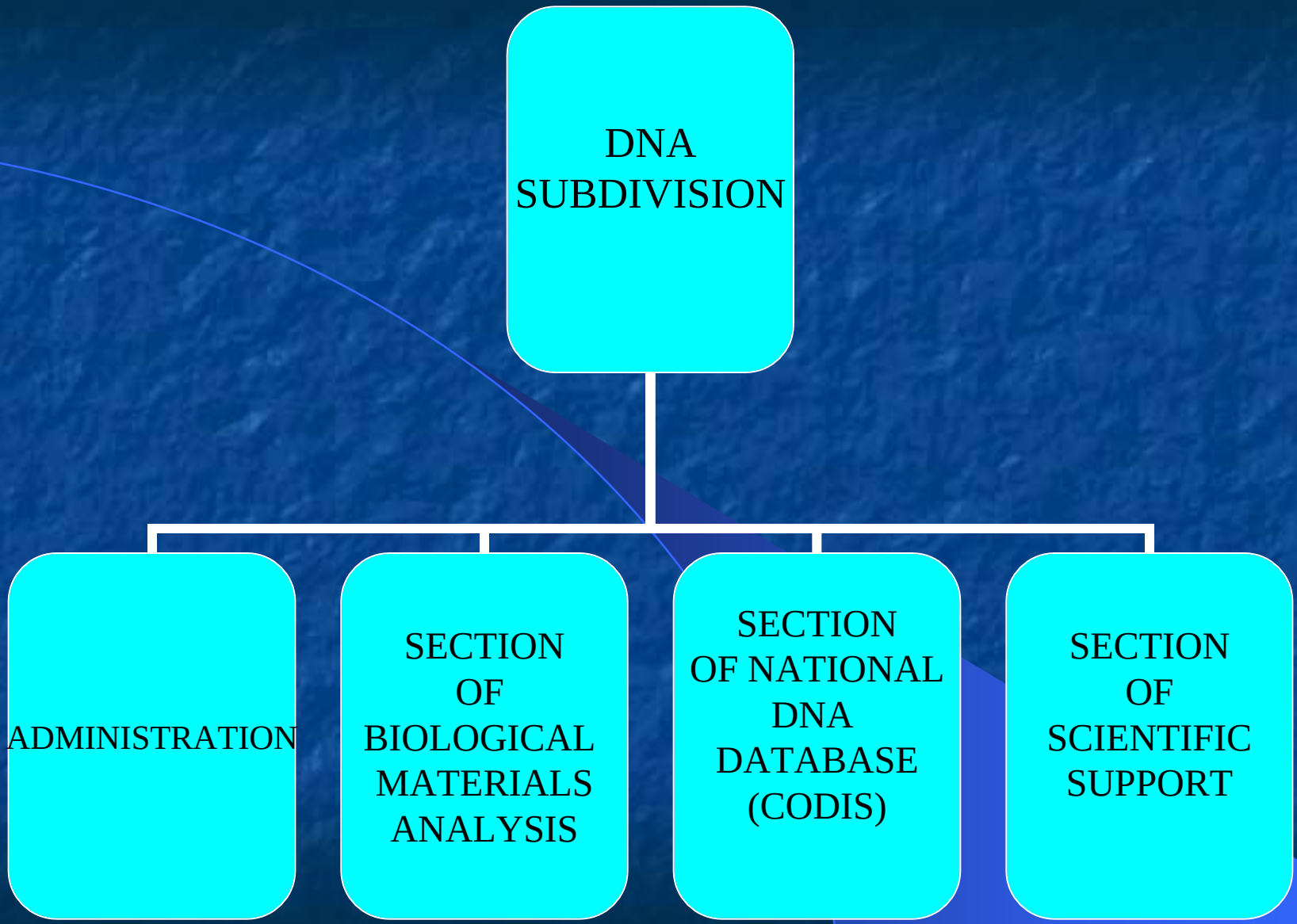
Dr. Penelope MINIATI, Ph.D.


ΕΙΣΟΔΟΣ ΠΡΟΣΩΠΙΚΟΥ
ΕΙΣΟΔΟΣ ΕΠΙΣΚΕΠΤΩΝ
ΑΠΑΓΟΡΕΥΕΤΑΙ Η ΕΙΣΟΔΟΣ
ΑΡΧΗΓΕΙΟ
ΕΛΛΗΝΙΚΗΣ ΑΣΤΥΝΟΜΙΑΣ
ΔΙΕΥΘΥΝΣΗ
ΕΓΚΛΗΜΑΤΟΛΟΓΙΚΩΝ
ΕΡΕΥΝΩΝ

Subdivision of Biological & Biochemical Examination & Analysis

- The First DNA Laboratory established in Greece in1994.(STRIDE)
- 45 Police Officers – Scientists serve in the Subdivision.
- Among them specialized scientists in Biology and Biochemistry and also well trained specialized technicians.
- Up to date more than 15.000 forensic cases have been undertaken.
- More than 120.000 forensic samples have been examined.
- The capacity of the Lab is appr. 100 samples/day




DNA
SUBDIVISION
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SECTION
OF
BIOLOGICAL
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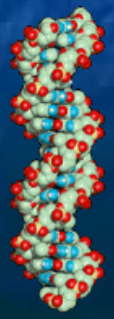
ANALYSIS
SECTION
OF NATIONAL
DNA
DATABASE
(CODIS)
SECTION
OF
SCIENTIFIC
SUPPORT

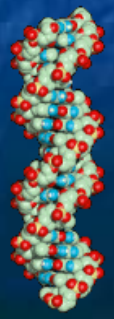
DNA extraction



Quantification /PCR

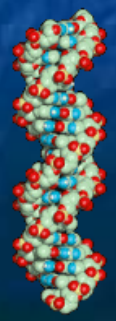
Subdivision's Activities:

- Crime scene investigation and sampling of appropriate biological substances.
- Examination of samples
- Databasing - CODIS
- Consulting - from Police Officers to Judges, from Politicians to the Law makers e.t.c.

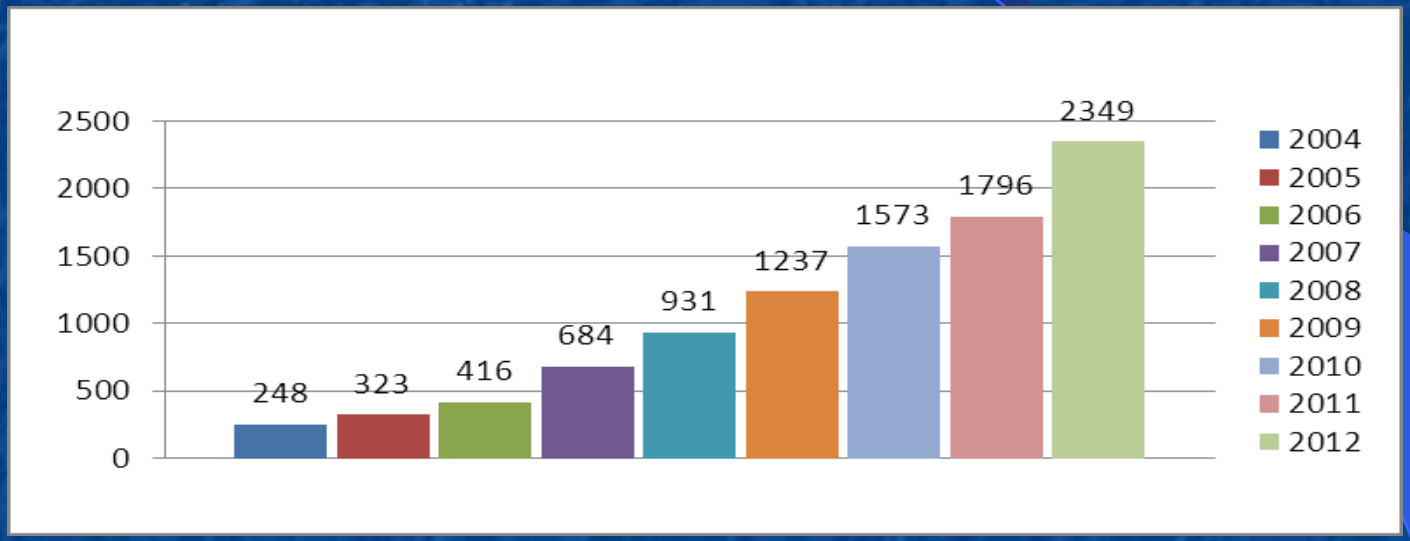


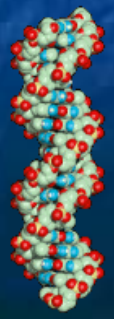
Subdivision's Activities:

- Identification of bodies/ body parts unidentified by all other known ways – Identification of Victims of Mass Disasters.
- Court Presentation – Reporting Officers
- Education (Police/ Army/ Navy/Coastal Guard Officers, Judges & Prosecutor's Office Personnel / Continuous Education of the Staff)

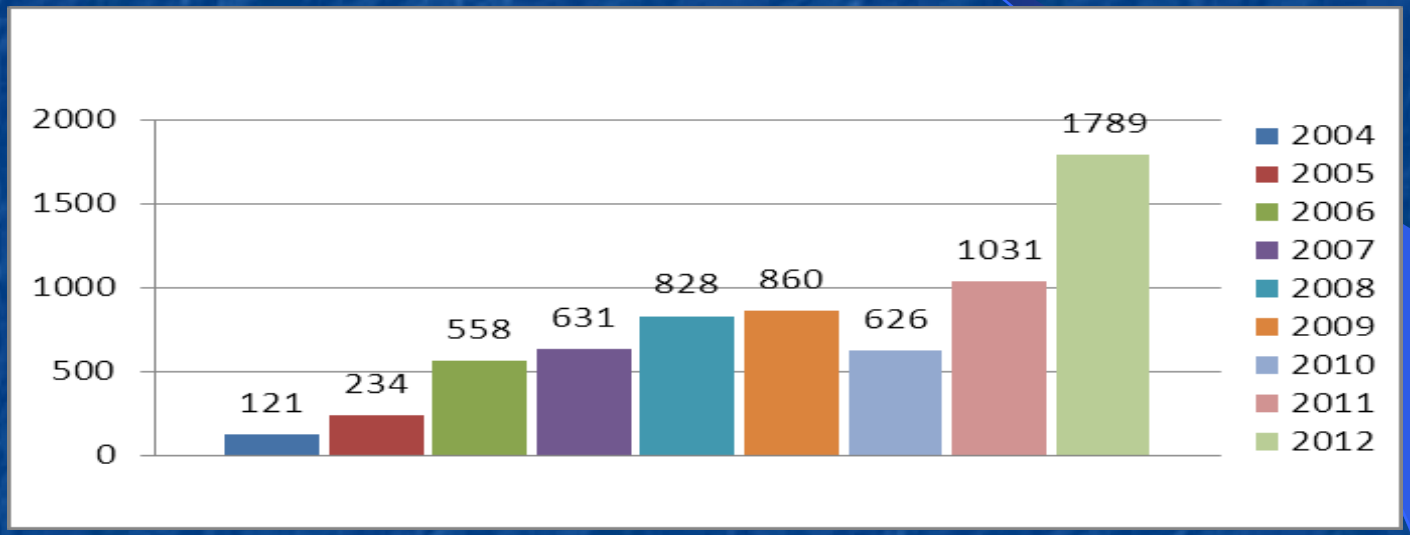


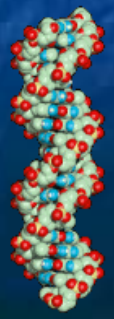
Number of Incoming Cases





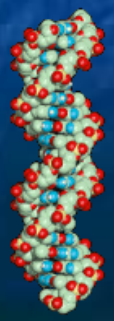
Number of Examined Cases





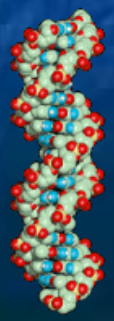
Types of cases examined in our Lab

- Homicides
- Rapes
- Robberies

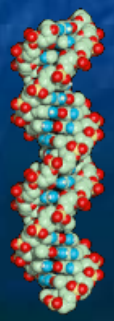


Types of cases examined in our Lab

- Car accidents
- Trafficking
- Kidnapping
- Drugs dealing
- Bodies identification
- Terrorism activities



Evidence



Contribution to Greek major **DVI** cases

HELIOS Air Crash

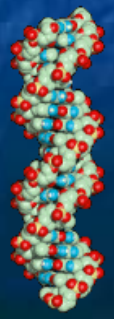
August 2005

(121 victims)

Massive Fires

August 2007

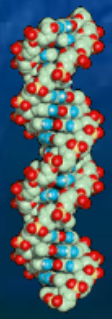
(64 victims)



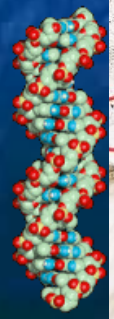
HELIOS Air Crash

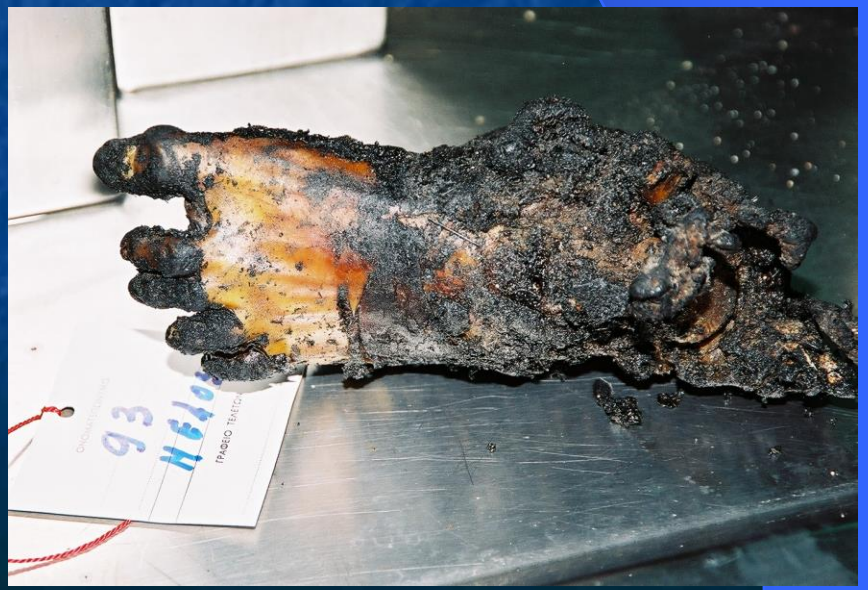
- ✓ *On August 14th, 2005 in Grammatiko village, a place very close to Athens.*
- ✓ *All 121 people on board were killed.*

HELIOS Air Crash



HELIOS Air Crash





HELIOS Air Crash

24 Aug

86 IDs

6 IDs

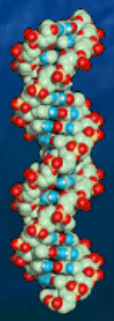
14 Aug

27 Aug

92 DNA Identifications

+ 184 Reference Samples

+ 7 body parts



2007 - Massive Fires

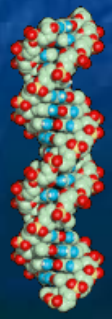
Greek President:

"This is a national catastrophe"

Greek Prime Minister:

"Unspeakable tragedy"

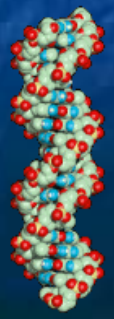
2007 - Massive Fires



2007 - Massive Fires

Identification via DNA analysis:

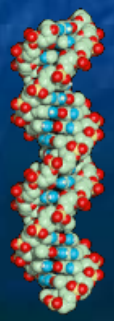
- 53 victims
- 5 misidentified bodies by visual recognition



2007 - Massive Fires

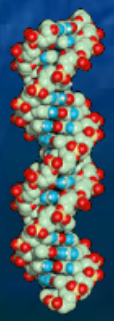
Completion of DNA identification

within less than 15 days!



Future Goals

- Implementation of Prum Treaty – Decisions JHA/615/2008 & JHA/616/2008 of the European Commission
- Automation - ISEC
- Develop further the R&D Section



Thank you!!!!! ☺

E-mail: dna@astynomia.gr

FORENSIC SCIENCE DIVISION

BIOLOGICAL & BIOCHEMICAL EXAMINATIONS

AND ANALYSES SUBDIVISION

Dr. Metheniti Aristea

Police Major - Biologist

*Maria's Case

a DNA analysis with global impact

The story

* During a routine check at a Romani camp at Farsala, the police found a blond, blue-eyed four-year-old girl.
* The couple was detained for questioning by police.
* They reportedly kept changing their testimonies about how the girl came to live with them on the camp.

*The story

*The only way for the truth to come out was:

DNA analysis

The story

- Samples from the alleged parents and Maria arrived at the lab late at night and in a few hours we had the results.

Maria was not their child!

The story

- The finding had just opened the **Pandora's box**.
- DNA findings confirmed that the young girl was not on Interpol's list of 610 missing children.

International search

- 190 countries were asked to check for a possible match for Maria's DNA

International search

International interest

Two high profile disappearances of British blonde-haired children:

- Madeleine McCann in Portugal (2007)
- Ben Needham on the Greek island of Kos (1991)
  - ✓A few days after, a fair-haired 23-year-old man brought up in a Roma settlement in central Greece, was DNA tested to determine whether he could be Ben Needham.
  - ✓There was **NO DNA match**.

Police suspicions

Social benefit scam:

- the couple who registered Maria used falsed identification and claimed to have had 14 children, six of them in under 10 months.
- the couple allegedly received more than €2,500 a month in social benefits.

Illegal adoption ring

- the child was intended for adoption by a Greek couple
- after illegal adoption rings were busted, the adoption fell through and the Roma couple was left with the child.

Identification of biological parents

* Sasha Ruseva and Atanas Rusev, a Bulgarian Roma couple, were identified as the biological parents of Maria.

International Impact

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23 October 2013 Last updated at 18:16 GMT

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Roma case in Greece raises child concerns

By Paul Kirby
BBC News

Police in 190 countries have been asked to check Maria's DNA

theguardian

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Maria case exposes extent of child trafficking in crisis-hit Greece

Growing black market revealed as Bulgaria names mother of girl found in Roma camp and Athens couple accused of buying baby

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Helena Smith in Athens
The Guardian, Friday 25 October 2013 20.40 BST
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Article history

*International Impact

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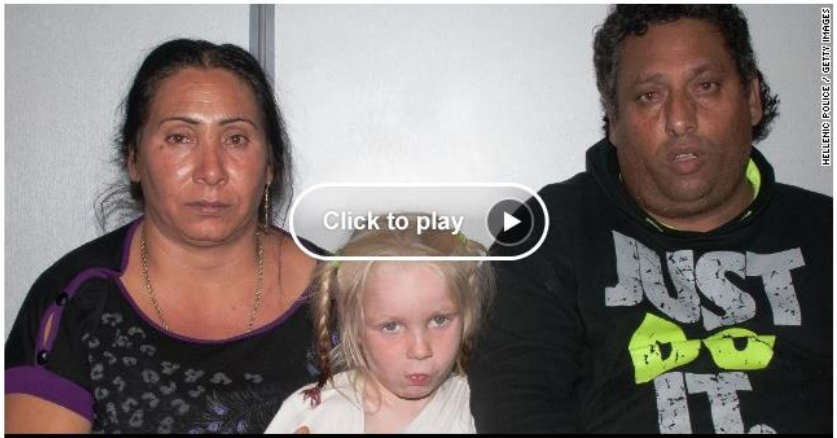
Greece mystery girl: Attorney for Roma couple says they adopted 'Maria'

By David Simpson, CNN
October 21, 2013 -- Updated 1803 GMT (0203 HKT)



Search for mystery blond girl's parents

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Roma Abduction Case Prompts Birth Certificate Review in Greece

By NIKI KITSANTONIS and DAN BILEFSKY
Published: October 22, 2013

ATHENS — A powerful Greek prosecutor ordered an emergency nationwide investigation on Tuesday into birth certificates issued over the past six years, after a Roma couple were jailed on accusations of abducting a child found at a camp in central Greece.

Enlarge This Image

The prosecutor, Efterpi Koutzamani, ordered the inquiry after reviewing news media reports that families were fraudulently securing benefits by declaring births in several regions of the country.

Last week, the police became

FACEBOOK
TWITTER
GOOGLE+
SAVE
E-MAIL
SHARE
PRINT
REPRINTS

*Unfortunately…

The case revealed:

* weaknesses in Greek birth registering system (localised and out-of-date).
* theories for child trafficking network especially inside Roma communities.
* widespread Roma discrimination in Europe.

*Conclusion

*International co-operation and exchange of data

*Significance of the common-used STRs

Leading in direct and accurate results!

FORENSIC SCIENCE DIVISION
BIOLOGICAL & BIOCHEMICAL EXAMINATIONS AND ANALYSES SUBDIVISION
2-6, Antigonis Str.
104 42 - Athens

Tel: +30 210 5103407 -09
Fax: +30 210 5103408
e-mail: dna@astynomia.gr

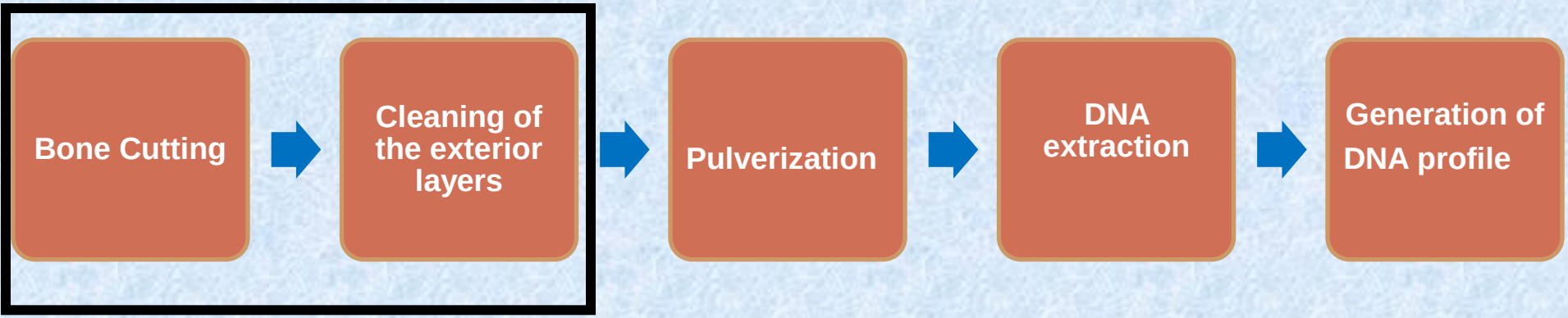
*Thank you

Preparing Bones for DNA Analysis
A Home Made System

Maria Vouropoulou, PhD
Emmanouel Palaigeorgiou, BSc
Georgios Koukouvinos, MSc
DNA Subdivision
Forensic Sciences Division
Hellenic Police Headquarters

One of the greatest challenges forensic laboratories face is the preparation of bones for DNA extraction.

A Typical Flow Chart



Cleaning of the external layers is mostly accomplished with mechanical methods:

Soft tissue is removed with a surgical scraper and then sanding erases external layers.

Risks of cutting and cleaning bones by mechanical methods:

High Health Hazard for the Operator

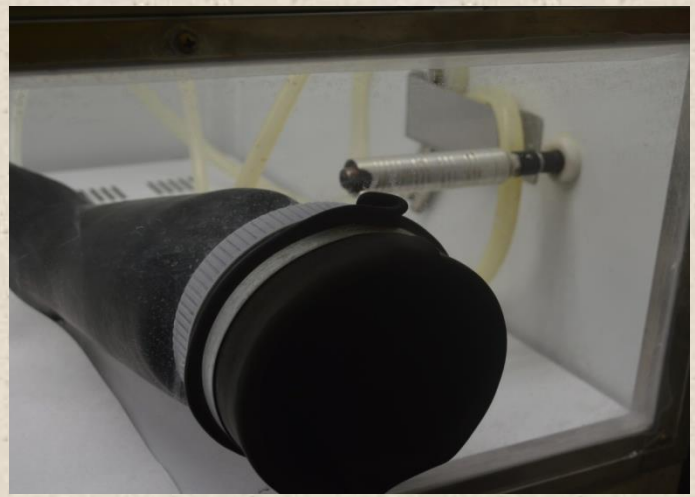
High Possibility to Cross Contaminate Between Samples

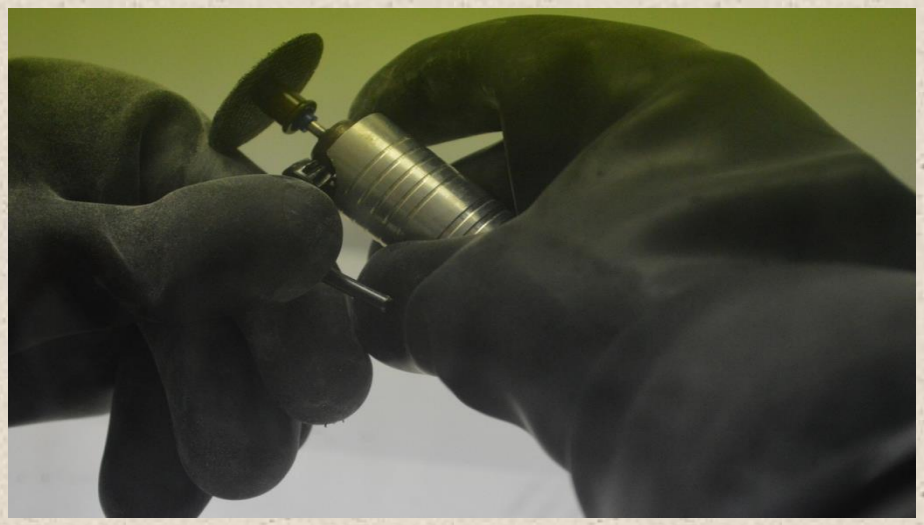

EUREKA !!!

The idea was to make a cabinet that closes hermetically and the sample is processed totally isolated.

THE OSTEOCRACKER

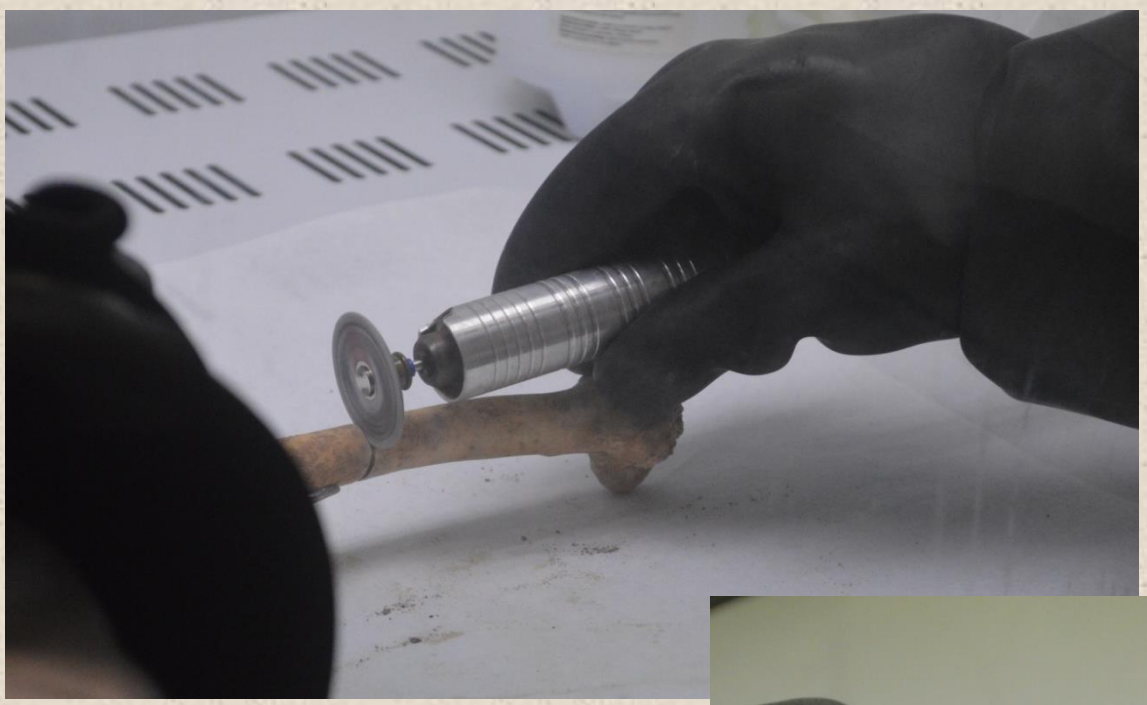
The Dremel Rotary Tool



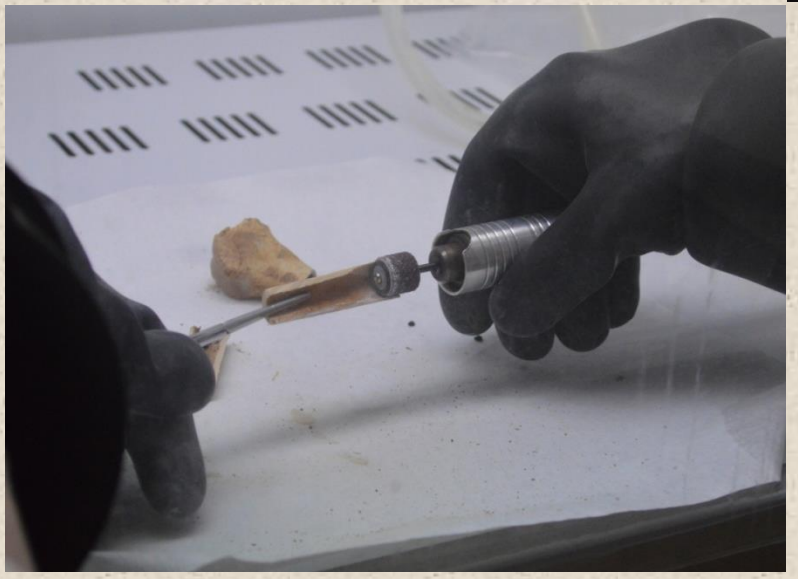


Seeing Things In Action...

Seeing Things In Action... Cutting

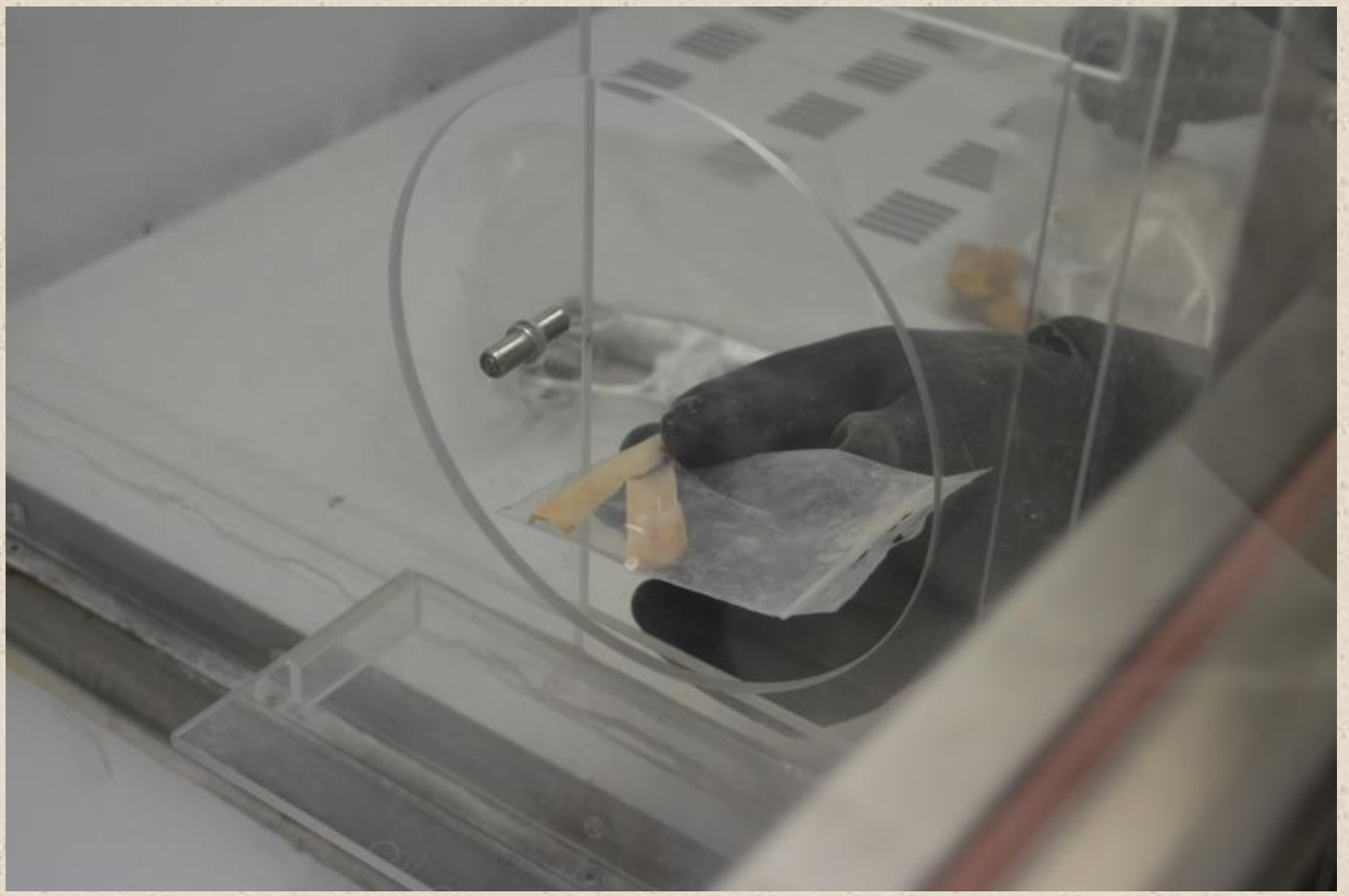


Seeing Things In Action... Sanding

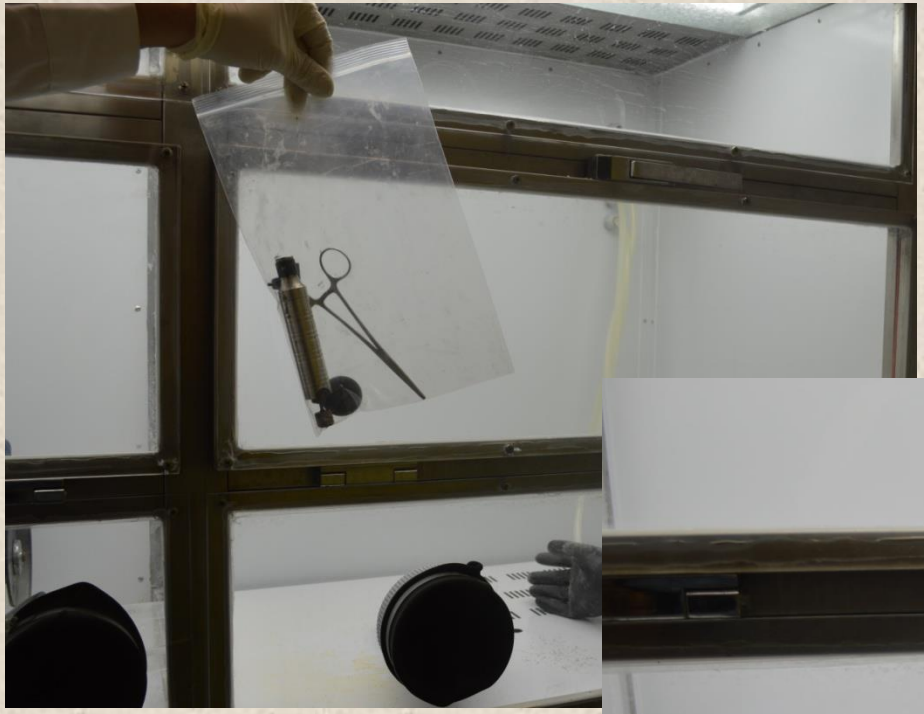


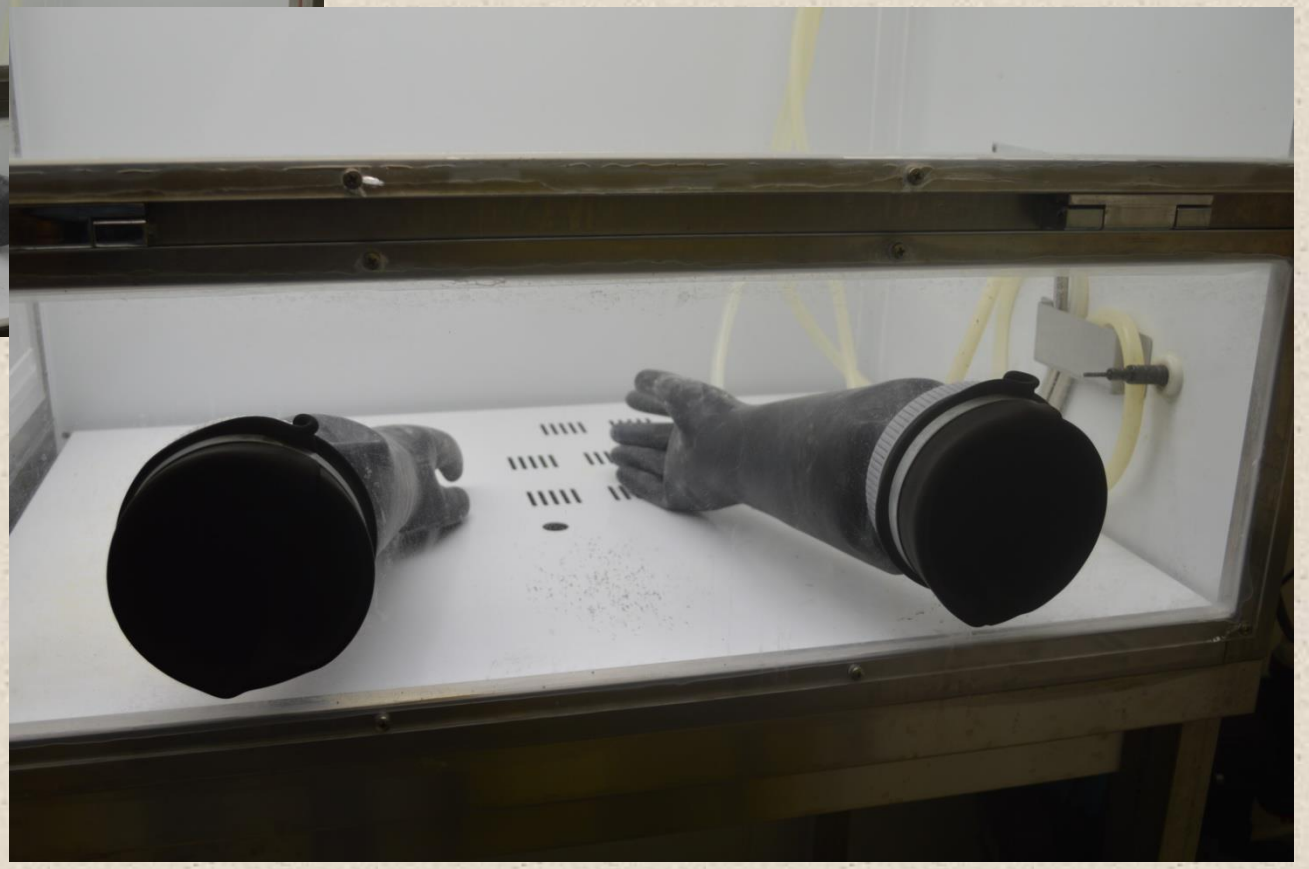
Seeing Things In Action...

Treated bone fragments being transferred to the compartment on the left

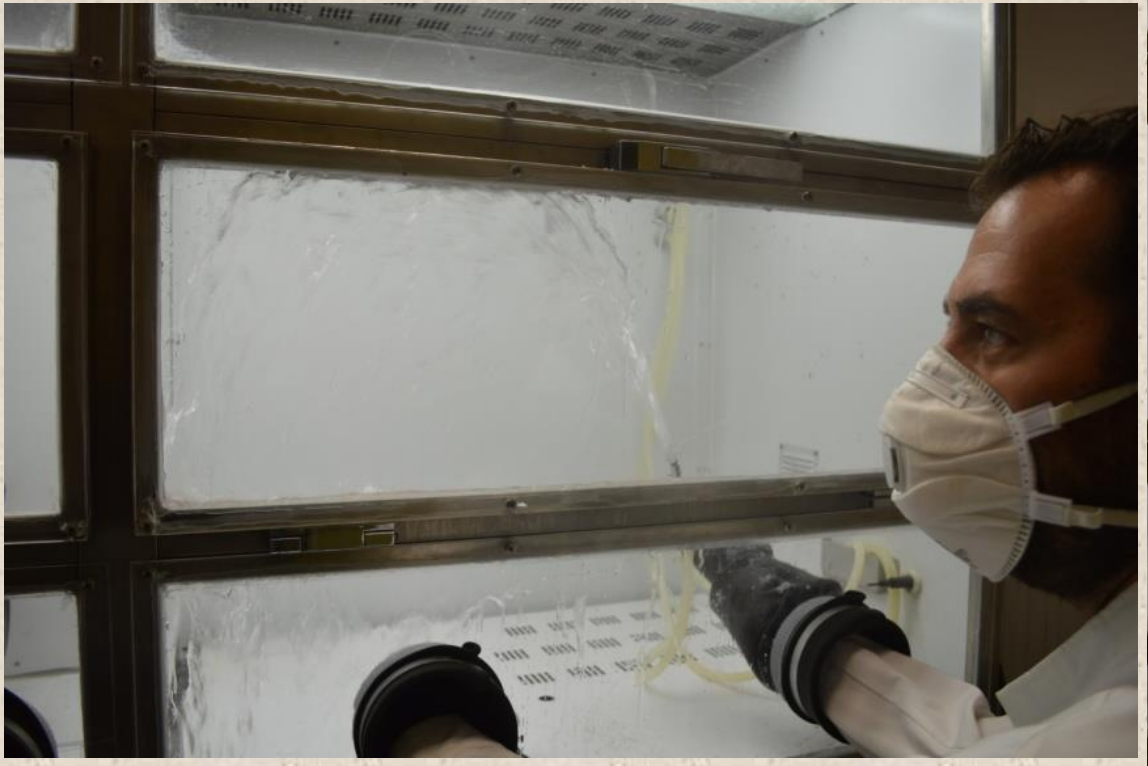


Seeing Things In Action... Ready to Clean





Seeing Things In Action... Cleaning



Seeing Things In Action...Bleaching & Drying

Seeing Things In Action... UV treatment

- Bone fragments once cleaned are pulverized in a SPEX 6700 Freezer Mill in the presence of liquid nitrogen

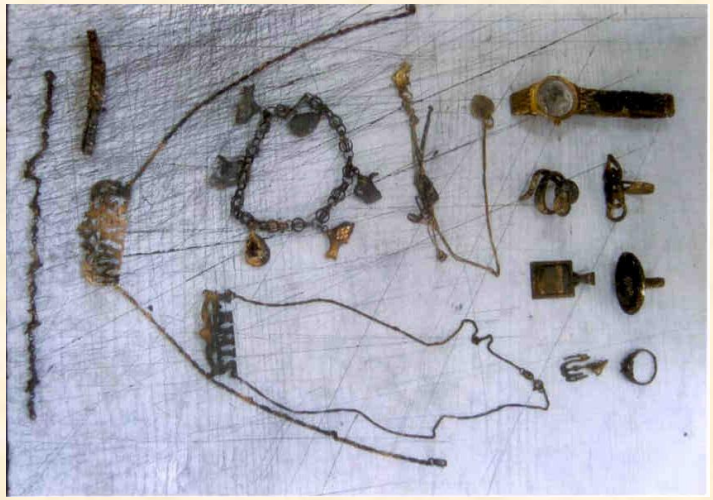
- 250mg of powder goes for demineralization with Bone Incubation Buffer (Promega)

- Lysis takes place with a modified DNA IQ protocol (Promega)

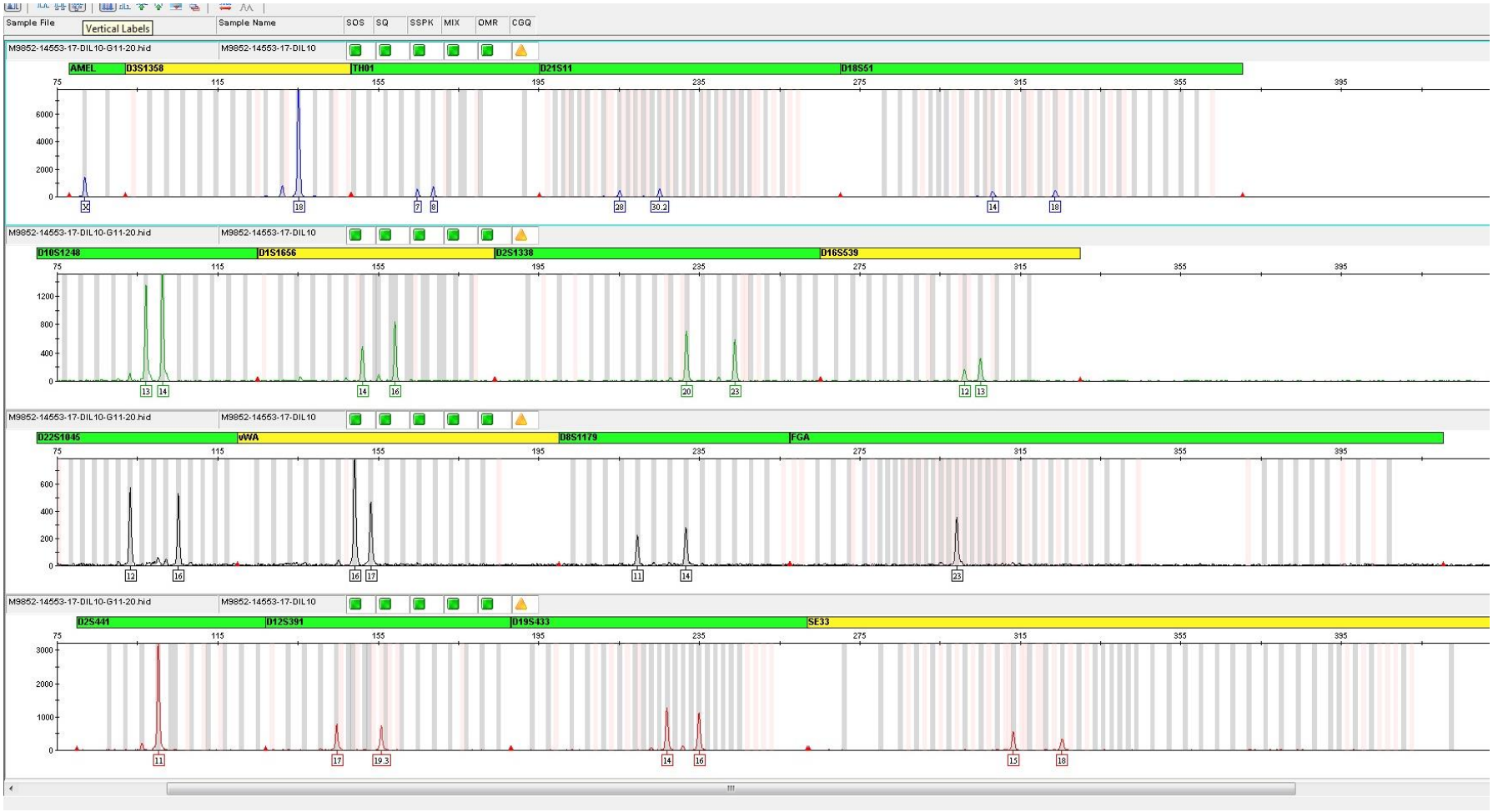
Demonstrating the Efficiency of our System through a Real Case

Identification of skeletal remains from a severely burned woman in a case of illegal immigration at the island of Samos

Demonstrating the Efficiency of our System through a Real Case

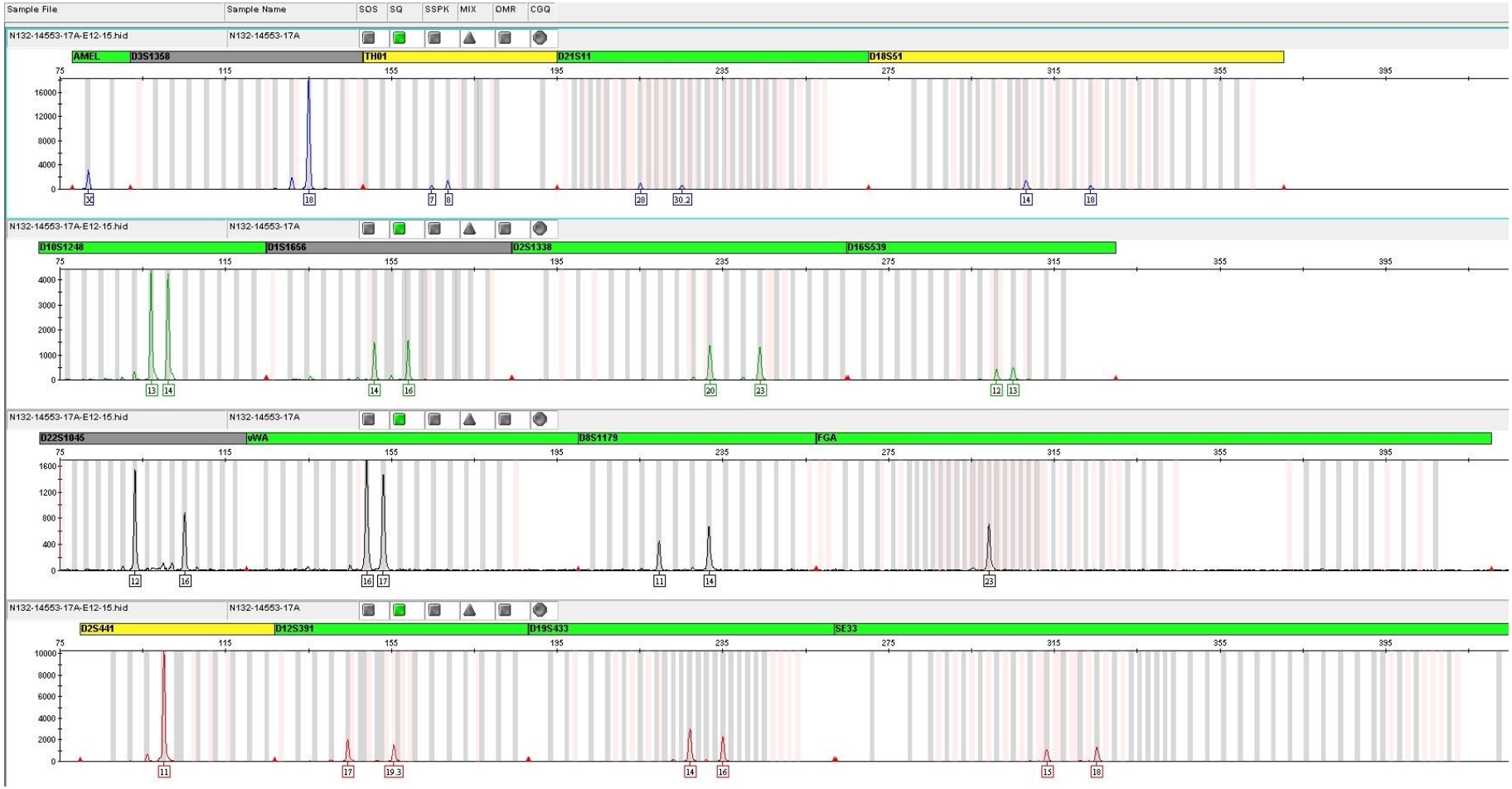


PowerPlex ESX 17 profile from the above bones generated in an ABI 3500*xl* genetic analyser



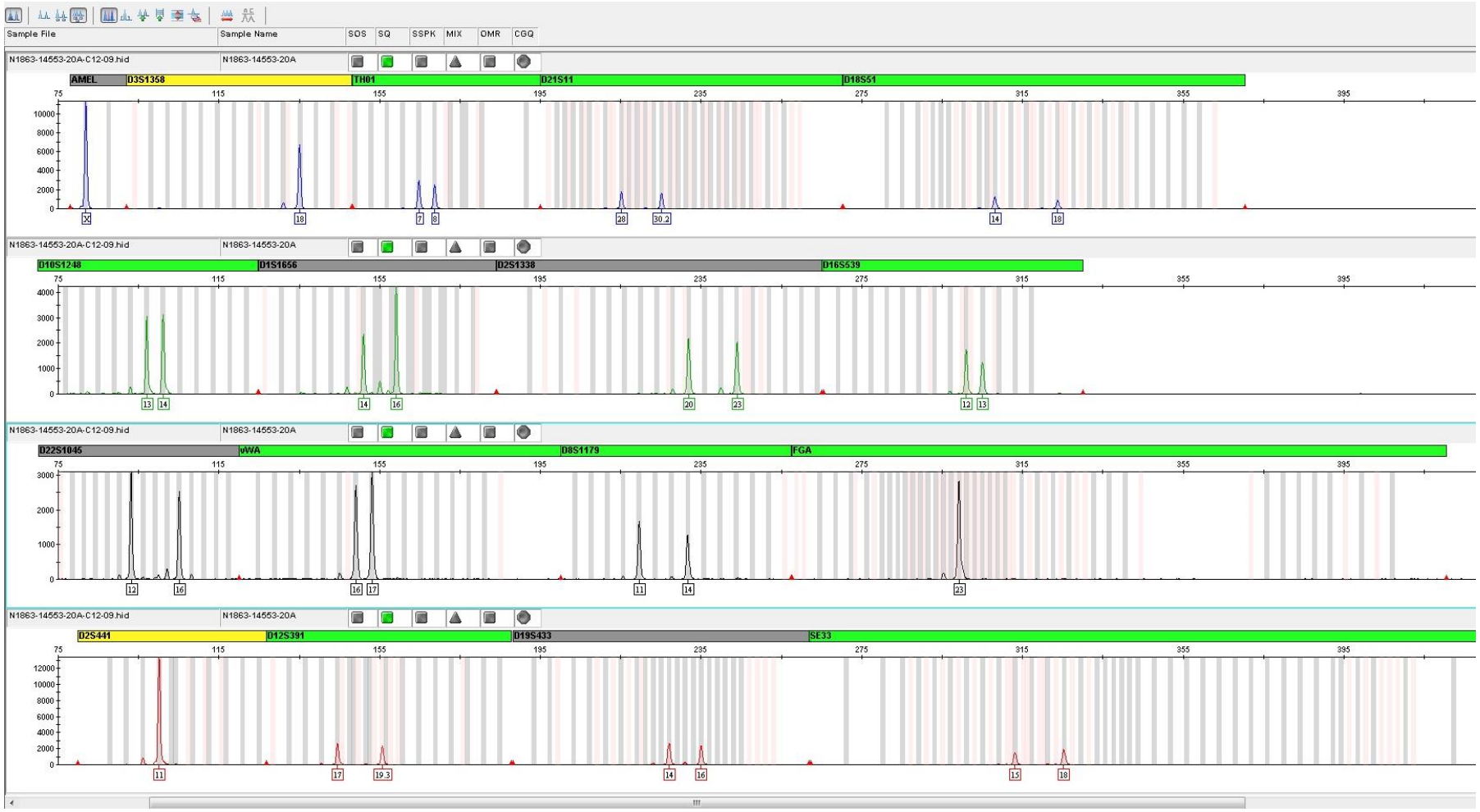
1st PCR, 0.5ng total DNA in a 25ul reaction. Injection: 1.2kV, 15sec

PowerPlex ESX 17 profile from the above bones generated in an ABI 3500*xl* genetic analyser



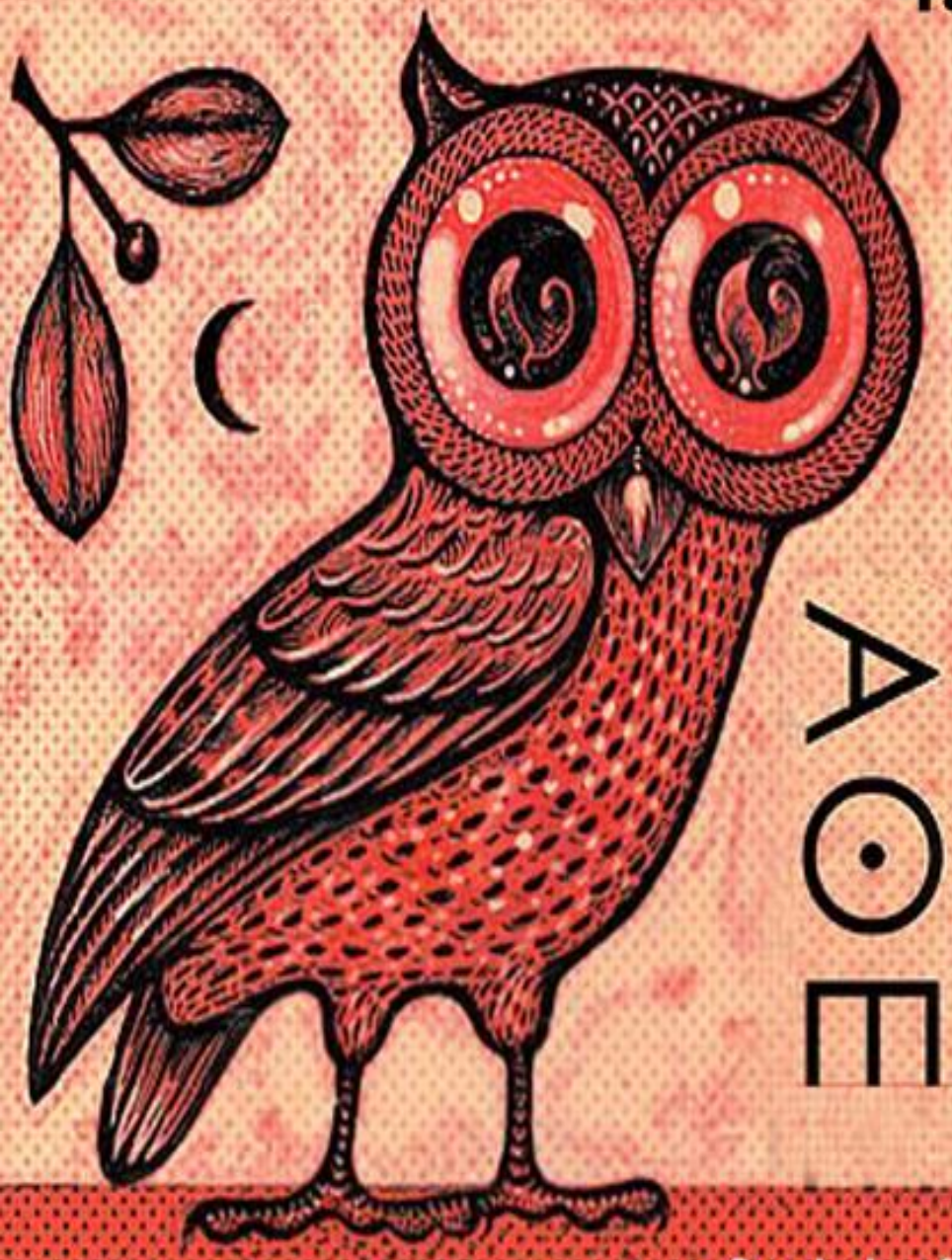
2nd PCR, 0.7ng total DNA in a 25ul reaction. Injection: 1.2kV, 15sec

PowerPlex ESX 17 profile from the victim's lipstick generated in an ABI 3500*xl* genetic analyser



0.5ng total DNA in a 25ul reaction. Injection: 1.2kV, 15sec

45th EDNAP Meeting
Athens 2013



THANK YOU !

The Owl and the Olive Tree,
Symbols of Athens, as inspired from Ancient Silver Coins.

University of Zurich[UZH]

Institute of Legal Medicine

EDNAP
mRNA profiling exercise 6

Cordula Haas / Erin Hanson / Jack Ballantyne

20. November 2013, Athens

EDNAP mRNA profiling exercises 4 + 5

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

C. Haas[a,*], E. Hanson[b], M.J. Anjos[k], K.N. Ballantyne[w], R. Banemann[r], B. Bhoelai[e], E. Borges[j], M. Carvalho[k], C. Courts[h], G. De Cock[d], K. Drobnic[p], M. Dötsch[r], R. Fleming[f], C. Franchi[x], I. Gomes[n], G. Hadzic[p], S.A. Harbison[f], J. Harteveld[e], B. Hjort[v], C. Hollard[i], P. Hoff-Olsen[g], C. Hüls[l], C. Keyser[i], O. Maroñas[t], N. McCallum[m], D. Moore[q], N. Morling[v], H. Niederstätter[o], F. Noël[d], W. Parson[o], C. Phillips[t], C. Popielarz[j], A.D. Roeder[s], L. Salvaderi[y], E. Sauer[h], P.M. Schneider[n], G. Shanthan[g], D. Syndercombe Court[c], M. Turanská[u], R.A.H. van Oorschot[w], M. Vennemann[l], A. Vidaki[c], L. Zatkalíková[u], J. Ballantyne[b]

EDNAP mRNA profiling exercise 6: Identification of skin

- mRNA markers
- Task
- Samples
- Participating laboratories
- Questionnaire
- Results
- Next steps

Evaluated mRNA markers

Skin markers:

- LCE1C
- IL1F7
- LCE1D
- LCE2D
- CCL27

skin 5-plex
Hanson, Ballantyne
(FSI Genetics, 2012)

- LOR
- KRT9
- CDSN

skin 3-plex
Visser, Kayser
(Int J Legal Med, 2011)

Housekeeping genes:

- UBC
- B2M
- UCE

HKG 3-plex

Extraction methods

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

Arcturus® PicoPure® RNA Isolation Kit

Task

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

Extraction:
- any RNA/DNA kit or manual method
- *RNA quantification (optional)*

Reverse transcription:
- any kit/protocol

PCR:
- RNA: Multiplex mixes (Skin 5-plex, Skin 3-plex, HKG 3-plex) provided (standardized protocol)
- *post PCR purification (optional)*
- *DNA (optional): any commercial STR kit*

Samples

- dilution series of skin RNA on swabs:
  200, 50, 12, 3, 0.8 ng skin RNA

- 8 mock casework samples:
  1: small swab fom palm
  2: hand print on paper, glossy side
  3: 1 key from computer keybord
  4: finger print on glass slide
  5: small swab with urine
  6: small swab from palm + 1ul blood
  7: small swab with saliva
  8: scraped skin from back of hand

- *optional:*
  - own casework / mock casework samples
  - non skin samples
  - non-human samples

Participating laboratories

- BKA (Germany)
- Rome (Italy)
- Coimbra (Portugal)
- Bonn (Germany)
- Ljubljana (Slovenia)
- NCFS (USA)
- NFI (The Netherlands)
- Copenhagen (Denmark)
- Santiago de Compostela (Spain)
- Strasbourg (France)

Innsbruck (Austria)
Brussels (Belgium)
Köln (Germany)
London (England)
Strathclyde (Scotland)
Slovenska Lupca (Slovakia)
Hannover (Germany)
ESR (New Zealand)
Oslo (Norway)
Zurich (Switzerland)

Summary Questionnaire

- Delivery time Fedex (samples+primers): 18 labs within 1-2 days (max: 7 days to Italy)

- 11x Allprep RNA/DNA mini kit, 5x Artcurus PicoPure kit, 5x others
- 4x RNA quantification (RiboGreen, Nanodrop)
- RT: 14x Superscript III, 7x others
- PCR: 21x according to protocol

- DNA quantification (8x Quantifiler, 2x other, 11x no)
- DNA-kits used: Identifiler(+), NGM (SElect), SGM+, SEfiler+, PP ESI, PP ESX, PP 16HS
- 28-32 cycles

RNA results: skin RNA dilution series

	skin1 5plex					skin2 3plex			HKG 3plex		
sample	LCE1C	IL1F7	LCE1D	LCE2D	CCL27	LOR	KRT9	CDSN	B2M	UBC	UCE
200 ng	18/19	18/19	18/19	17/19	19/19	18/19	12/19	17/19	19/19	19/19	17/19
50 ng	18/19	19/19	18/19	17/19	18/19	19/19	7/19	19/19	19/19	19/19	18/19
12 ng	17/19	15/19	16/19	16/19	15/19	17/19	2/19	16/19	17/19	17/19	16/19
3 ng	17/19	13/19	12/19	13/19	9/19	17/19	0/19	12/19	17/19	17/19	10/19
0.8 ng	16/19	10/19	13/19	8/19	6/19	17/19	0/19	7/19	17/19	16/19	4/19

more than 1/2 of the labs detected this marker
1/2 to 1/4 of the labs detected this marker
less than 1/4 of the labs detected this marker

RNA results: Stains

samples	skin1 5plex					skin2 3plex			HKG 3plex		
	LCE1C	IL1F7	LCE1D	LCE2D	CCL27	LOR	KRT9	CDSN	B2M	UBC	UCE
1: small swab fom palm	16/20	5/20	10/20	4/20	0/20	18/20	2/20	0/20	9/20	3/20	0/20
	0/20	0/20	0/20	0/20	0/20	1/20	0/20	0/20	1/20	0/20	0/20
2: hand print on paper, glossy side	10/20	1/20	3/20	2/20	0/20	6/20	2/20	0/20	9/20	4/20	0/20
	0/20	0/20	0/20	0/20	0/20	1/20	0/20	0/20	0/20	0/20	0/20
3: 1 key from computer keybord	7/20	0/20	0/20	0/20	0/20	5/20	0/20	0/20	6/20	0/20	0/20
	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20	0/20
4: finger print on glass slide	7/20	0/20	0/20	0/20	0/20	3/20	1/20	1/20	1/20	1/20	0/20
	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
5: small swab with urine	4/20	1/20	0/20	0/20	0/20	3/20	0/20	0/20	9/20	4/20	1/20
	0/20	0/20	0/20	0/20	0/20	1/20	0/20	0/20	0/20	0/20	0/20
6: swab from palm + 1ul blood	15/20	9/20	14/20	10/20	1/20	17/20	8/20	4/20	19/20	15/20	13/20
	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
7: small swab with saliva	8/20	5/20	1/20	2/20	0/20	13/20	3/20	5/20	19/20	19/20	10/20
	0/20	0/20	0/20	0/20	0/20	1/20	0/20	1/20	2/20	1/20	0/20
8: scraped skin from back of hand	16/20	15/20	17/20	16/20	8/20	17/20	6/20	13/20	17/20	18/20	10/20
	1/20	0/20	0/20	0/20	0/20	1/20	0/20	1/20	0/20	0/20	0/20

more than 1/2 of the labs detected this marker
1/2 to 1/4 of the labs detected this marker
less than 1/4 of the labs detected this marker

RNA results: Stains (peakheights)

		skin1 5plex					skin2 3plex			HKG 3plex		
		LCE1C	IL1F7	LCE1D	LCE2D	CCL27	LOR	KRT9	CDSN	B2M	UBC	UCE
AllPrep	1+	6580	65	550	149	0	4734	15	8	3276	75	0
10 labs	2+	1211	22	53	0	5	273	11	0	378	55	0
3x GA3500	3+	142	0	0	0	0	118	0	0	190	0	0
	4+	46	0	0	0	0	41	0	14	0	0	0
	5+	52	27	0	0	0	166	0	0	1464	329	101
	6+	11370	472	2607	1734	0	7575	174	53	10208	2502	2275
	7+	997	127	262	233	0	1908	0	186	14885	9298	542
	8+	7203	2319	2962	3532	430	5319	1198	1477	4569	3153	690
	mean	**3450**	**379**	**804**	**706**	**54**	**2517**	**175**	**217**	**4371**	**1926**	**451**
Arcturus	1+	13756	232	6477	0	0	15880	638	0	11297	352	13
5 labs	2+	8239	0	9751	2806	0	7124	1564	0	11766	2461	14
2x GA3500	3+	2526	0	0	0	0	60	0	0	1385	0	0
	4+	2906	0	0	0	0	103	495	0	132	54	0
	5+	448	0	0	6	0	971	0	0	1654	528	0
	6+	11379	2765	13318	2372	0	13177	121	407	16646	11558	10183
	7+	3151	3639	0	0	0	9099	942	1761	14471	15927	2471
	8+	4429	10218	10208	10116	1301	12591	0	15226	16581	16338	9818
	mean	**5854**	**2107**	**4969**	**1913**	**163**	**7376**	**470**	**2174**	**9242**	**5902**	**2812**
other	1+	3333	71	57	0	0	3300	0	0	672	129	0
5 labs	2+	1771	0	0	0	0	133	0	0	1796	0	0
0x GA3500	3+	365	0	0	0	0	45	0	0	101	0	0
	4+	550	0	0	0	0	289	0	0	0	0	0
	5+	0	0	0	0	0	0	0	0	411	0	0
	6+	4388	182	1639	93	177	7044	37	121	8405	2490	486
	7+	15	28	0	0	0	599	0	0	8321	3379	83
	8+	4050	1945	3307	4000	961	7058	56	5089	6114	5544	673
	mean	**1809**	**278**	**625**	**512**	**142**	**2308**	**12**	**651**	**3227**	**1443**	**155**

Other:
-RNAqueous micro kit
-miRVana miRNA isol. kit
-EZ1 RNA univ. tissue kit
-Zymo Quick RNA mini Prep

RNA results: Stains

samples	skin1 5plex					skin2 3plex			HKG 3plex		
	LCE1C	IL1F7	LCE1D	LCE2D	CCL27	LOR	KRT9	CDSN	B2M	UBC	UCE
8: scraped skin from back of hand	16/20	15/20	17/20	16/20	8/20	17/20	6/20	13/20	17/20	18/20	10/20
6: swab from palm + 1ul blood	15/20	9/20	14/20	10/20	1/20	17/20	8/20	4/20	19/20	15/20	13/20
1: small swab fom palm	16/20	5/20	10/20	4/20	0/20	18/20	2/20	0/20	9/20	3/20	0/20
2: hand print on paper, glossy side	10/20	1/20	3/20	2/20	0/20	6/20	2/20	0/20	9/20	4/20	0/20
3: 1 key from computer keybord	7/20	0/20	0/20	0/20	0/20	5/20	0/20	0/20	6/20	0/20	0/20
4: finger print on glass slide	7/20	0/20	0/20	0/20	0/20	3/20	1/20	1/20	1/20	1/20	0/20
5: small swab with urine	4/20	1/20	0/20	0/20	0/20	3/20	0/20	0/20	9/20	4/20	1/20
7: small swab with saliva	8/20	5/20	1/20	2/20	0/20	13/20	3/20	5/20	19/20	19/20	10/20

DNA results: Stains

	D10	vWA	D16	D2S1338	AML	D8	D21	D18	D22	D19	TH01	FGA	D2S441	D3	D1S1656	D12	SE33
8	8/8	12/12	12/12	11/11	12/12	12/12	12/12	12/12	8/8	11/11	12/12	12/12	8/8	12/12	8/8	8/8	5/5
6	8/8	12/12	12/12	11/11	12/12	12/12	12/12	12/12	8/8	11/11	12/12	12/12	8/8	12/12	8/8	8/8	5/5
1	6/8	11/12	10/12	10/11	8/12	11/12	9/12	8/12	6/8	10/11	11/12	8/12	6/8	10/12	5/8	5/8	2/5
2	5/8	6/12	8/12	4/11	9/12	5/12	8/12	4/12	5/8	7/11	5/12	4/12	5/8	7/12	4/8	3/8	2/5
3	1/8	4/12	1/12	2/11	4/12	4/12	3/12	3/12	4/8	5/11	4/12	3/12	3/8	5/12	2/8	1/8	1/5
4	3/8	6/12	5/12	4/11	6/12	4/12	6/12	5/12	4/8	4/11	6/12	6/12	4/8	6/12	3/8	2/8	2/5
5	6/8	9/12	6/12	7/11	7/12	7/12	6/12	6/12	5/8	6/11	6/12	7/12	6/8	8/12	5/8	4/8	3/5
7	8/8	12/12	12/12	11/11	12/12	12/12	12/12	12/12	8/8	11/11	12/12	12/12	8/8	12/12	8/8	8/8	5/5

more than 1/2 of the labs detected this marker
1/2 to 1/4 of the labs detected this marker
less than 1/4 of the labs detected this marker

Quantification

RNA results

Lab	4	6	15	15
Method	RiboGreen	RiboGreen	RiboGreen	Nanodrop
	stains			
8	0.0	0.0	0.0	110.0
6	0.0	0.0	0.0	115.0
1	0.0	90.0	0.0	100.0
2	0.0	0.0	0.0	60.0
3	0.0	0.0	0.0	75.0
4	0.0	26.0	0.0	60.0
5	0.0	28.0	0.0	120.0
7	270.0	8.0	270.0	575.0

	Dilution series skin RNA			
200 ng	0.0	16.0	25.6	150.0
50 ng	0.0	58.0	0.0	95.0
12 ng	0.0	0.0	0.0	115.0
3 ng	0.0	60.0	0.0	105.0
0.8 ng	0.0	0.0	0.0	185.0

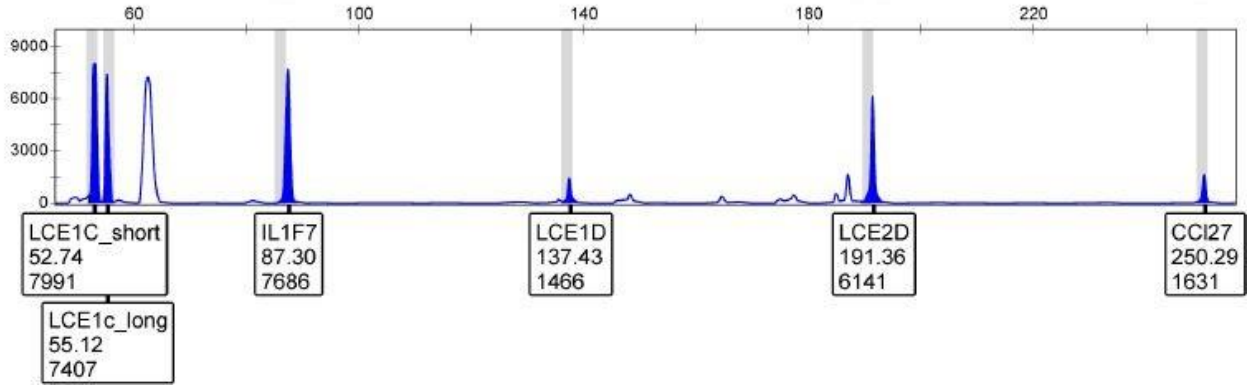
DNA results

	Mean	Stdev
	stains	
8	21.39	25.67
6	15.21	11.39
1	1.76	1.31
2	0.74	1.07
3	0.25	0.52
4	0.44	0.92
5	0.26	0.33
7	158.13	148.59

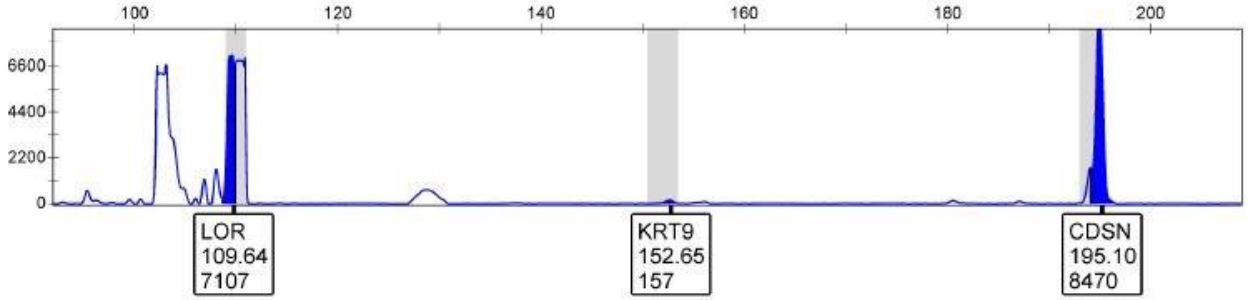
RNA results: Stains

#8: scraped skin (lab 15, EZ1 RNA Universal Tissue kit)

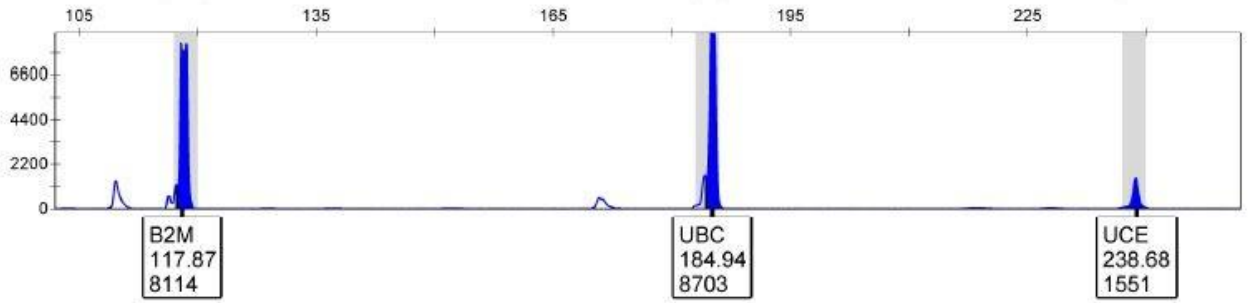
Skin 5-plex



Skin 3-plex



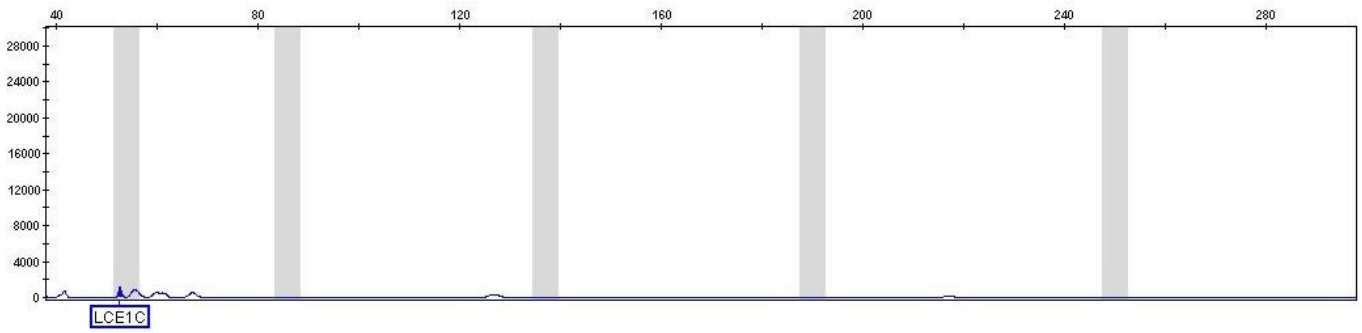
HKG 3-plex



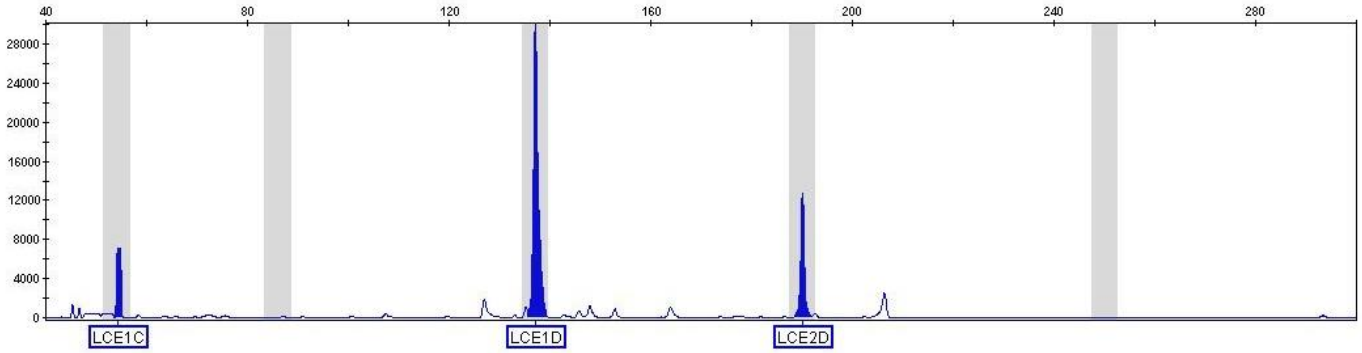
RNA results: Stains

#2: hand print on paper (lab 23)

AllPrep

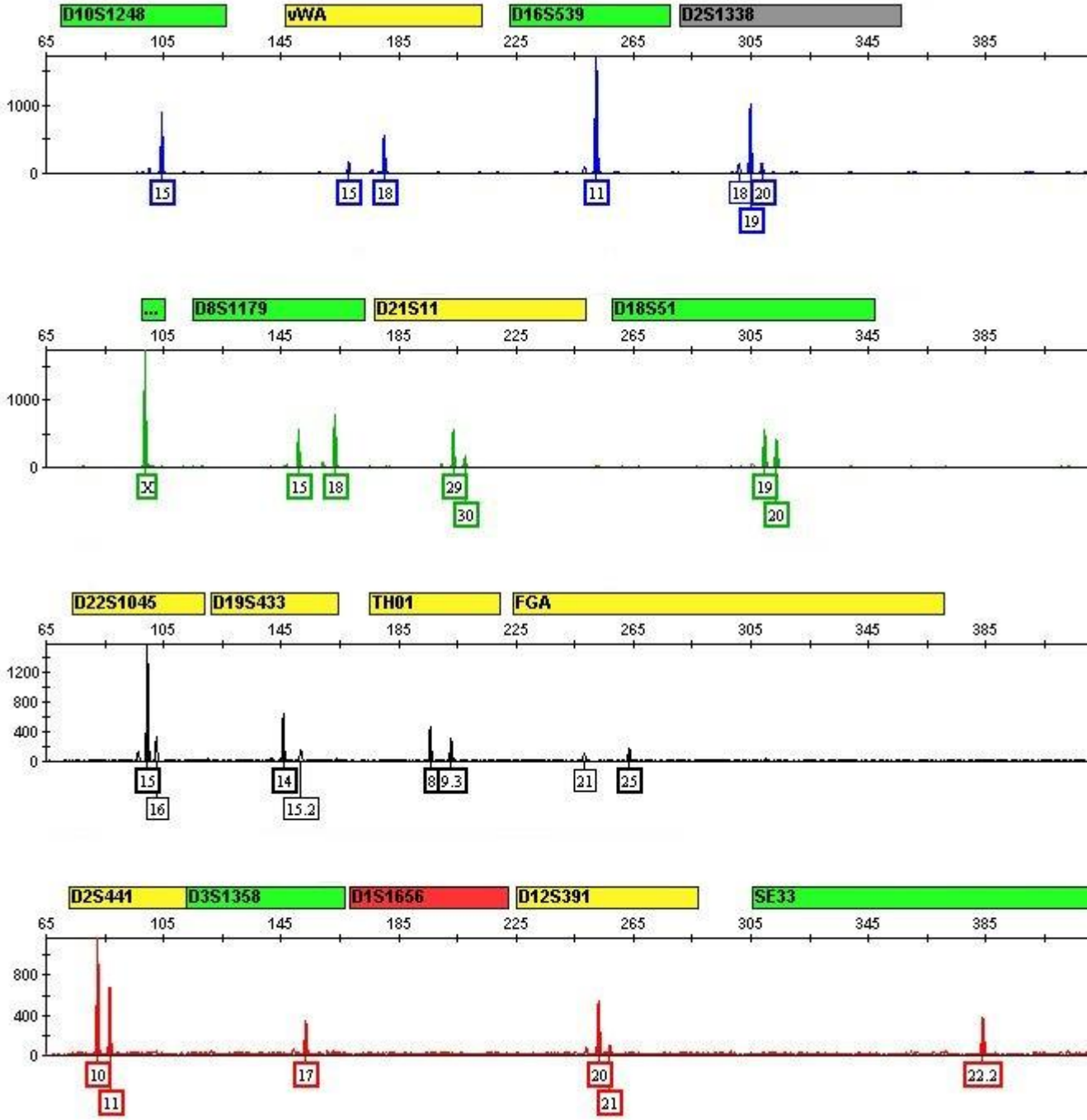


Arcturus



DNA results: Stains

#2: hand print on paper (lab 23)



RNA results:

additional own skin samples

		skin1 5plex					skin2 3plex			HKG 3plex		
Lab	sample	LCE1C(rfu)	IL1F7(rfu)	LCE1D(rfu)	LCE2D(rfu)	CCL27(rfu)	LOR(rfu)	KRT9(rfu)	CDSN(rfu)	B2M(rfu)	UBC(rfu)	UCE(rfu)
4	Skin Swab Arm	4739 / 1224	-	180	-	-	3445	-	-	1831	256	-
4	Swab Doorhandle	4552 / 505	-	-	-	-	2244	-	-	1771	180	-
4	Skin Swab Forehead	7220 / 7076	492	1417	1070	-	7592	-	67	7281	4885	-
5	swab-doorknob	-	-	-	-	-	-	-	-	-	-	-
6	surface swab of female forearm	-	-	-	-	-	-	-	-	247	-	-
6	swab of fingertips on one hand (female donor)	420	-	-	-	-	126	-	-	487	-	-
6	surface swab of coffee cup (where hand would grip to drink)	-	-	-	-	-	-	-	-	-	-	-
6	swab of lab coat collar, inside	-	-	-	-	-	-	-	-	-	-	-
6	skin positive control (RT)	8788/7480	8902	669	8966	9060	7877	1979	8996	8818	8952	8836
6	skin positive control (amp)	8265/8178	8987	1949	4500	9131	7938	-	8530	8853	9043	9024
7	Swab skin back	8643/589	1134	702	-	-	8555	-	-	1231	172	-
7	Swab skin foot	5631/-	-	383	-	-	7101	381	1309	-	-	241
7	Swab skin penis	1255/-	-	-	-	-	587	-	-	-	-	-
7	Swab skin unwashed	5096/-	259	270	-	-	3505	-	-	3742	-	-
8	Human 1+	1427	-	-	-	-	1858	786	-	8509	-	-
8	Human 2+	5711	-	130	-	-	568	-	-	-	-	-
8	Human 3+	2304	-	273	239	-	8862	-	-	7689	328	-
8	Human 4+	2850	-	-	-	-	-	-	-	-	-	-
9	nails of a woman+epitelium cells from a man´s head	30444	-	384	644	-	289	-	218	32344	16219	-
9	cotton tape which was tied to the wrist of the hand of a man for 3 days	4971	-	829	-	-	2719	-	-	32514	4677	-
15	(1+2) post mortem skin tissue samples (epidermis)	6758/5748	7704	386	4201	8293	7518	8511	8243	8356	5520	8399
15	(1+2) post mortem skin tissue samples (epidermis)	5139/1284	8427	261	7726	8477	7984	5821	8505	8198	7101	8380
15	(3) post mortem skin tissue sample (epidermis) from wounded area	7547	4134	339	7874	8632	7127	8406	7893	8047	8419	8264
15	(4) dry swab from telephone mouth piece	-	-	-	-	-	-	-	-	177	-	174
15	(5) dry swab from pen	-	-	-	-	-	-	-	-	39	-	-
15	(6+7) dry swab from hand palms	-	-	-	-	-	-	-	-	32	-	-
15	(6+7) dry swab from hand palms	-	-	-	-	-	-	-	-	711	-	-
16	Hand swab	7408/2339	467	1859	359	-	8120	-	143	288	154	-
17	Swab of surface of skin	7842	2497	2066	1025	-	8725	-	436	-	971	-
17	Tablet computer screen	1100	-	-	-	-	-	-	-	1261	-	-
17	Touchscreen mobile phone	1209	-	-	-	-	3060	-	-	-	-	-
17	Mug handle	-	-	-	-	-	-	-	2503	-	-	-
17	Steering wheel	2376	820	-	-	-	-	-	-	-	-	-
17	Computer keyboard	1337	-	-	-	-	-	-	-	-	-	-
17	Computer mouse	8444	-	-	-	-	4367	-	-	-	-	-
18	M (cell phone swab)	2889	958?	-	-	502	185	-	-	4381	-	86
18	B (PET bottle closure swab)	2939/1891	-	-	-	-	186	-	-	1940	-	-
18	T (wallet tape)	-	-	-	-	-	-	-	-	-	-	-
19	1a+ (blue pipet tip, handling for 5 mins)	?1426?	74	79	-	-	5878	1927	-	6054	1031	-
19	1b+ (blue pipet tip, handling for 5 mins)	?6355?	-	2171	-	-	6155	-	1009	4315	1894	-
19	2a+ (blue pipet tip, firm grip 15 sec)	?6398?	-	-	-	-	6660	-	1112	3164	-	-
19	2b+ (blue pipet tip, firm grip 15 sec)	?4925?	-	-	-	-	1506	-	-	1194	-	-
19	3+ (moist swab from forearm)	-	-	-	-	-	5741	-	-	2070	-	-
19	4+(dry swab from forearm)	?6670?	5008	3403	611	-	6219	-	852	2998	1434	-
23	8+ (T-Shirt)	1283	-	-	-	-	520(2ul)	-	-	-	-	-
23	9+ Kl. Swab ab Handschuh	12267	-	340	-	-	-	-	-	-	-	-

RNA results:

non-skin samples

		skin1 5plex					skin2 3plex			HKG 3plex		
Lab	sample	LCE1C(rfu)	IL1F7(rfu)	LCE1D(rfu)	LCE2D(rfu)	CCL27(rfu)	LOR(rfu)	KRT9(rfu)	CDSN(rfu)	B2M(rfu)	UBC(rfu)	UCE(rfu)
4	Buccal Swab	- / 232	61	-	151	-	-	-	-	7371	7446	754
5	spittle in tube	-	-	-	-	-	-	-	-	8226	665	-
6	whole buccal swab (female donor); fresh	-	-	-	-	-	-	-	-	2706	652	-
7	Tongue sampling	-	-	-	-	-	740	-	-	6823	6359	873
7	Drink simulation	-	-	-	-	-	-	-	-	-	-	-
16	5ul saliva	-	-	-	-	-	209	-	-	8450	1700	-
20	Buccal sample	396	-	-	-	-	n.d.	n.d.	n.d.	1321	135	-
4	Vaginal Secretions	- / 480	-	-	147	-	378	140	532	6945	7446	7336
6	1/2 vaginal swab; stored frozen	8788/3186	-	968			9127	-	-	9020	9096	8076
7	Vaginal mucosa swab 1	156/-	-	-	-	-	1864	-	-	9000	1738	789
7	Vaginal mucosa swab 2	5524/293	-	-	-	-	7140	-	452	8940	5742	1599
16	Vaginal swab	7684/3646	136	1361	825	-	7907	-	1075	8404	8131	8276
7	Menstrual secretion swab	3491/488	758	-	175	714	3083	-	-	8355	8698	8465
20	menstrual blood	471	-	-	-	-	n.d.	n.d.	n.d.	7031	8022	8047
20	semen	483	-	-	-	-	n.d.	n.d.	n.d.	839	237	-
20	blood	398	-	-	-	-	476	-	-	7144	8078	7995
5	nose mucus	-	-	-	-	-	-	-	-	-	-	-
5	urine	-	-	-	-	-	-	-	-	-	-	-
16	Hair	419/-	-	(40)	-	-	-	-	-	-	84	-
8	Rabbit+	4347	-	-	-	-	-	-	-	1104	-	-
8	Cat+	1028	-	-	-	-	-	-	-	-	-	-
8	Dog1+	-	-	-	-	-	-	-	-	-	-	-
8	Dog2+	-	-	-	-	-	-	-	-	-	-	-

negative controls

6	extraction blank	-	-	-	-	-	-	-	-	-	-	-
6	RT blank (nuc free water)	-	-	-	-	-	-	-	-	-	-	-
6	nuclease free water	-	-	-	-	-	-	-	-	-	-	-
9	H2O	-	-	-	-	-	-	-	-	-	-	-
17	Extraction blank	-	-	-	-	-	-	-	-	-	-	-
17	RT blank	-	-	-	-	-	-	-	-	-	-	-
17	PCR negative	-	-	-	-	-	-	-	-	-	-	-
19	Extraction negative	-	-	-	-	-	-	-	-	-	-	-
19	RT negative	-	-	-	-	-	-	-	-	-	-	-
19	PCR negative	-	-	-	-	-	-	-	-	-	-	-

RNA results:

1 ng RNA into 20ul RT, 1ul cDNA into 12.5ul PCR

human tissues (Lab 23)

	skin 5plex					skin 3plex		
Tissues	**LCE1C**	**IL1F7**	**LCE1D**	**LCE2D**	**CCL27**	**LOR**	**KRT9**	**CDSN**
Adipose	0	0	0	0	0	0	0	0
Bladder	0	0	0	0	0	0	0	0
Brain	0	0	0	0	0	0	0	0
Cervix	0	0	0	0	0	1951	0	0
Colon	0	0	0	0	0	0	0	0
Esophagus	0	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0	0
Kidney	0	0	0	0	0	0	0	0
Liver	0	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0	0
Ovary	0	0	0	0	456	0	0	0
Placenta	0	0	0	0	0	0	0	2663
Prostate	0	0	0	0	0	0	0	0
Skeletal Muscle	0	0	0	0	0	0	0	0
Small Intestine	0	0	0	0	0	0	0	0
Spleen	0	0	0	0	0	0	0	0
Testes	0	0	0	0	1229	0	0	0
Thymus	0	0	125	0	0	823	795	0
Thyroid	0	0	0	0	0	565	0	0
Trachea	0	0	0	0	0	0	0	0
Positive control	6602	8198	5495	8373	8493	6776	496	8084

Next steps

Prepare manuscript on EDNAP RNA exercise 6?

Additional RNA exercises?
- -RNA quantification
- -others?

Future tasks
- -Interpretation of RNA results, especially partial and mixed profiles
- -miRNAs

Thank you for your attention!

Cordula Haas / Erin Hanson / Jack Ballantyne

20. November 2013, Athens

Manuscript send to all co-authors on Nov 18

Collaborative EDNAP Exercise on the IrisPlex system for DNA based prediction of human eye colour

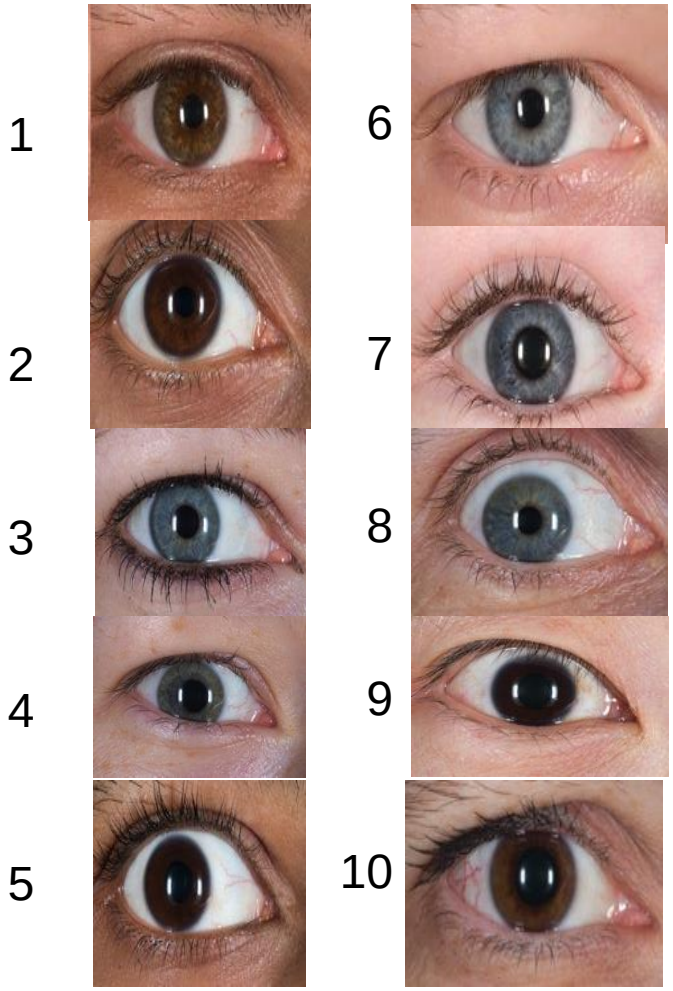
Manfred Kayser

Dept. Forensic Molecular Biology
Erasmus MC University Medical Center Rotterdam, The Netherlands

21 Participating Labs

First name	Last name	Adsress 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- IrisPlex eye colour prediction from biological samples with eye colour knowledge



- Each group received 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 was included.

What was done for task 1:

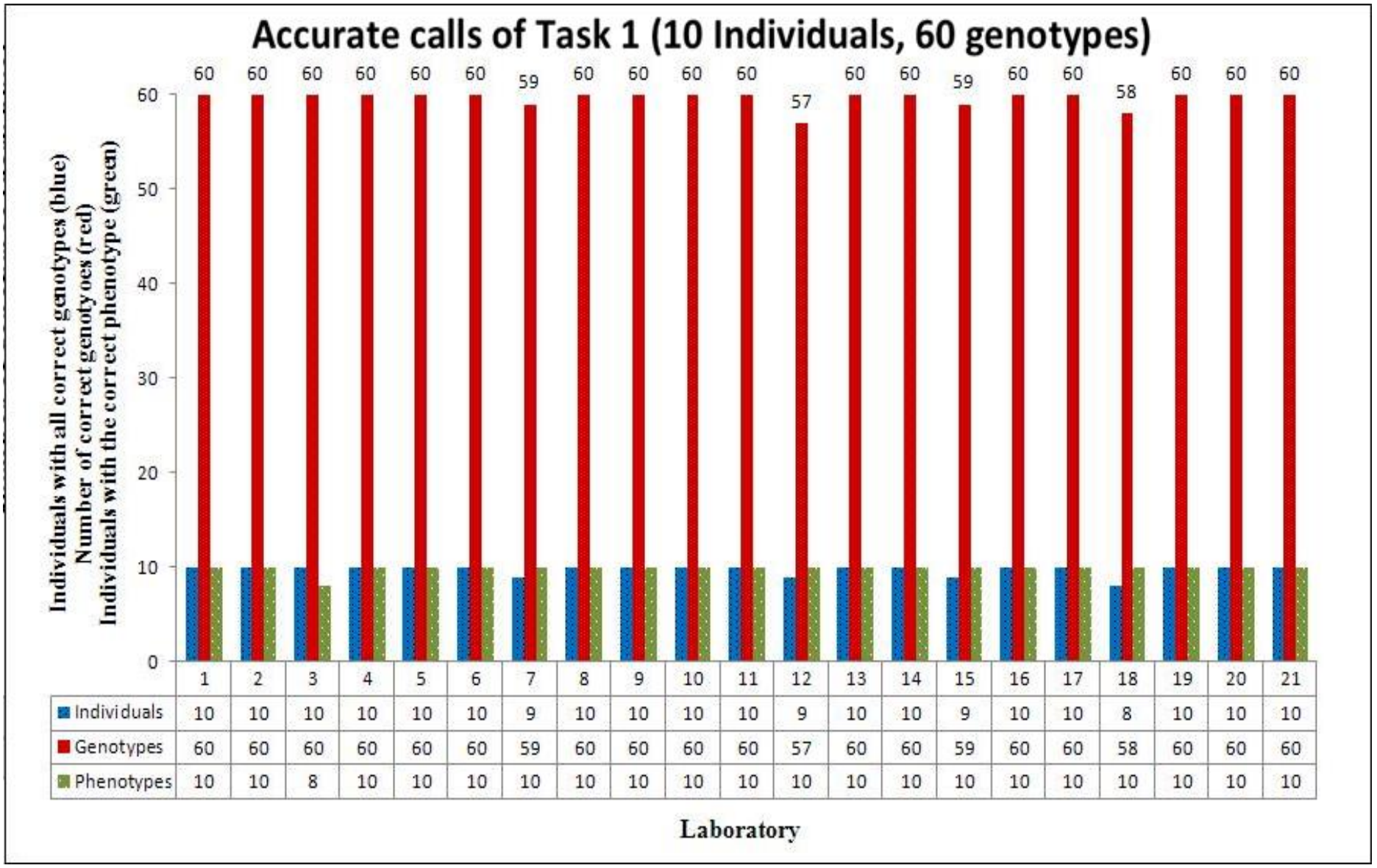
- Groups were asked to extract DNA from the samples and make to a concentration recorded by the lab
- They were asked to produce a IrisPlex genotype profile for each individual sample
- They were asked to state what the most likely eye color prediction outcome and its likely accuracy is based on IrisPlex.

OVERALL PREDICTION RESULTS TASK 1

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

20 labs predicted the correct eye color

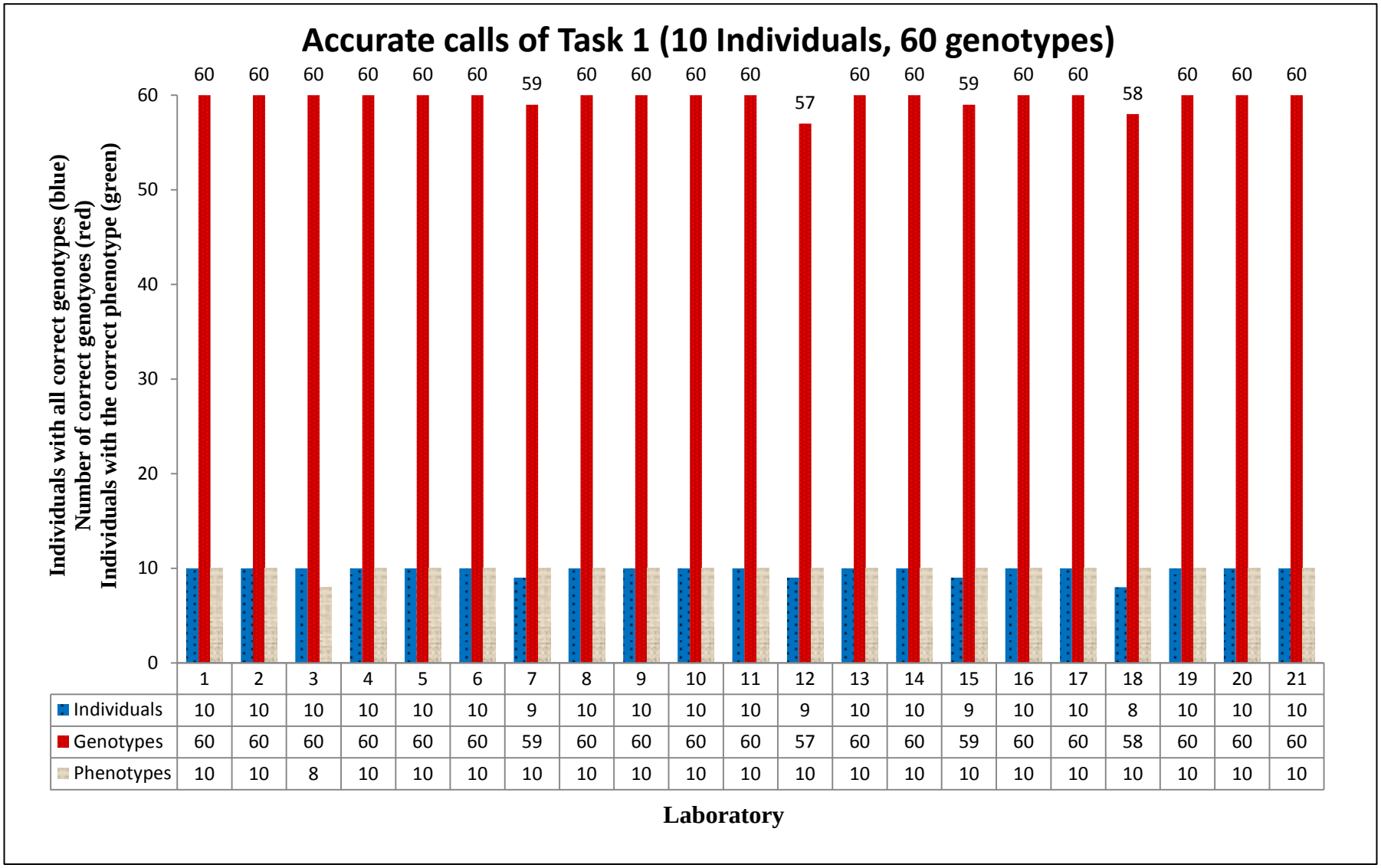
Some explanations for drop in/out are concentration, each lab ran the 10 individuals at varying extracted concentrations



	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	9	10	10	8	10	10	10
Genotypes	60	60	60	60	60	60	59	60	60	60	60	57	60	60	59	60	60	58	60	60	60
Phenotypes	10	10	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

17 out of 21 labs reported the correct 6-SNP IrisPlex profiles for all 10 samples.

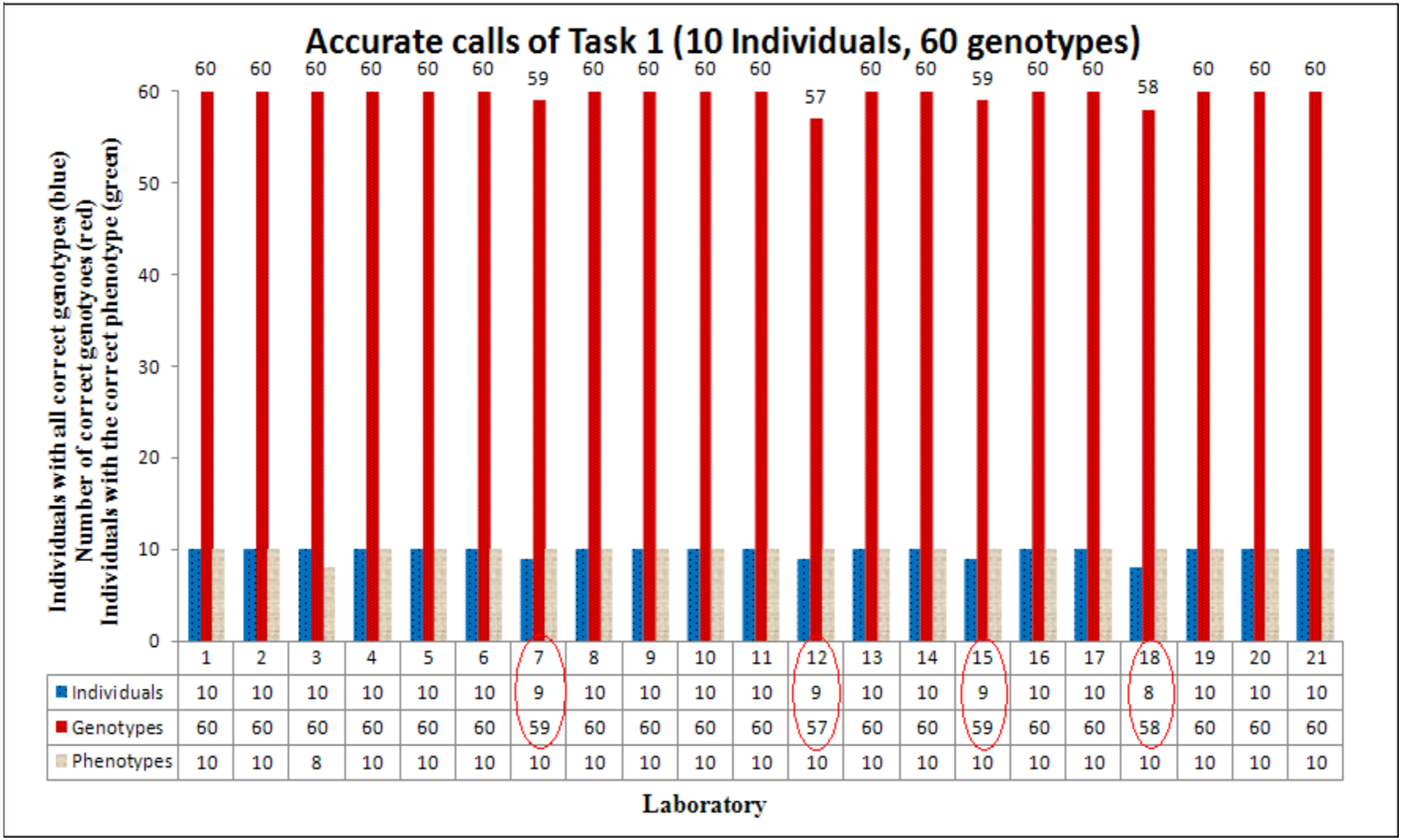
17 out of 21 labs reported the correct 6-SNP IrisPlex profiles for all 10 samples.



	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	9	10	10	8	10	10	10
Genotypes	60	60	60	60	60	60	59	60	60	60	60	57	60	60	59	60	60	58	60	60	60
Phenotypes	10	10	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

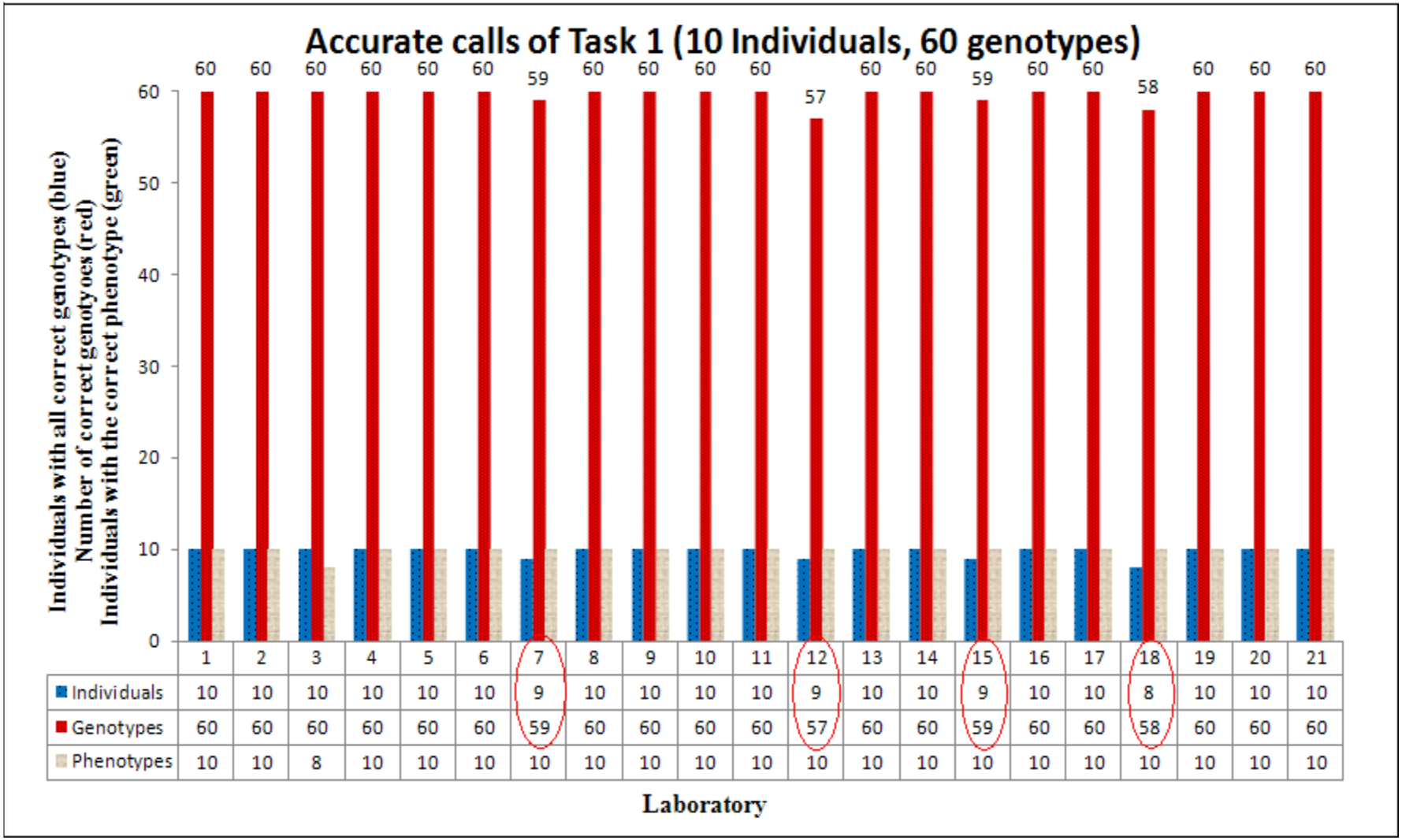
1253 out of 1260 genotype calls correct = 99.4% accuracy

1253 out of 1260 genotype calls correct = 99.4% accuracy



The 7 incorrect genotypes were reported by only 4 of the 21 labs

1253 out of 1260 genotype calls correct = 99.4% accuracy

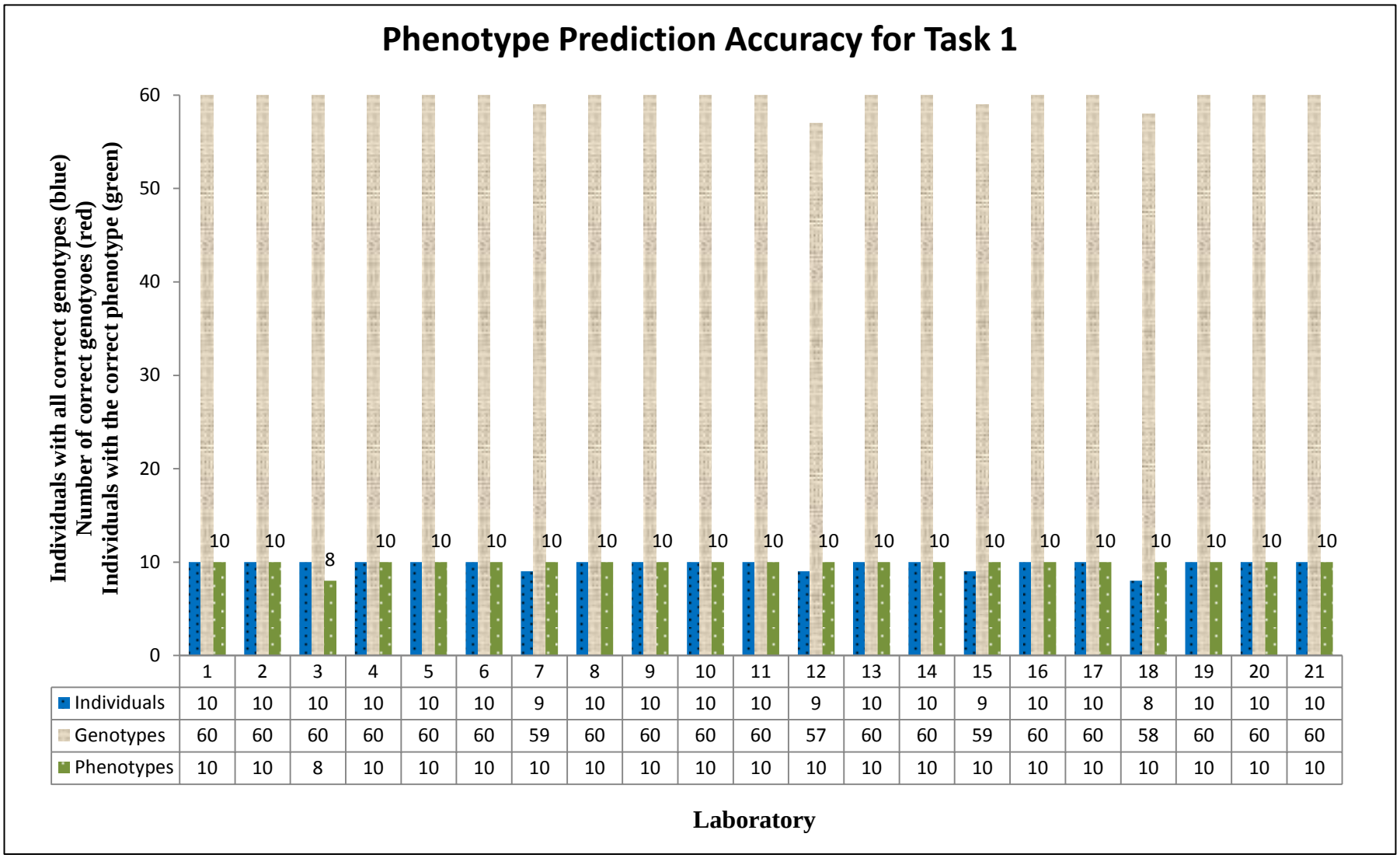


	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Individuals	10	10	10	10	10	10	9	10	10	10	10	9	10	10	9	10	10	8	10	10	10
Genotypes	60	60	60	60	60	60	59	60	60	60	60	57	60	60	59	60	60	58	60	60	60
Phenotypes	10	10	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

The 7 incorrect genotypes were reported by only 4 of the 21 labs

However these incorrect genotypes did not influence the final eye colour phenotype prediction

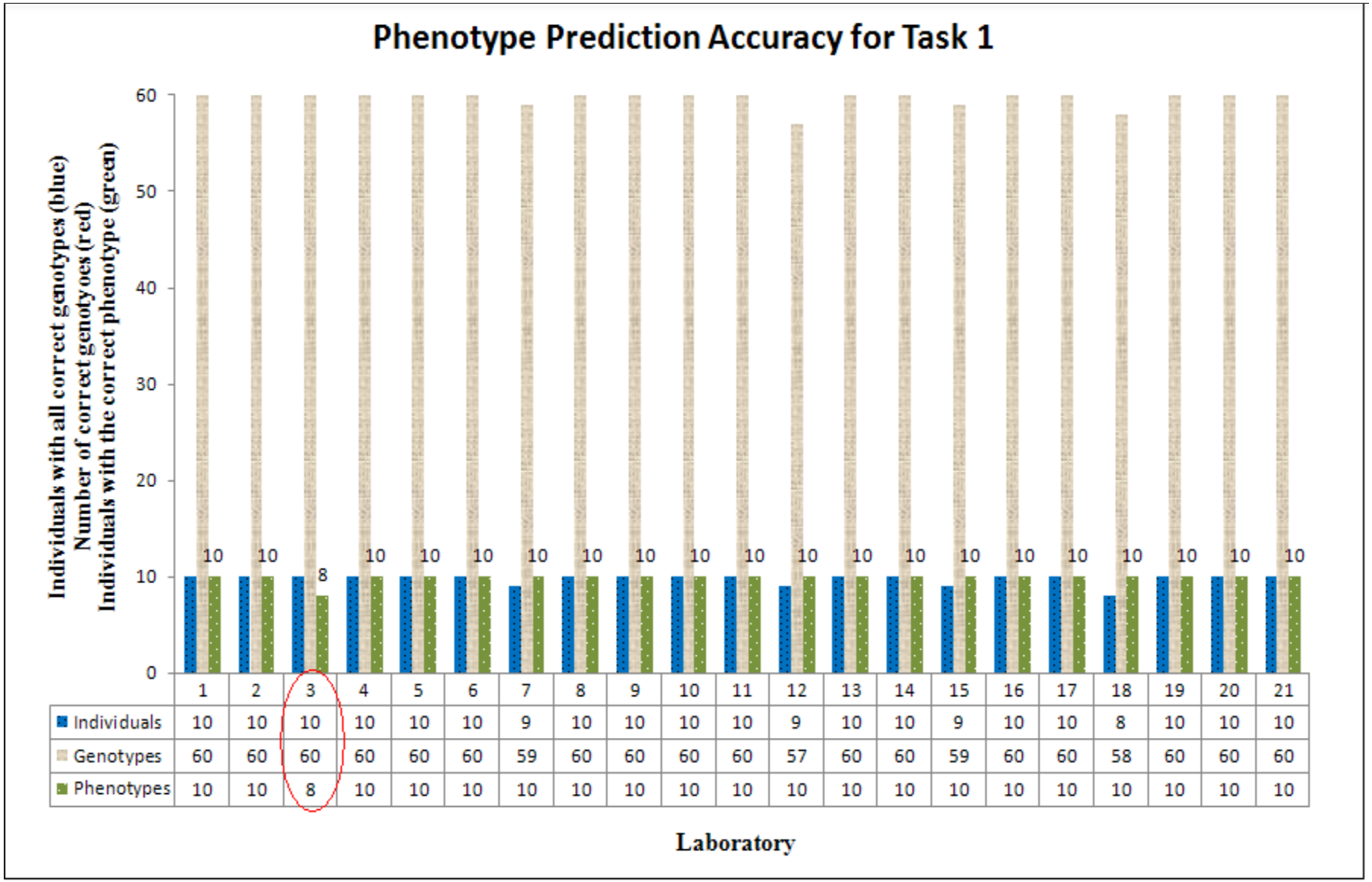
Out of 21 labs **20** of them predicted the phenotypes accurately



	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	9	10	10	8	10	10	10
Genotypes	60	60	60	60	60	60	59	60	60	60	60	57	60	60	59	60	60	58	60	60	60
Phenotypes	10	10	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

208 **(99%)** of the 210 samples analysed by all the 21 labs were reported with the correct eye colour prediction.

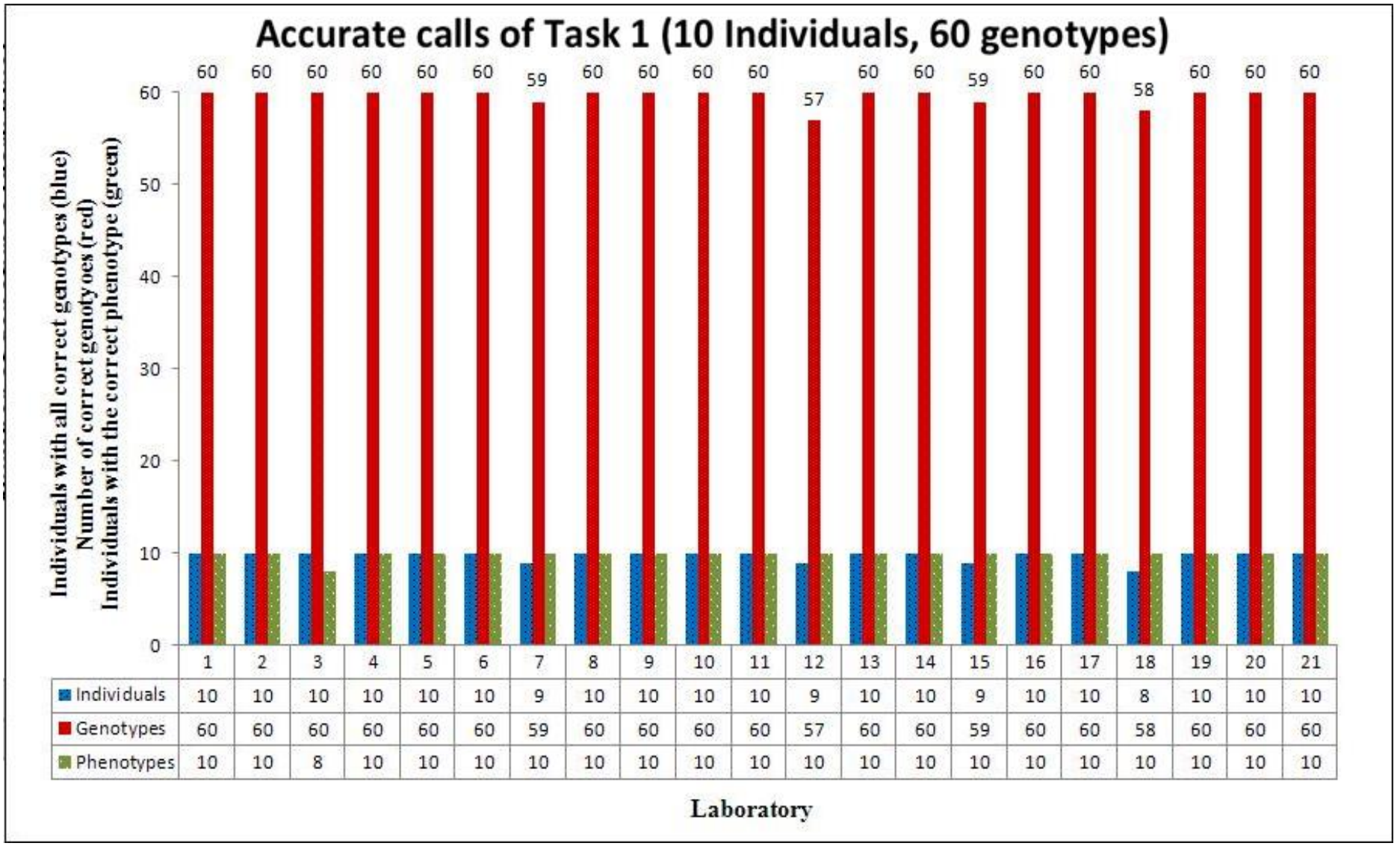
Out of 21 labs **20** of them predicted the phenotypes accurately



	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	9	10	10	8	10	10	10
Genotypes	60	60	60	60	60	60	59	60	60	60	60	57	60	60	59	60	60	58	60	60	60
Phenotypes	10	10	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

The phenotype was reported as inconclusive, although the correct IrisPlex genotypes were obtained and reported.

OVERALL PREDICTION RESULTS 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	9	10	10	8	10	10	10
Genotypes	60	60	60	60	60	60	59	60	60	60	60	57	60	60	59	60	60	58	60	60	60
Phenotypes	10	10	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

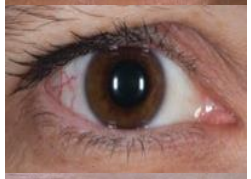
1253 out of 1260 genotype calls correct = 99.4% accuracy

208 out of 210 samples predicted with correct phenotype = 99% accuracy

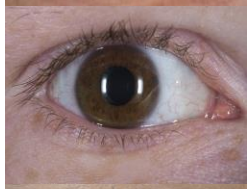
Task 2 - IrisPlex eye colour prediction from DNA of simulated casework samples without eye colour knowledge

1

2



3



4

5

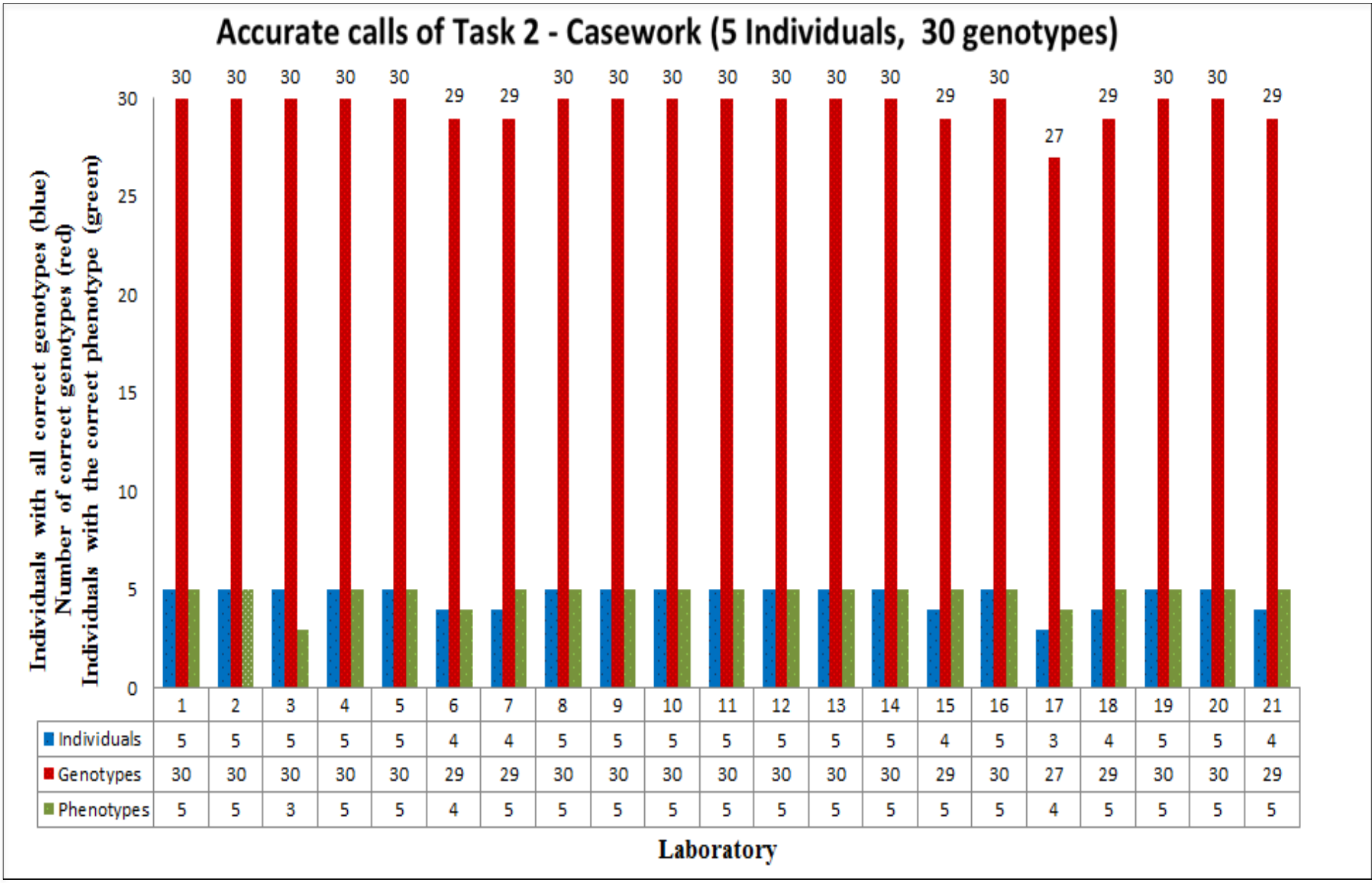
- Each group received 5 additional samples of already extracted DNA that have been subject to simulated casework conditions
- Should have been run as is by laboratory using 1µl sample for IrisPlex assay
- No eye colour phenotype information was provided to the groups.

Sample #	Sample Type	Treatment	Concentration (ng/µl)
CW1	Buccal Swab	UV for 1 min	0.5
CW2	Buccal Swab	UV for 1 min	0.1
CW3	Saliva on Slide	RT for 1 week	0.25
CW4	Blood on slide	RT for 1 week	2
CW5	Semen	-	50

What was done for task 2:

- Groups were asked to produce a IrisPlex genotype profile for each individual sample

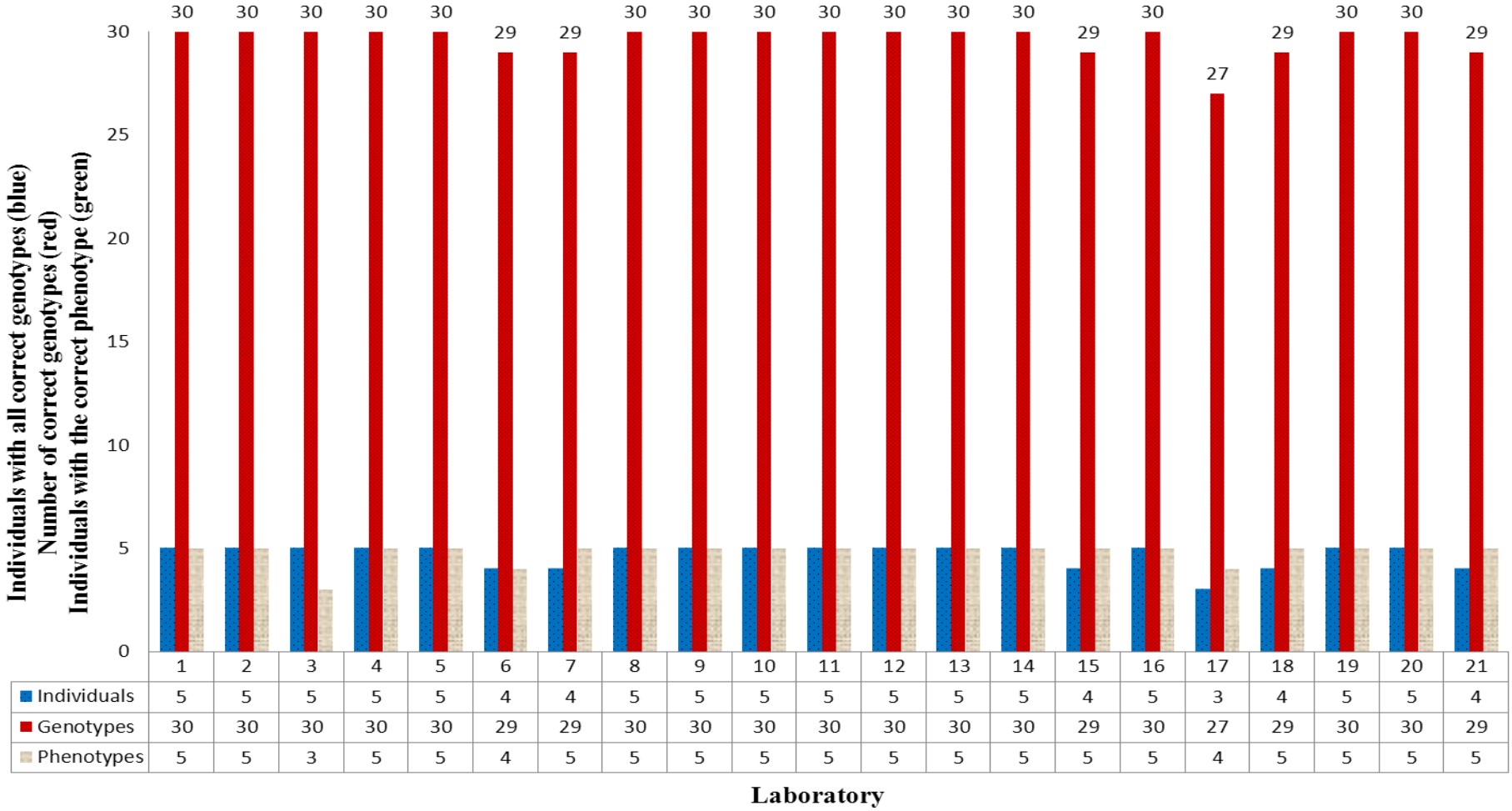
- They were asked to state what the most likely eye color prediction outcome and its likely accuracy is based on IrisPlex.



	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
Phenotypes	5	5	3	5	5	4	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5

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

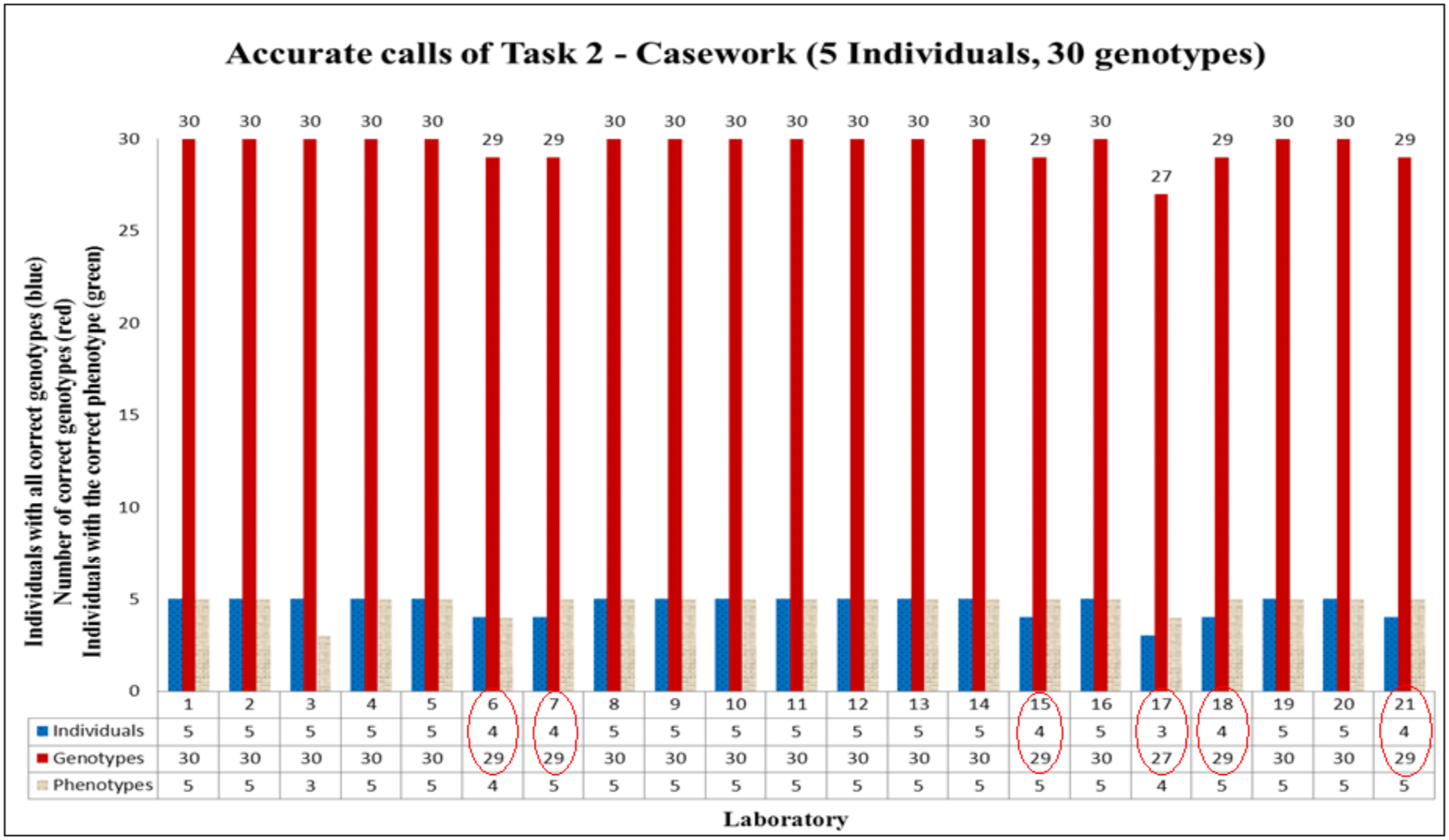
15 out of 21 Labs did not have any problems with Task 2



	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
Phenotypes	5	5	3	5	5	4	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5

622 out of 630 genotype calls correct = 98.7% accuracy

622 out of 630 genotype calls correct = 98.7% accuracy

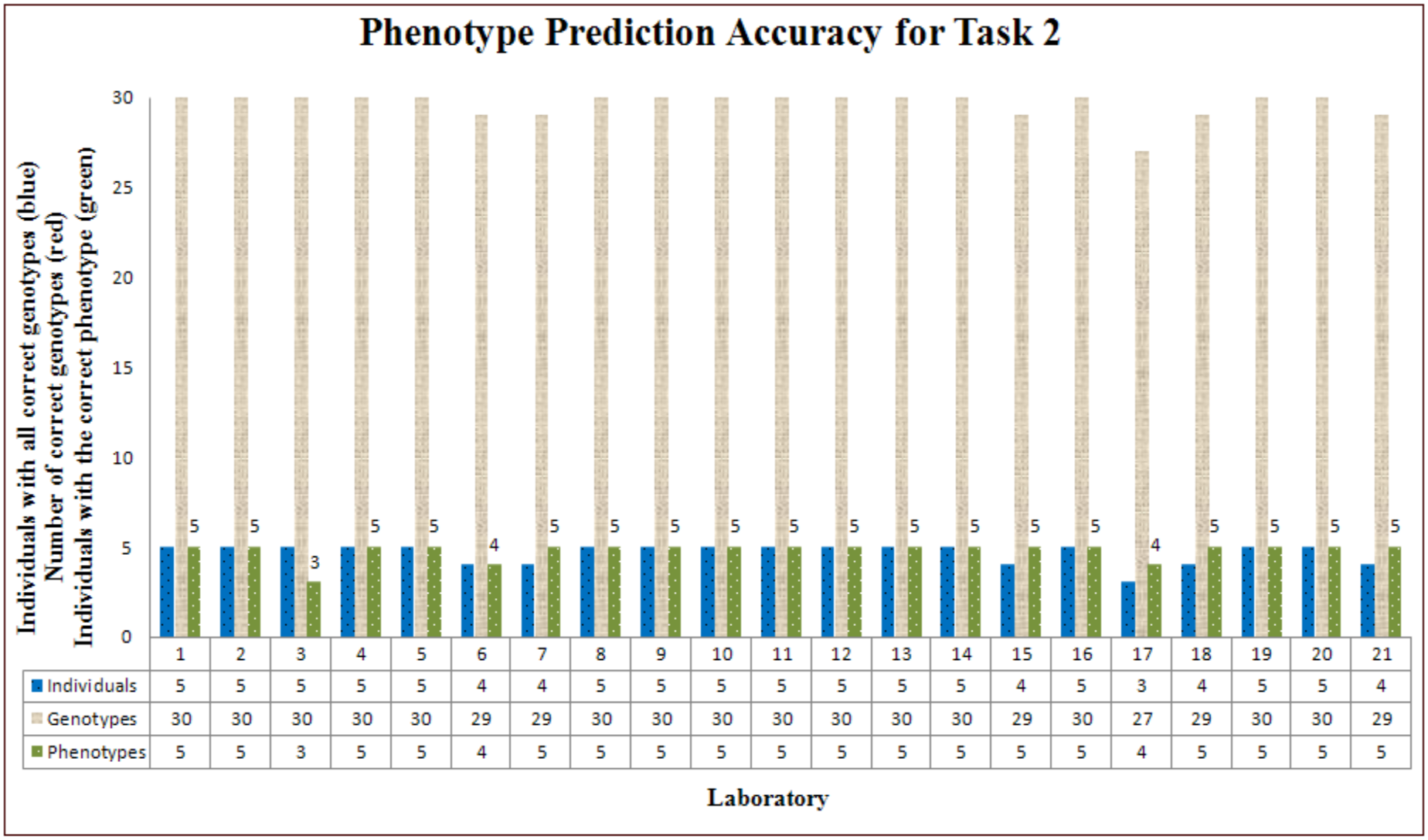


	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Individuals	5	5	5	5	5	4	4	5	5	5	5	5	5	5	4	5	3	4	5	5	4
Genotypes	30	30	30	30	30	29	29	30	30	30	30	30	30	30	29	30	27	29	30	30	29
Phenotypes	5	5	3	5	5	4	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5

The 8 incorrect genotypes were produced in 2 samples (CW2 and CW3) by 6 labs

6 incorrect genotypes (75%) did not have any impact on the phenotype accuracy.

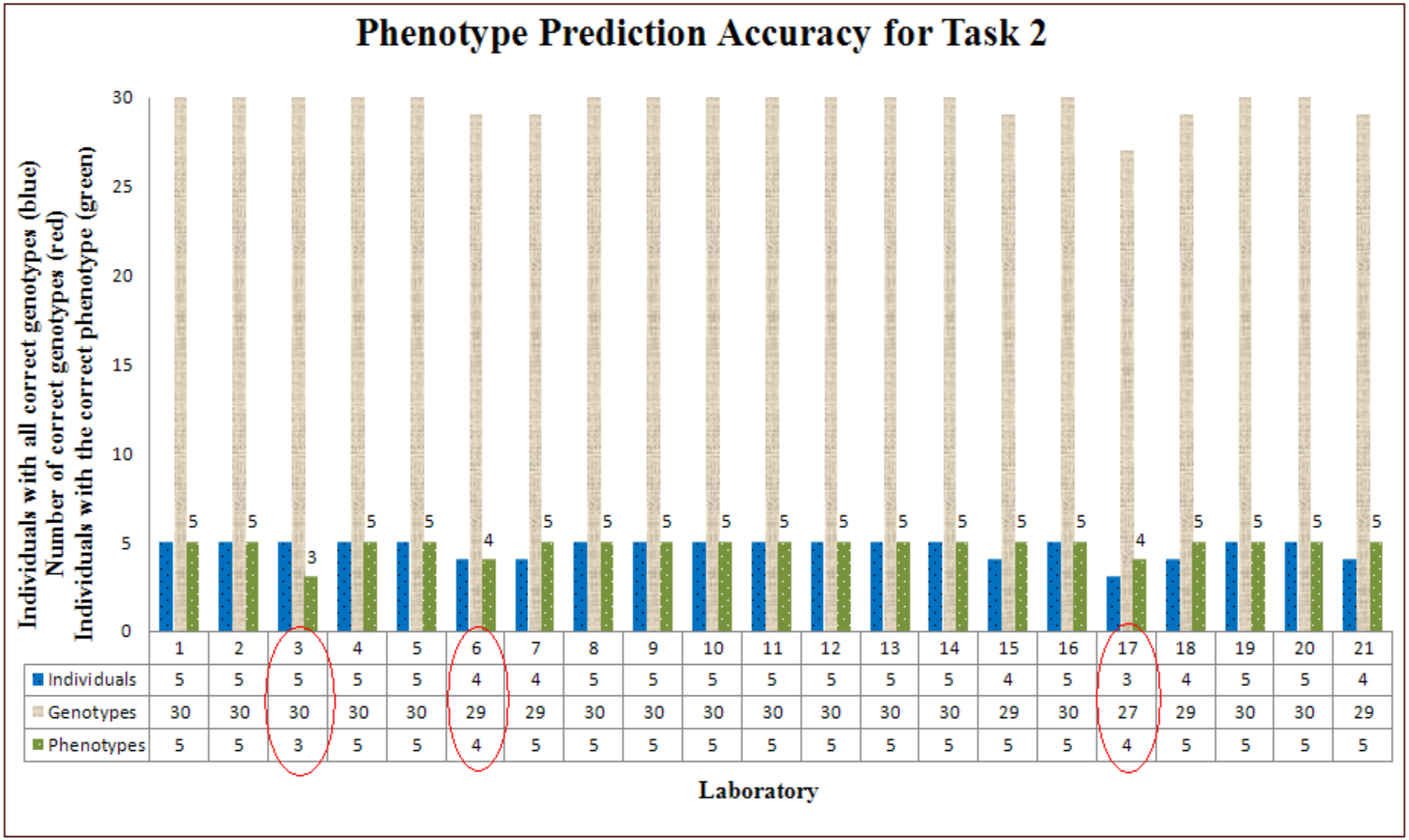
Out of 21 labs **18** of them predicted the phenotypes accurately



	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
Phenotypes	5	5	3	5	5	4	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5

101 **(96.2%)** of the 105 samples analysed by all the 21 labs were reported with the correct eye colour prediction.

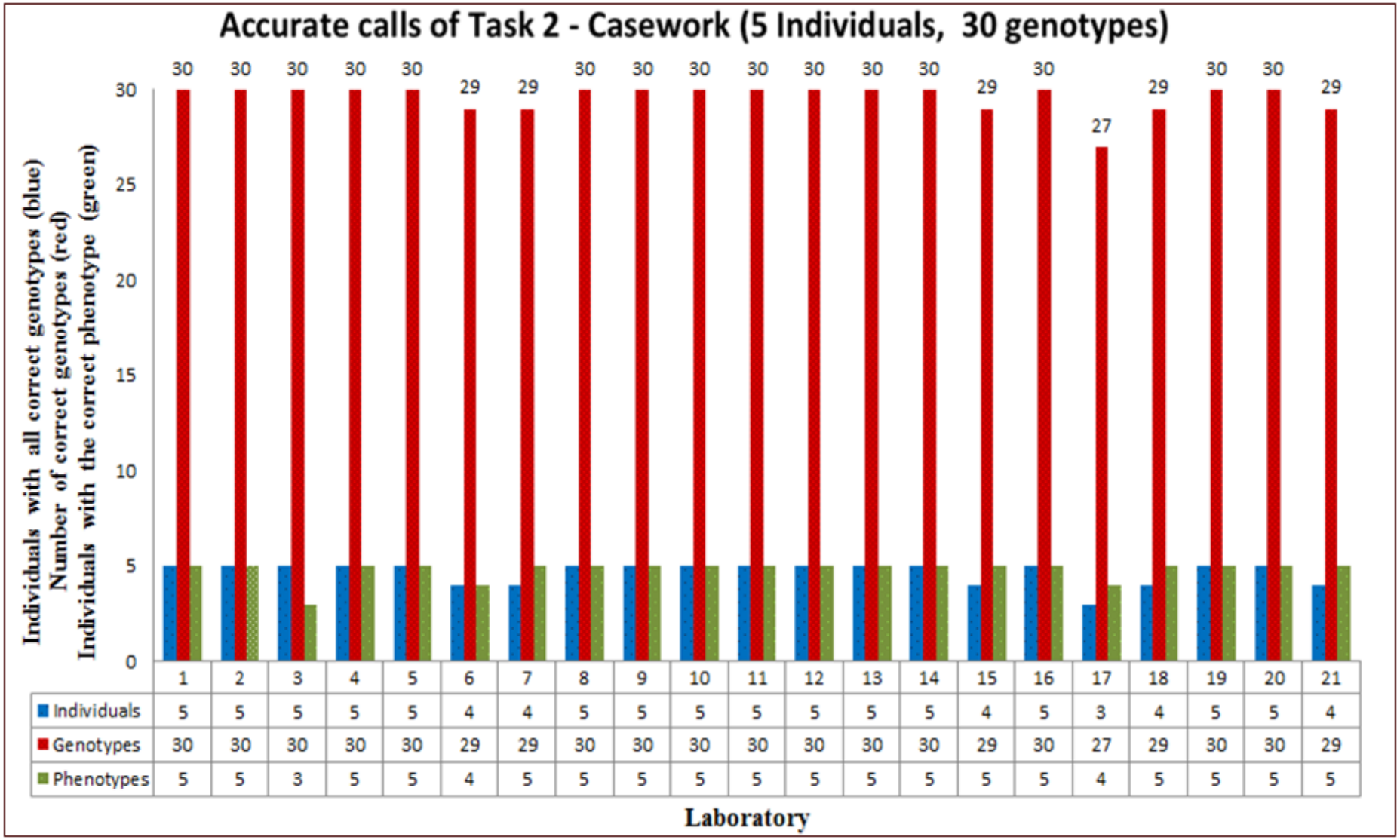
Out of 21 labs **18** of them predicted the phenotypes accurately



	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
Phenotypes	5	5	3	5	5	4	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5

Only **3** labs reported incorrect eye colour phenotype

OVERALL PREDICTION RESULTS TASK 2



	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
Phenotypes	5	5	3	5	5	4	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5

622 out of 630 genotype calls correct = 98.7% accuracy

101 out of 105 samples predicted with correct phenotype = 96.2% accuracy

Task 3 –Participant driven IrisPlex testing

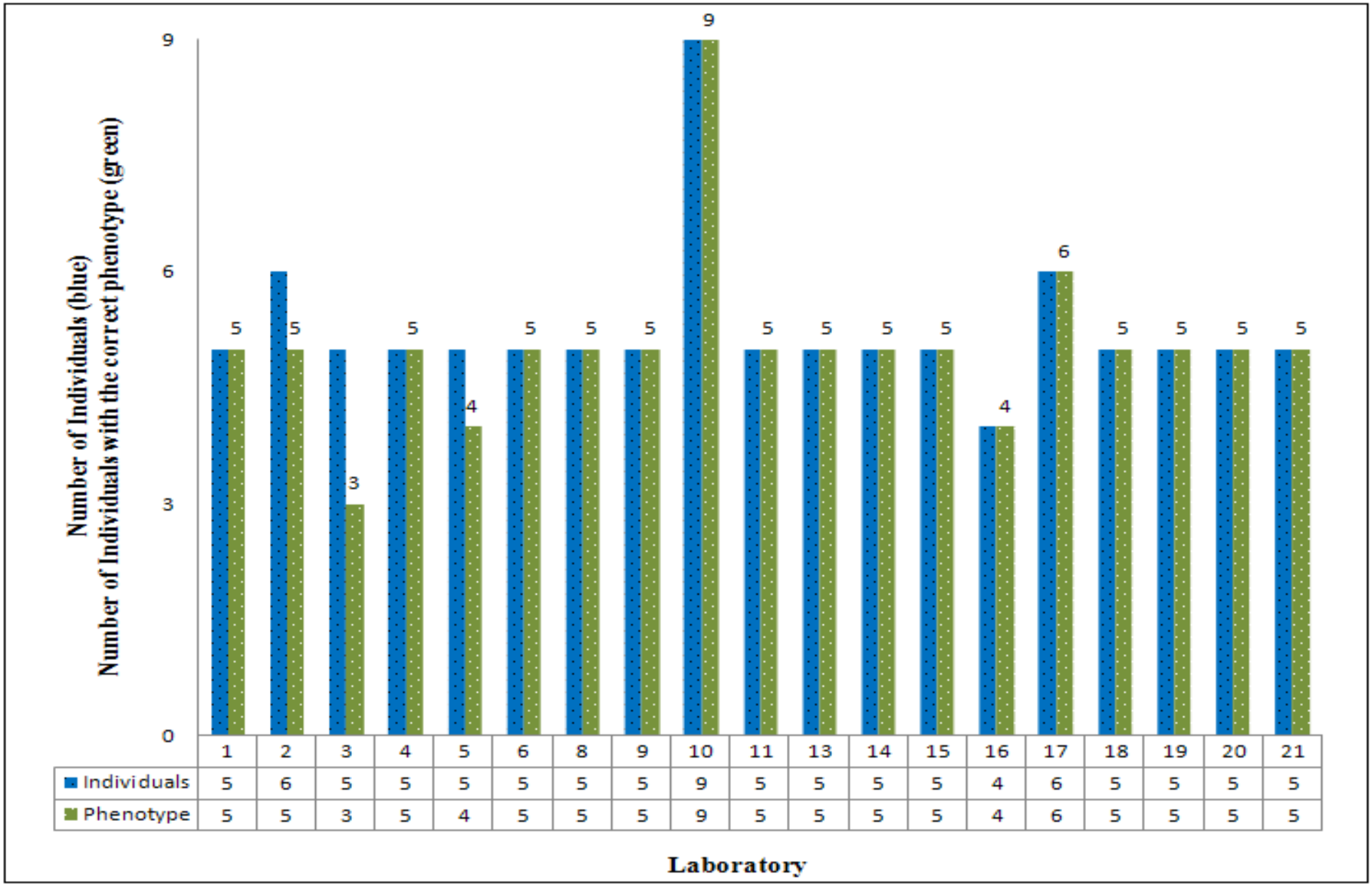
- Each group was asked to collect 5 additional DNA samples from 5 individuals in their own group of any eye color.

- 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

What to do for task 3:

- Groups 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.
- Take a digital high-resolution photo of both eyes.

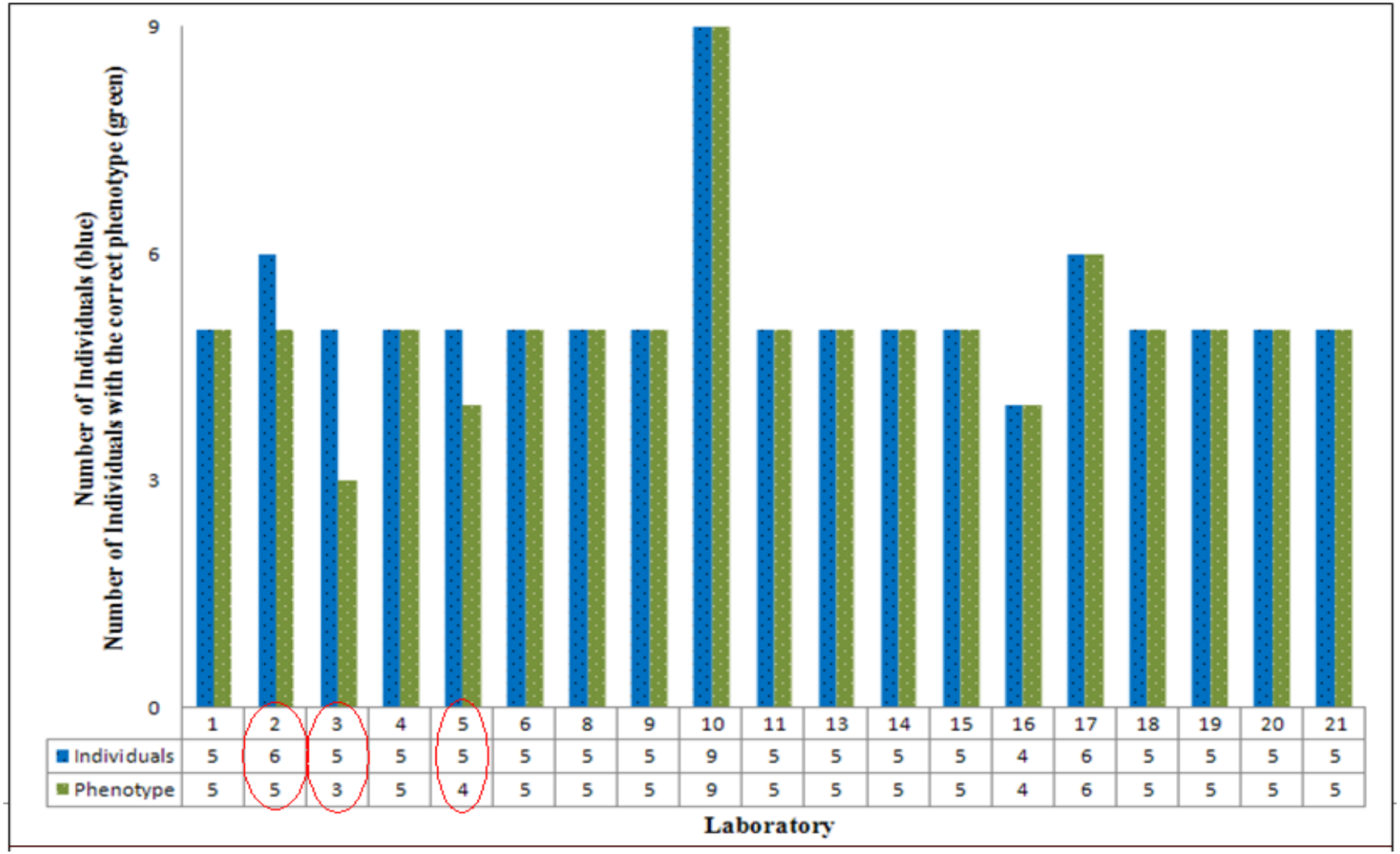
OVERALL PREDICTION RESULTS TASK 3



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

16 of the 19 labs predicted the eye colour of all analysed individual samples correctly

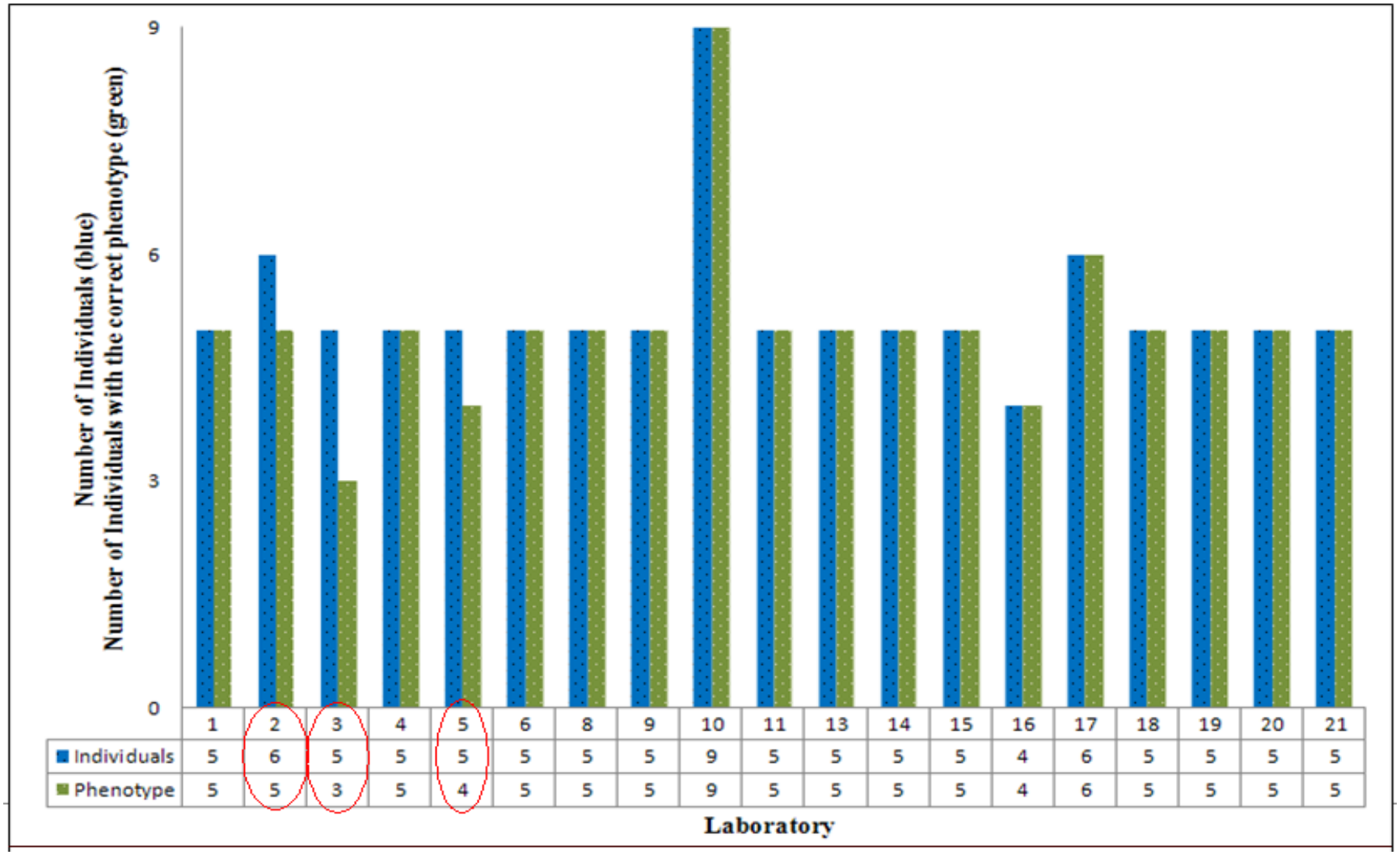
16 of the 19 labs predicted the eye colour of all analysed individual samples correctly



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

Only **3** labs faced difficulties in some samples in concluding the correct eye colour from the estimated probability combinations.

16 of the 19 labs predicted the eye colour of all analysed individual samples correctly



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

Overall, **96** of the 100 samples analysed by the 19 labs were reported with the correct eye colour prediction = **96%** accuracy

Summary Results

- Combining task 1, 2 and 3, **seventeen** of the participating laboratories did not experience any problems in inferring the correct eye colour phenotypes from the IrisPlex genotypes

- For 406 of the 415 samples in task 1-3 eye colour was correctly predicted = **97.83% phenotype prediction accuracy**

- 1875 of the 1890 genotypes generated in task 1 and 2 were correct: **99.2% genotype accuracy**

- In tasks 1 and 2 combined, only **2** wrong genotypes (0.1%) resulted in an **incorrect eye colour phenotype**, while the remaining 1873 (99.9%) incorrect genotypes had no consequence on the phenotype prediction accuracy.

Please provide feed-back to the manuscript until December 1

EMPOP Update

Walther Parson
Institute of Legal Medicine
Innsbruck Medical University
Austria

EMPOP update

1. New EMPOP related publications
   - Phylogenetics
   - SWGDAM adapted alignment guidelines to EMPOP nomenclature
2. Past meetings
3. EMPOP Trainings
4. EMPOP database update

1. New publications

Evaluation of next generation mtGenome sequencing using the Ion Torrent Personal Genome Machine (PGM).
Parson W, Strobl C, Huber G, Zimmermann B, Gomes SM, Souto L, Fendt L, Delport R, Langit R, Wootton S, Lagacé R, Irwin J.
Forensic Sci Int Genet. 2013 7(6):632-9
Update in Bratislava

Mass spectrometric base composition profiling: Implications for forensic mtDNA databasing.
Eduardoff M, Huber G, Bayer B, Schmid D, Anslinger K, Göbel T, Zimmermann B, Schneider PM, Röck AW, Parson W.
Forensic Sci Int Genet. 2013 7(6):587-92

Concept for estimating mitochondrial DNA haplogroups using a maximum likelihood approach (EMMA).
Röck AW, Dür A, van Oven M, Parson W.
Forensic Sci Int Genet. 2013 7(6):601-9

Relevance of mitochondrial phylogeny for forensics

Ancestry informative marker

Alignment and reporting of haplotypes

Estimation for the mitochondrial haplogroup

Quality control mechanisms

Reporting mtDNA sequences

Control Region sequence WP (16024-576; 1124 bp)

TTCTTTCATGGGGAAGCAGATTTGGGTACCACCCAAGTATTGACTCACCCATCAACAACCGCTATGTATTTCGTACATTACTGCCAGCCACCATGAATATTGTACGGTACCATAA
ATACTTGACCACCTGTAGTACATAAAAACCCAATCCACATCAAAACCCCCCCCCCCATGCTTACAAGCAAGTACAGCAATCAACCCTCAACTATCACACATCAACTGCAACTCCA
AAGCCACCCCTCACCCACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAGTACATAAAGCCATTTACCGTACATAGCACATTACAGTCAAATCCCCCTCTCCCCCCA
TGGATGACCCCCCTCAGATAGGGGTCCCTTGACCACCATCCTCCGTGAAATCAATATCCCGCACAAGAGTGCTACTCTCCTCGCTCCGGGCCCATAACACTTGGGGGTAGCTA
AAGTGAACTGTATCCGACATCTGGTTCCTACTTCAGGGCCCATAAAGCCTAAATAGCCCACACGTTCCCCTTAAATAAGACATCACGATGGATCACAGGTCTATCACCCTATTAAC
CACTCACGGGAGCTCTCCATGCATTTGGTATTTTCGTCTGGGGGGTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTC
CTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTTGTAGGACATRATAATAACAATTGAATGTCTGCAC
AGCCGCTTTCCACACAGACATCATAACAAAAAATTTCCACCAAACCCCCCCTCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAAACAAAGAACCCT
AACACCAGCCTAACCAGATTTCAAATTTTATCTTTTGGCGGTATGCACTTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCACTCCCATACTACTAATCTCATCAATA
CAACCCCCGCCCATCCTACCCAGCACACACACC--CGCTGCTAACCCCATACCCCGAACCAACCAAACCCCAAAGACACCCCCCCCACA

rCRS-coded

16024-576
16189C 16193.1C 16356C 16362C 16519C 234R 263G 315.1C 523del 524del 573.1C 573.2C

rCRS-coded alignment is straight forward except for indels, because they can be aligned in multiple ways and we have no experimental approach to determine which is the correct one

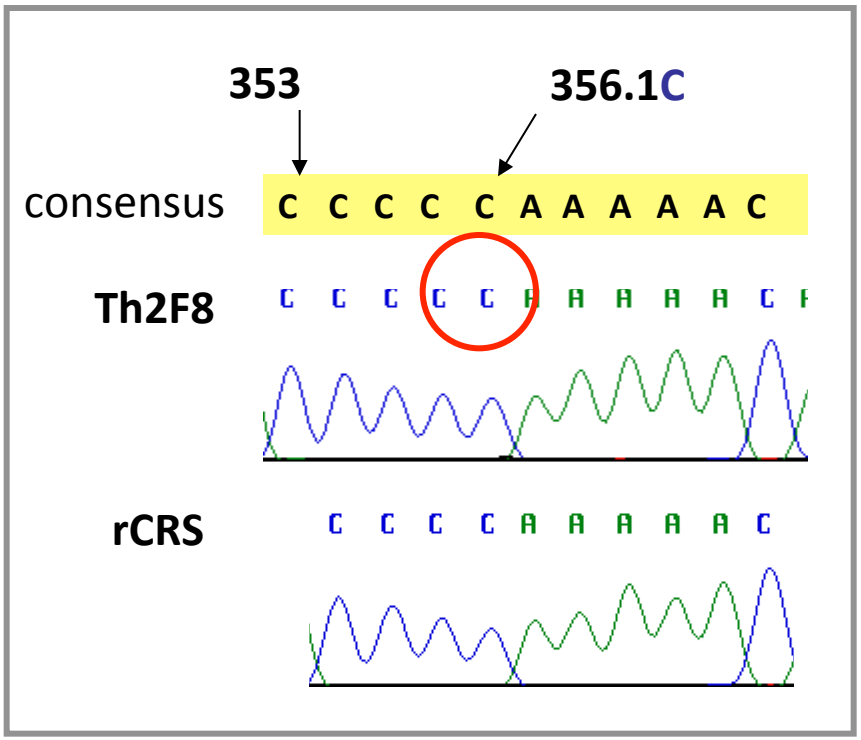
Problem
Alignment of indels

Alignment rules (length variants)

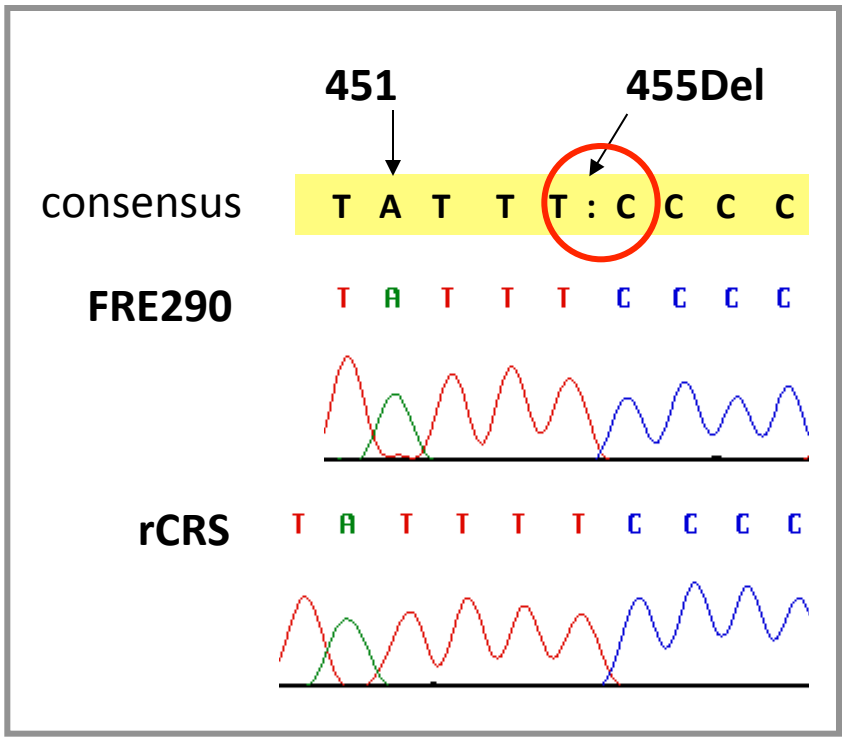
ISFG guidelines (Bär et al 2000, Carracedo et al 2000)

3’ convention for indels in length variants - still multiple notations possible

365.1C (Th2F8, Thailand, EMPOP)



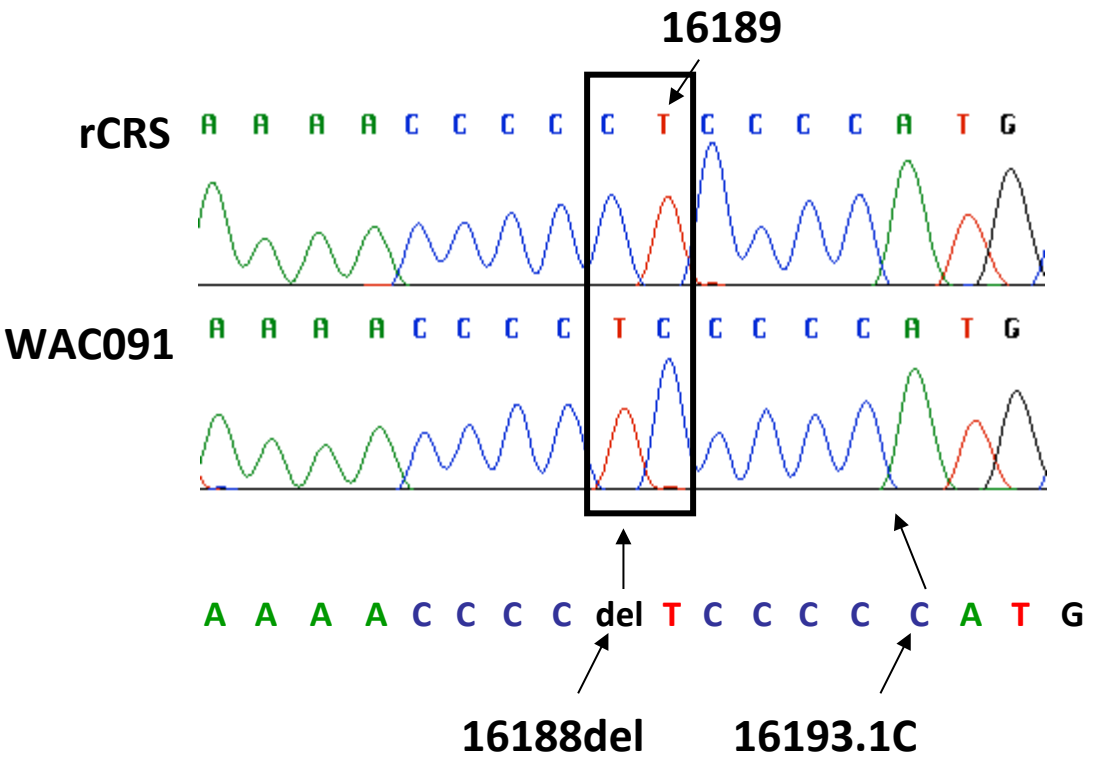
455Del (FRE390, Germany, EMPOP)



Multiple possible alignments

16189C 16193.1C 16356C 16362C 16519C 234R 263G 315.1C 523del 524del 573.1C 573.2C

16188T 16189C 16356C 16519C 263G 315.1C - WAC091 (difference at 16188T)



„jumping alignment“

mutation rates

16188T 16189C (1 diff to WP)

16188Del 16193.1C (2 diff to WP)
Wilson et al 2002

1) parsimony
2) indels>transitions>transversions
3) 3‘ alignment

phylogenetic alignment

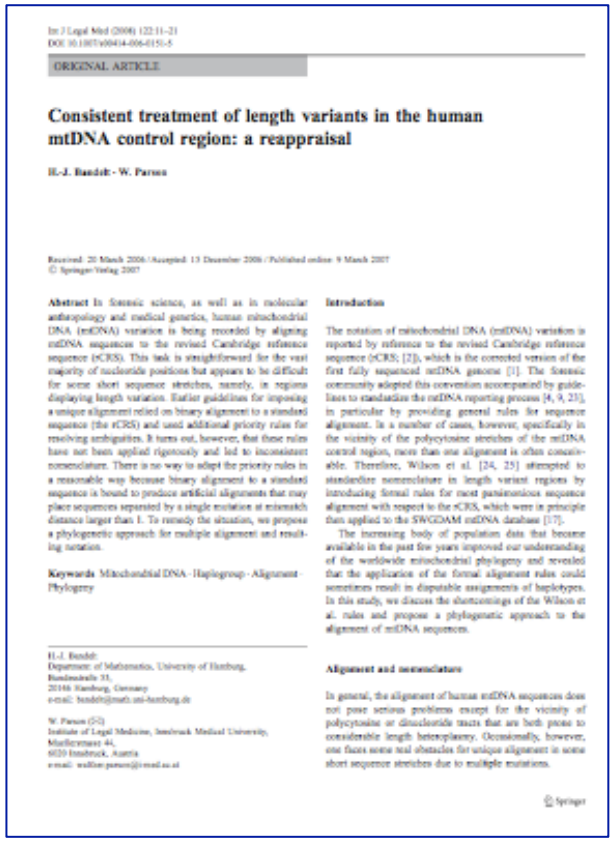
EMPOP uses phylogenetic alignment

Bandelt and Parson (2008) Consistent treatment of length variants in the human mtDNA control region: a reappraisal, Int J Legal Med 122:1-21

Rule 1. Phylogenetic rule

Rule 2. Anchor 16189 and 310

Rule 3. 3' alignment



Int J Legal Med (2008) 122:11–21
DOI 10.1007/s00414-006-0151-5

ORIGINAL ARTICLE

Consistent treatment of length variants in the human mtDNA control region: a reappraisal

H.-J. Bandelt · W. Parson

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Abstract In forensic science, as well as in molecular anthropology and medical genetics, human mitochondrial DNA (mtDNA) variation is being recorded by aligning mtDNA sequences to the revised Cambridge reference sequence (rCRS). This task is straightforward for the vast majority of nucleotide positions but appears to be difficult for some short sequence stretches, namely, in regions displaying length variation. Earlier guidelines for imposing a unique alignment relied on binary alignment to a standard sequence (the rCRS) and used additional priority rules for resolving ambiguities. It turns out, however, that these rules have not been applied rigorously and led to inconsistent nomenclature. There is no way to adopt the priority rules in a reasonable way because binary alignment to a standard sequence is bound to produce artificial alignments that may place sequences separated by a single mutation at mismatch distance larger than 1. To remedy the situation, we propose a phylogenetic approach for multiple alignment and resulting notation.

Keywords Mitochondrial DNA · Haplogroup · Alignment · Phylogeny

H.-J. Bandelt
Department of Mathematics, University of Hamburg,
Bundesstraße 55,
20146 Hamburg, Germany
e-mail: bandelt@math.uni-hamburg.de

W. Parson (✉)
Institute of Legal Medicine, Innsbruck Medical University,
Müllerstrasse 44,
6020 Innsbruck, Austria
e-mail: walther.parson@i-med.ac.at

Introduction

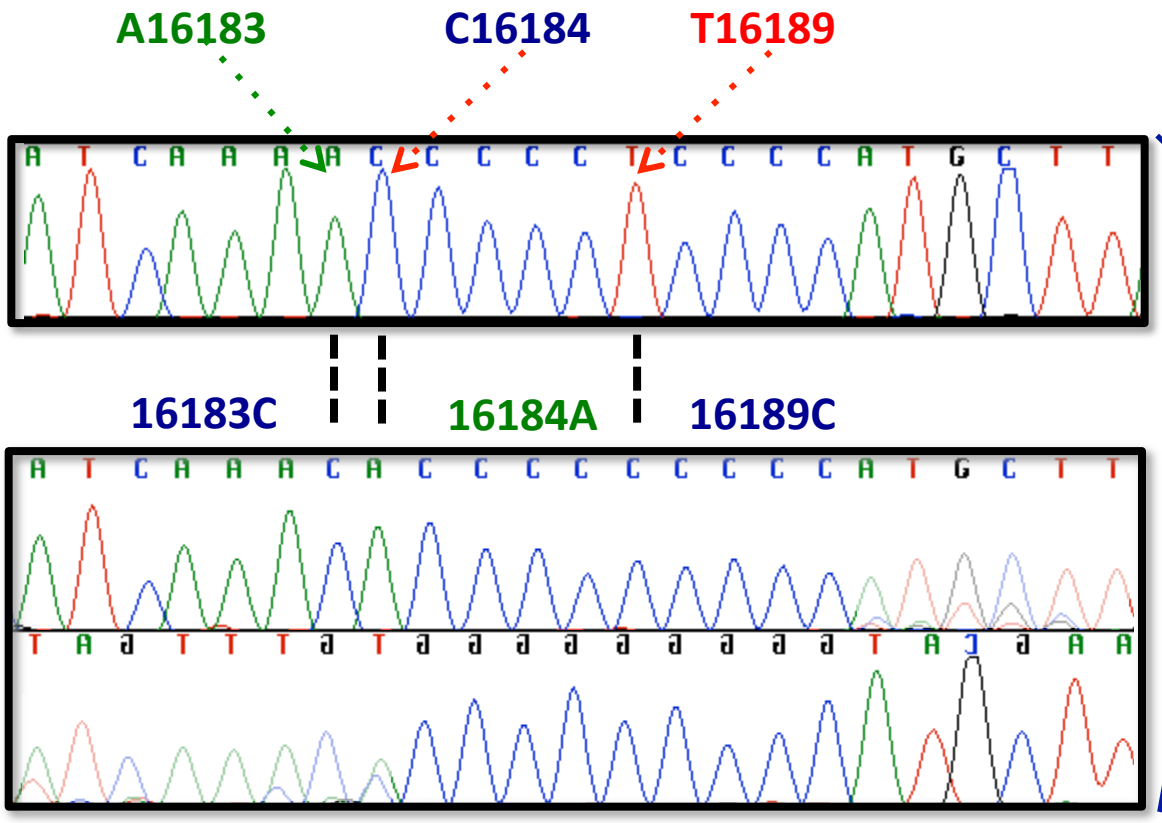
The notation of mitochondrial DNA (mtDNA) variation is reported by reference to the revised Cambridge reference sequence (rCRS; [2]), which is the corrected version of the first fully sequenced mtDNA genome [1]. The forensic community adopted this convention accompanied by guidelines to standardize the mtDNA reporting process [4, 9, 23], in particular by providing general rules for sequence alignment. In a number of cases, however, specifically in the vicinity of the polycytosine stretches of the mtDNA control region, more than one alignment is often conceivable. Therefore, Wilson et al. [24, 25] attempted to standardize nomenclature in length variant regions by introducing formal rules for most parsimonious sequence alignment with respect to the rCRS, which were in principle then applied to the SWGDAM mtDNA database [17].

The increasing body of population data that became available in the past few years improved our understanding of the worldwide mitochondrial phylogeny and revealed that the application of the formal alignment rules could sometimes result in disputable assignments of haplotypes. In this study, we discuss the shortcomings of the Wilson et al. rules and propose a phylogenetic approach to the alignment of mtDNA sequences.

Alignment and nomenclature

In general, the alignment of human mtDNA sequences does not pose serious problems except for the vicinity of polycytosine or dinucleotide tracts that are both prone to considerable length heteroplasmy. Occasionally, however, one faces some real obstacles for unique alignment in some short sequence stretches due to multiple mutations.

Parsimony and alignment



MAS019
16147T
16183C
16184A
16189C B
16217C B4
16235G
16294T
16519C
73G
263G
309.1C
315.1C
523DEL
524DEL

16183C 16184A 16189C
16182.1C 16189DEL

phylogenetic alignment
parsimonious alignment

most parsimonious call may result in jumping alignment

History of forensic alignment rules

ISFG guidelines (Bär et al. 2000)

3’ convention for indels in length variants - still multiple notations possible

Hierarchical Wilson rules (Wilson et al. 2002)

1) minimal number of differences (unweighted parsimony)
2) indels > transitions > transversions
3) 3’ convention for indels

problem - 150T 151C would need to be called 150del 151.1C

Phylogenetic alignment (Bandelt and Parson, 2008)

1) alignment according to mtDNA phylogeny
2) C-tract conventions
3) 3’ convention for indels except phylogeny suggests otherwise

“MitoTyper” rules (Budowle et al. 2010)

Corrected and extended Wilson rules based on unweighted parsimony

problem – parsimony

cannot be performed manually – “black box”

New SWGDAM mtDNA guidelines use phylogenetic-based alignment (July 2013)

Parsimony and alignment

Parsimony

It seems to be a straight forward approach to determine the most parsimonious alignment because it uses the fewest number of differences to the rCRS, but…

CHN.ASN.000206 (24 differences to rCRS)

16181C 16182C 16183C 16189C 16213A 16217C 16242T 16261T 16292T 16301T 16519C

61A 62A 73G 183G 263G 309.1C 309.2C 309.3C 315.1C 323N 324N 523Del 524Del

How many possible alignments tolerating 24+1 mutations?

953,110

It is practically **impossible** to manually determine all alignments (and therefore the most parsimonious alignment)

Jumping alignments

Effect of jumping alignments on database searches (EMPOP, N=26.930)

Diff (16184-16194)	16188T 16189C	16188del 16193.1C
0	17	0
1	3,524	21
2	21,521	20,333
3	1,852	5,252

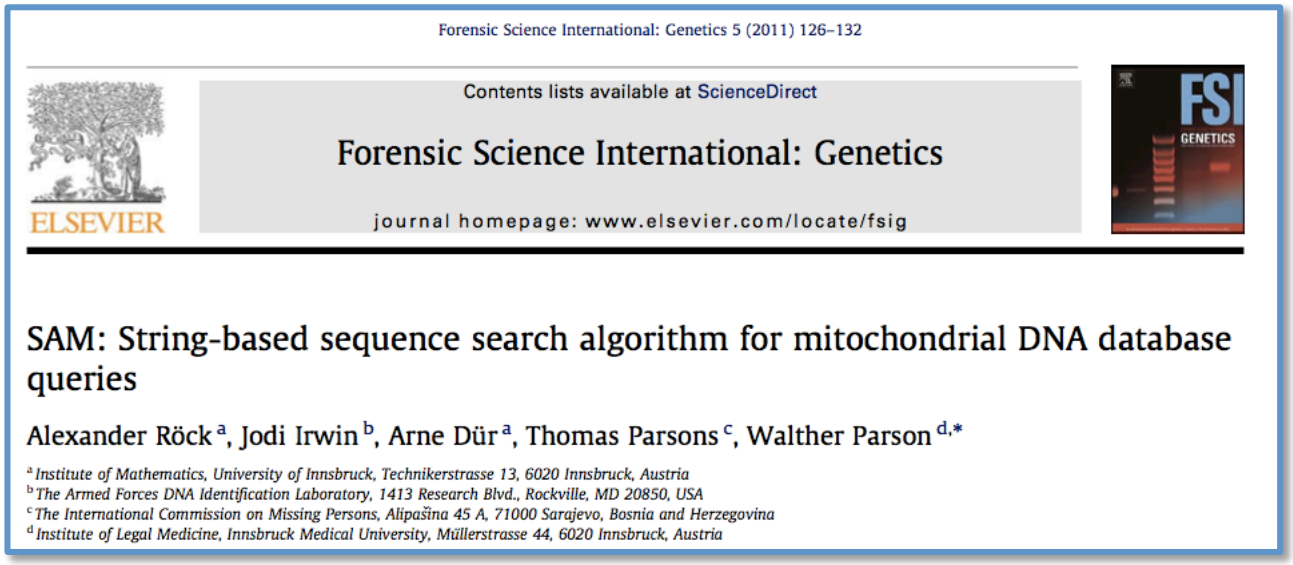
Jumping alignments arise when non-phylogenetic alignment is performed

⇒ implications for frequency estimates

Solution to the alignment dilemma

Translate all difference-coded haplotypes into **alignment-free** strings of nucleotides (query and database)

Thus, the database search becomes **independent** from alignment



Forensic Science International: Genetics 5 (2011) 126–132

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

SAM: String-based sequence search algorithm for mitochondrial DNA database queries

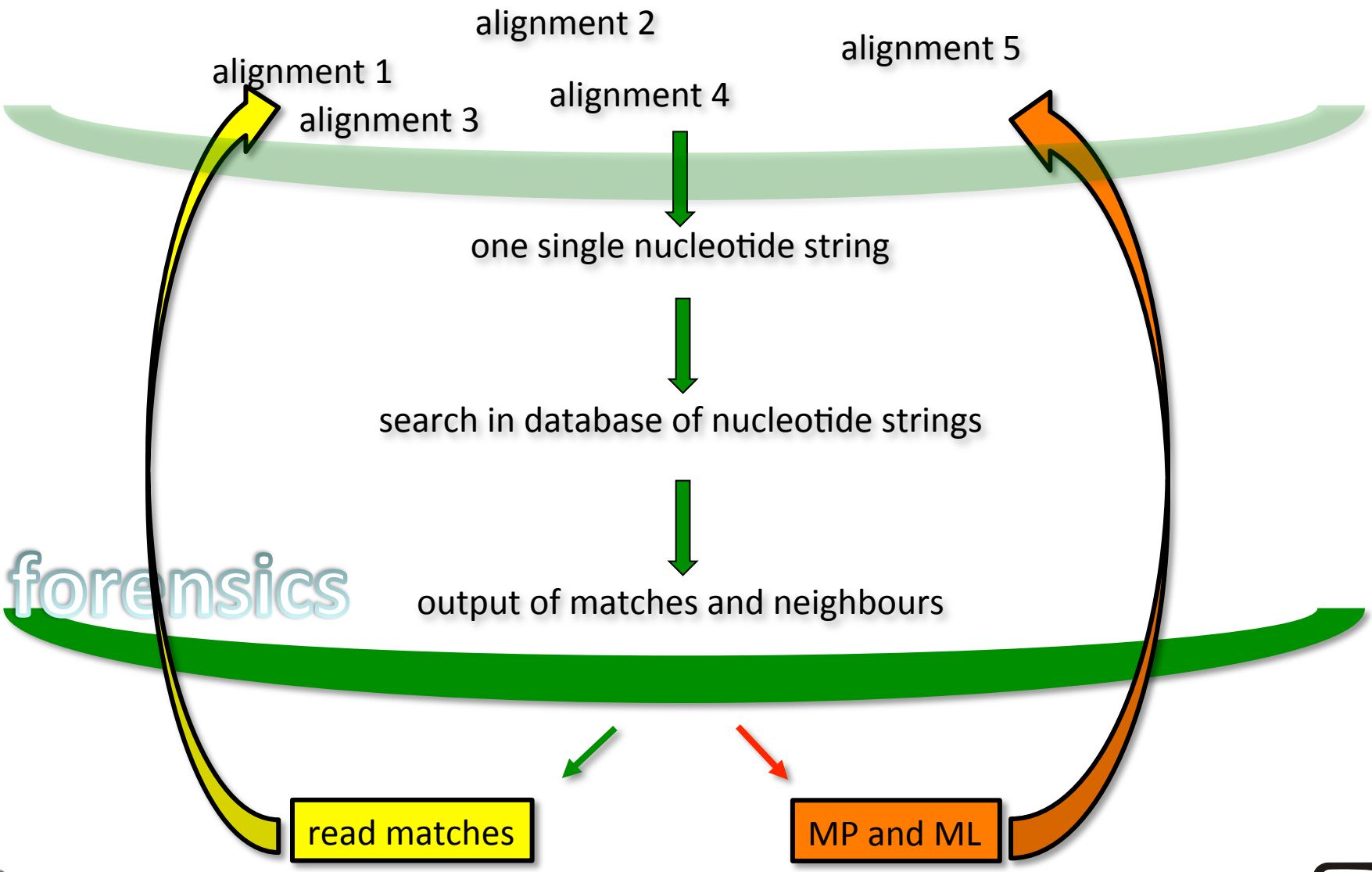
Alexander Röck [a], Jodi Irwin [b], Arne Dür [a], Thomas Parsons [c], Walther Parson [d,*]

[a] Institute of Mathematics, University of Innsbruck, Technikerstrasse 13, 6020 Innsbruck, Austria
[b] The Armed Forces DNA Identification Laboratory, 1413 Research Blvd., Rockville, MD 20850, USA
[c] The International Commission on Missing Persons, Alipašina 45 A, 71000 Sarajevo, Bosnia and Herzegovina
[d] Institute of Legal Medicine, Innsbruck Medical University, Müllerstrasse 44, 6020 Innsbruck, Austria

Haplotype not missed in a database search

Validation at http://stringvalidation.org

Scheme of string-based query



Relevance of mitochondrial phylogeny for forensics

Ancestry informative marker

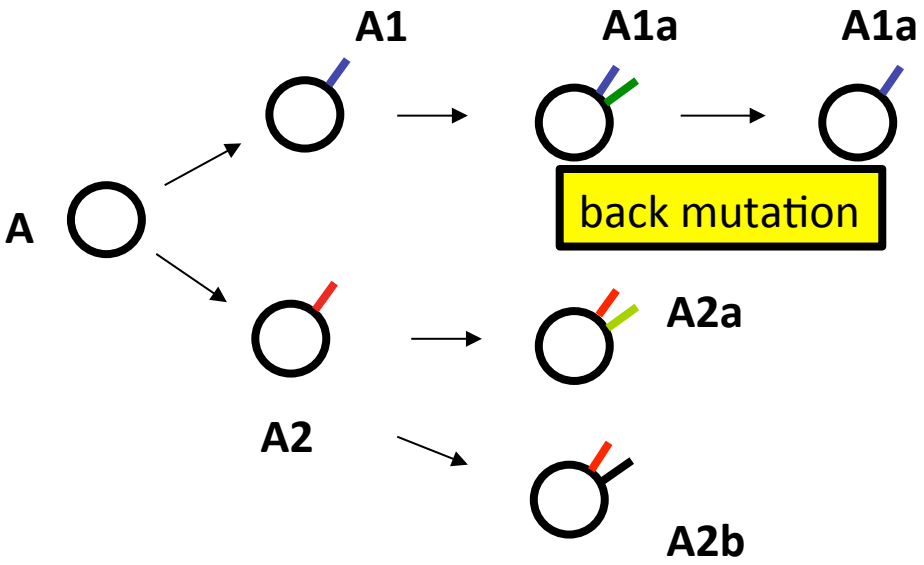
Alignment and reporting of haplotypes

Estimation for the mitochondrial haplogroup

Quality control mechanisms

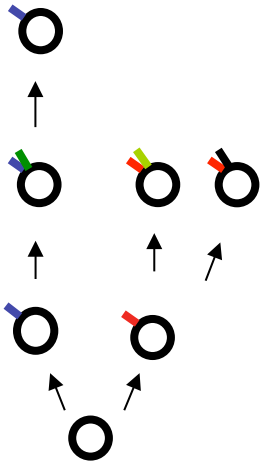
Evolution of maternal lineages: phylogeny

subsequently acquired mutations
addition onto precursor molecule
no recombination



evolution of maternal lineages

lineages are called haplogroups
groups of haplotypes with same diagnostic SNP pattern
hierarchical order in a **phylogenetic tree**



Haplogrouping

Haplogrouping

is the estimation of the haplogroup status of an (mtDNA) haplotype

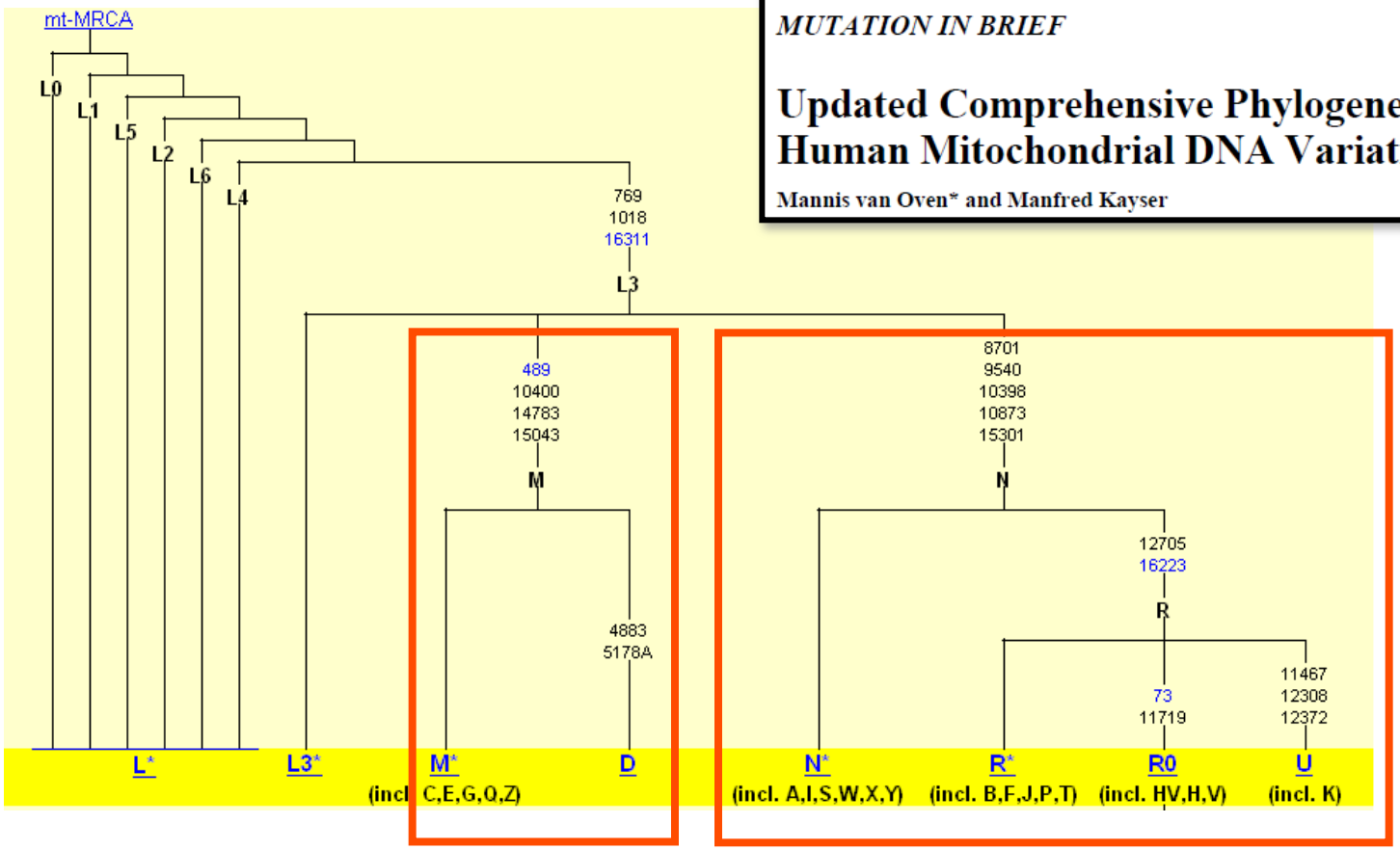
Phylotree (vanOven and Kayser, 2009)

phylogenetic tree of global human mitochondrial DNA variation, based on both coding- and control-region mutations, and including haplogroup nomenclature (www.phylotree.org)

most comprehensive human mtDNA tree

reference for haplogroup assignment

Phylotree



HUMAN MUTATION Mutation in Brief #1039, 30:E386-E394, (2008) Online

MUTATION IN BRIEF

Updated Comprehensive Phylogenetic Tree of Global Human Mitochondrial DNA Variation

Mannis van Oven* and Manfred Kayser

Haplogroup L
Africa
(America)

Haplogroup M
Eastern Eurasia
Native America

Haplogroup N
Eurasia
(Native)America

Searching Phylotree manually

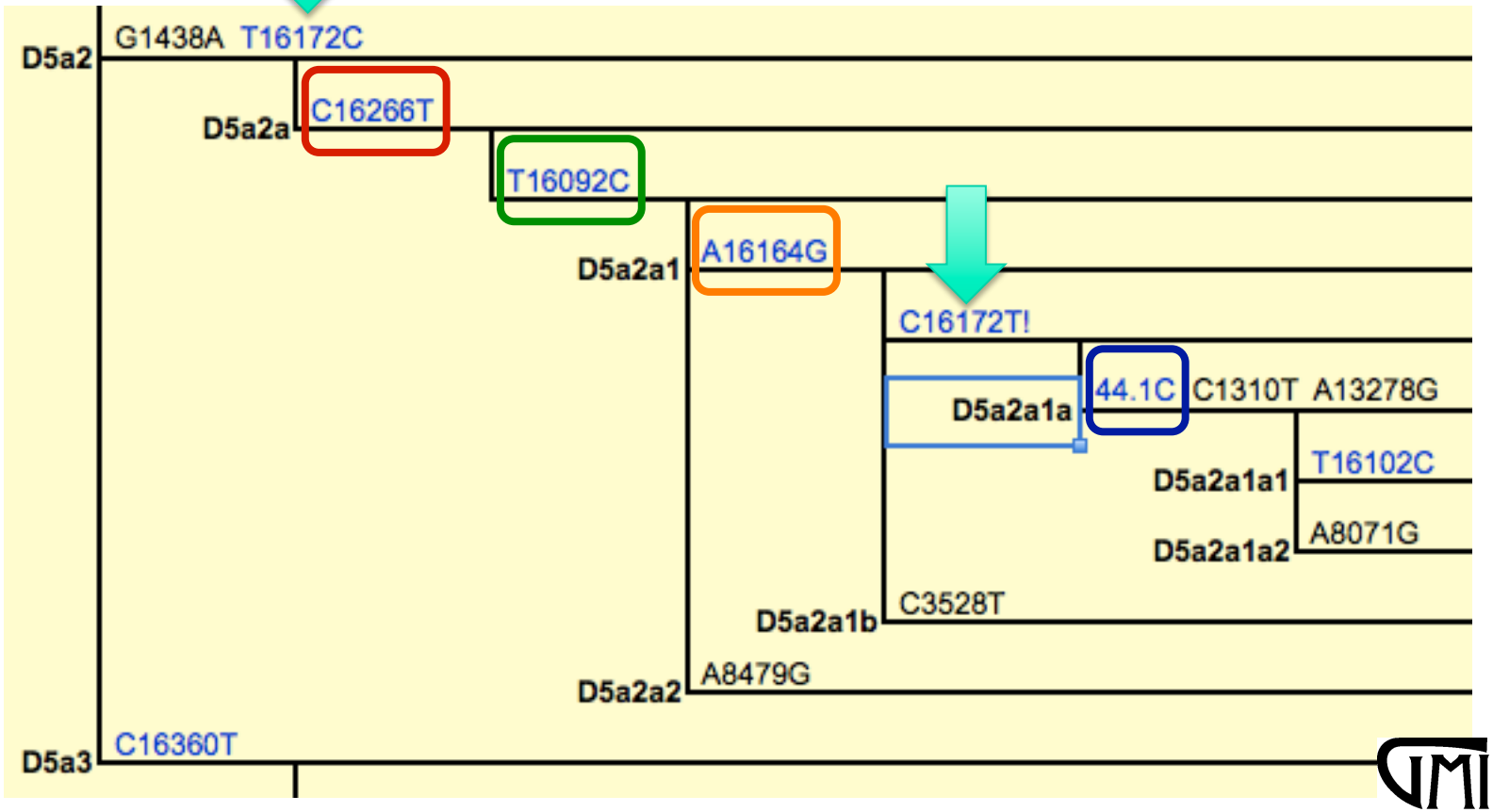
16024-576

D5 **M/N/L** **D**

16092C **16164G** 16182C 16183C 16189C 16223T **16266T** 16362C 16525G

44.1C 73G 150T 263G 315.1C 489C 523DEL 524DEL - **Haplogroup D5a2a1a**

M/N/L **D5** **M**

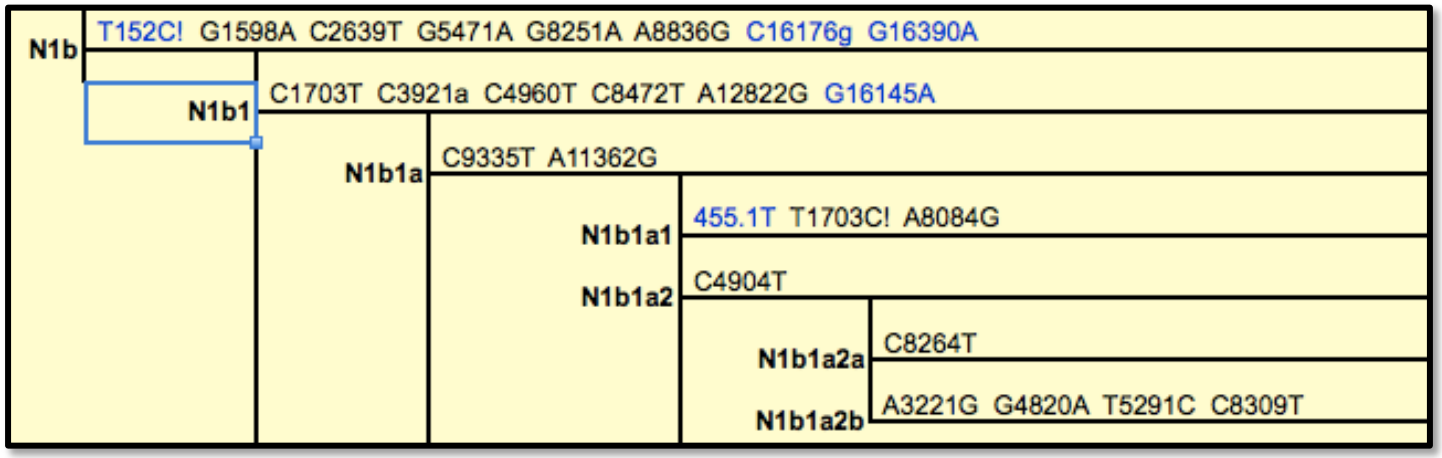


Example 1 - manual search

MtDNA haplotype from Macedonia; rCRS coded, 16024-576)

16145A	16176G	16223T	16390A	16519C	73G	152C	195C	263G	315.1C
25	1	5	28	n.a.	23	173	92	4	n.a.
many	N1b	L3/M/N*	many	many	all*	many	many	all*	all*

- with exceptions (recurrent mutations)



vanOven and Kayser, 2009; www.phylotree.org

Example 1 - manual search

MtDNA haplotype from Macedonia; rCRS coded, 16024-576)

16145A	16176G	16223T	16390A	16519C	73G	152C	195C	263G	315.1C
25	1	5	28	n.a.	23	173	92	4	n.a.
many	N1b	L3/M/N*	many	many	all*	many	many	all*	all*

- with exceptions (recurrent mutations)

Caveats:

manual haplogrouping requires experience

Phylotree haplotypes are virtual haplotypes as they miss

some mutations systematically (e.g. 16519, CA-repeat, 315.1C, …)

“private mutations”

Searching Phylotree manually

more difficult example

MtDNA haplotype from Argentina (Bobillo et al 2010, 16024-576)

16189C	16292T	16519C	71A	153G	204C	207A	263G	315.1C	373G
91	14	n.a.	0	10	29	33	4	n.a.	5
many	some**	all*	?	some**	many	many	all*	all*	some**

* with exceptions (back mutations)

**

373G - E1a2, H5n, R6a1a, R30b, U5b3a1a

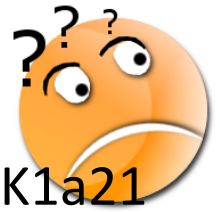
153G - M38b, C7a1a, M9a'b, M36b, M39b, A2, X1'2'3, H3b1b, H4b1, K1a21

haplogroup cannot be found manually with phylotree

Searching Phylotree manually

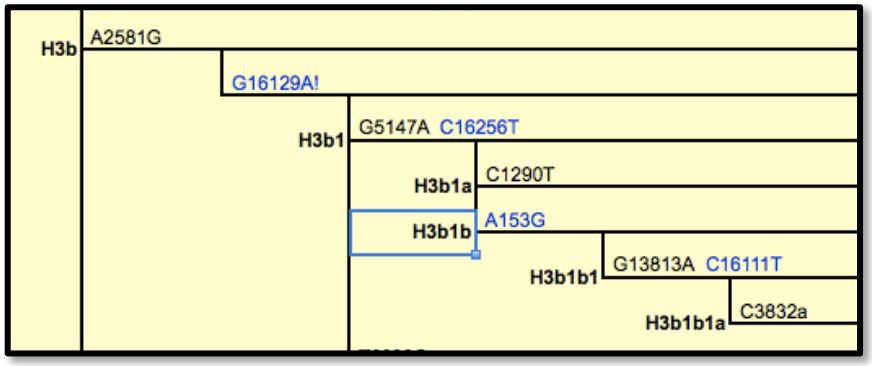
MtDNA haplotype from Argentina (Bobillo et al 2010, 16024-576)

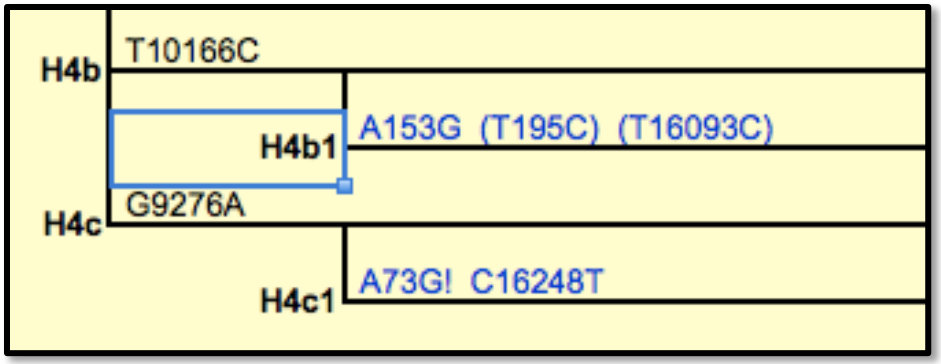
16189C	16292T	16519C	71A	153G	204C	207A	263G	315.1C	373G
91	14	n.a.	0	10	29	33	4	n.a.	5
many	some**	all*	?	some**	many	many	all*	all*	some**



373G - E1a2, H5n, R6a1a, R30b, U5b3a1a

153G - M38b, C7a1a, M9a'b, M36b, M39b, A2, X1'2'3, H3b1b, H4b1, K1a21





Searching full mtGenomes

MtDNA haplotype from Argentina (Bobillo et al 2010, 16024-576)

16189C 16292T 16519C 71A 153G 204C 207A 263G 315.1C 373G

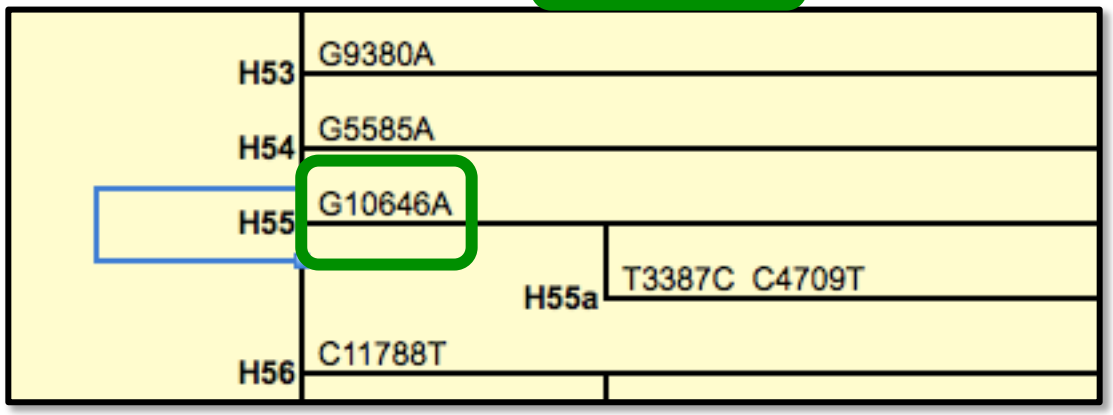
additional codR sequencing confirmed G10646A and therefore H55 (built 15)

search in database of full mtGenomes (real haplotypes)

best match: JQ705203 - hg H55

T16189C T16519C A153G T204C A263G -309.1C -309.2C -315.1C

A750G A1438G A4769G A8860G G10646A A15326G



Software-aided haplogrouping

published haplogrouping software

mtDNA manager (Lee et al 2008) - **not using** phylotree

HaploGrep (Kloss-Brandstätter et al 2011) - phylotree **motifs** only, no private mutations (**only virtual haplotypes**)

HaploFind (Vianello et al 2013) - phylotree, cannot query CR sequences (only full mtGenomes)

Automated haplogrouping

more difficult example (H55)

MtDNA haplotype from Argentina (Bobillo et al 2010, 16024-576)

16189C	16292T	16519C	71A	153G	204C	207A	263G	315.1C	373G
91	14	n.a.	0	10	29	33	4	n.a.	5
many	some**	all*	?	some**	many	many	all*	all*	some

mtDNA manager - H

HaploGrep - H1+16189

same limitation as manual search, motif lists or virtual haplotypes lead to biased or wrong haplogrouping results

EMMA

Forensic Science International: Genetics 7 (2013) 601–609

Concept for estimating mitochondrial DNA haplogroups using a maximum likelihood approach (EMMA)☆

Alexander W. Röck[a], Arne Dür[b], Mannis van Oven[c], Walther Parson[a,d,*]

[a] Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria
[b] Institute of Mathematics, University of Innsbruck, Innsbruck, Austria
[c] Department of Forensic Molecular Biology, Erasmus MC, University Medical Center Rotterdam, The Netherlands
[d] Penn State Eberly College of Science, University Park, PA, USA

EMMA uses

- phylotree haplogroup nomenclature
- virtual phylotree haplotypes (3,925; phylotree build 15)
- curated database of full mtGenomes (14,990; build 15)

more precise haplogroup estimates

e.g. MtDNA haplotype from Argentina (Bobillo et al 2010, 16024-576)

Two best matches assign hg H55

"weigh the mutations"

Mutation rates differ across mtGenome and within hgs

	worldwide	hg X1‘3	hg K	hg T
T16519C	in 18,362 of 40,246	in 8 of 8	in 893 of 931	in 1,124 of 1,171
%	45.6	100	95.5	95.9

We defined discernible control region haplogroups (CR-HGs), clusters of haplogroups that can be confidently determined by CR motifs

606 CR-HGs (phylotree build 15)

Manual haplogrouping of 19,171 high quality CR haplotypes according to phylotree build 12-15 (Nov 2011 - Sep 2012)

Requirements:

high quality sequences (EMPOP QC process)

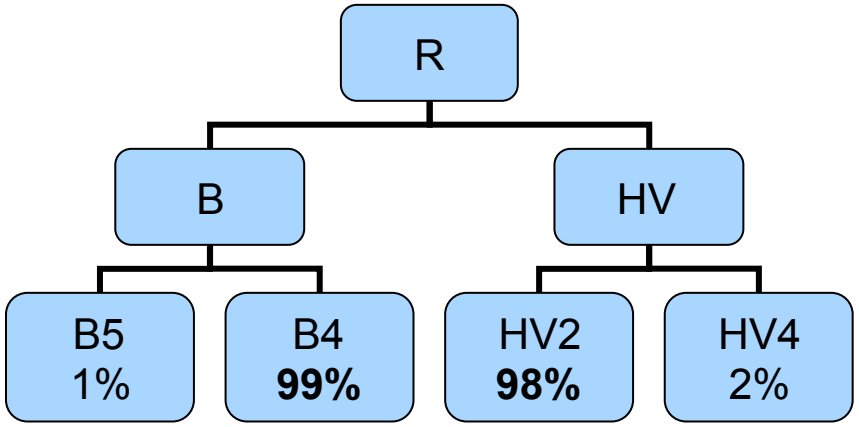
consistent phylogenetic alignment (Bandelt and Parson, 2008)

409 CR-HGs >4 haplotypes in EMPOP (R9)

Fluctuation rates

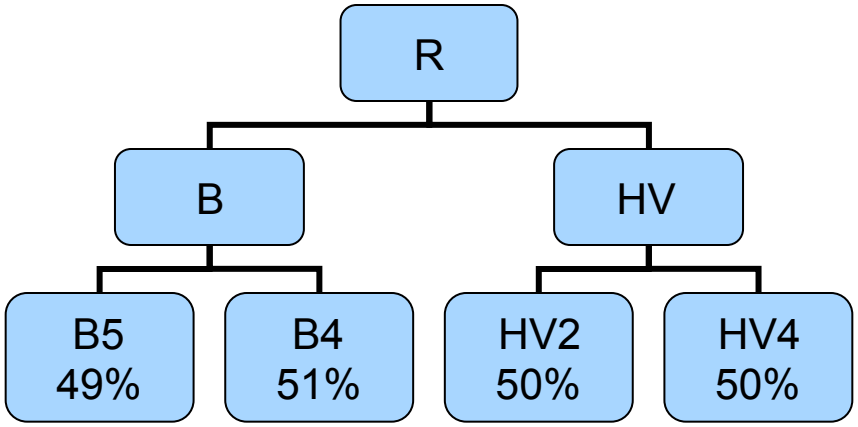
The fluctuation rate (r) is a measure for the stability of a “mutation” within a given haplogroup

Observations for T16217C



T16217C is a stable marker in hgs B4 and HV2 and therefore reliable for hg-estimation

Observations for T152C



T152C is strongly fluctuating in all 4 hgs and therefore of little relevance for hg-estimation

Fluctuation rates

The fluctuation rate (r) is a measure for the stability of a “mutation” within a given haplogroup

$$r_{\alpha\beta} = \frac{\sum_{\gamma} \min(n(\alpha, \gamma), n(\beta, \gamma))}{\sum_{\gamma} n(\gamma)}$$

α, β … A, C, G or T; α unequal β
γ … runs over all CR-hgs
n(x, γ) … denotes number of samples in CR-hg γ with symbol x
n(γ) … denotes total number of samples in CR-hg γ

Control region fluctuation rates

CR-HG	N	T16217C	Difference to majority	T152C	Difference to majority
A2	30	0	0	16	14
B2	40	39	1	19	19
C1	50	0	0	27	23
D1	40	2	2	12	12

$$r_{(T16217C)} = (0+1+0+2) / (30+40+50+40) = 3 / 160 = 0.01875$$

$$r_{(T152C)} = (16+19+27+12) / (30+40+50+40) = 74 / 160 = 0.4625$$

$$0 \leq r \leq 0.5$$

CR fluctuation rates - mtDNA specific conventions

If r=0, then minimum values are applied to

- "transitions" - 10^{-6}
- "transversions" - 10^{-9}
- "indels" - 10^{-9}

Heteroplasmies - split equally in represented bases

Zero weight is applied to indels around 16191, 16193, and 309

For insertions around 315(C), 455(T), 524(AC) and 573(C) and long indels (e.g. deletion between 106-111, 204 bp insertion at 563) only the first indel is weighted, additional insertions are weighted 0

Coding region fluctuation rates

CR fluctuation rates expanded to coding region using number of observations of coding region "mutations" following Sores et al AJHG 2009

Most frequent "mutation": 16519 (209 times)

$$r = r_{(T16519C)} \times n/209$$

Coding region conventions
Only first indels around 960, 965, 5899, 8276, 8278 and 8289 (9bp deletion) are weighted, additional indels are not weighted

Algorithm - maximum likelihood

Concept

Compare test haplotype to all database haplotypes by striving for maximum likelihood

$$L_t(b) = \prod_i r(b_i \rightarrow t_i)$$

b … database haplotype, t … test haplotype, i … positions

Calculation of the product is computationally intensive, therefore minimal costs are computed instead

Algorithm - minimal cost function

$$C_t(b) = \lg(\prod_i r(t_i \rightarrow t_i)/L_t(b)) \quad \text{where} \quad \lg(x) = \log 10(x)/3$$

For short motif lists, such as differences to rCRS between database and test haplotypes, the cost function can be efficiently evaluated by

$$C_t(b) = \sum_i c(b_i, t_i)$$

and

$$c(b_i,t_i) = \lg(r(t_i \rightarrow t_i)/r(b_i \rightarrow t_i))$$

are real numbers termed positional costs for the change from the base profile symbol to the test profile symbol

Average "mutations" yield value of approx. 1.0, unobserved transitions 2.0 and unobserved transversions 3.0

Ranking of haplotypes by total costs equals ranking by maximum likelihood

EMMA haplogrouping

MtDNA haplotype from Argentina (Bobillo et al 2010, 16024-576)

16189C	16292T	16519C	71A	153G	204C	207A	263G	315.1C	373G

Rank 1 Cost 3.39 Base profile: >JQ705203.1 Haplogroup: H55 (CR-R0)

Mutations: T16189C T16519C A153G T204C A263G -309.1C -309.2C -315.1C A750G A1438G A4769G A8860G G10646A A15326G

Missing mutations: -309.1C -309.2C

Private mutations: C16292T G71A G207A A373G

Rank 2 Cost 3.91 Base profile: >JQ704460.1 Haplogroup: H55 (CR-R0)

Mutations: T16189C T16519C A153G A263G -309.1C -309.2C -315.1C A750G A1438G A1603G A4769G A8860G G10646A A15326G

Missing mutations: -309.1C -309.2C

Private mutations: C16292T G71A T204C G207A A373G

EMMA haplogrouping

EMPOP 3 will include (CR-) haplogroups of all database haplotypes

Matches will be displayed with haplogroup status

Haplogroup of neighbors will be estimated by using EMMA

Estimated launch (next EDNAP Meeting)

Summary

Phylogenetic approach of mtDNA analysis

- provides information on geographic background of a lineage
- is essential for quality control
- dictates alignment and nomenclature
- allows haplogrouping of mtDNA haplotypes

2. Past meetings with EMPOP contribution

8th ISABS Conference, Split, Croatia (Jun 24-28, 2013)

25th ISFG Conference, Melbourne, Australia (Sep 02-07, 2013)

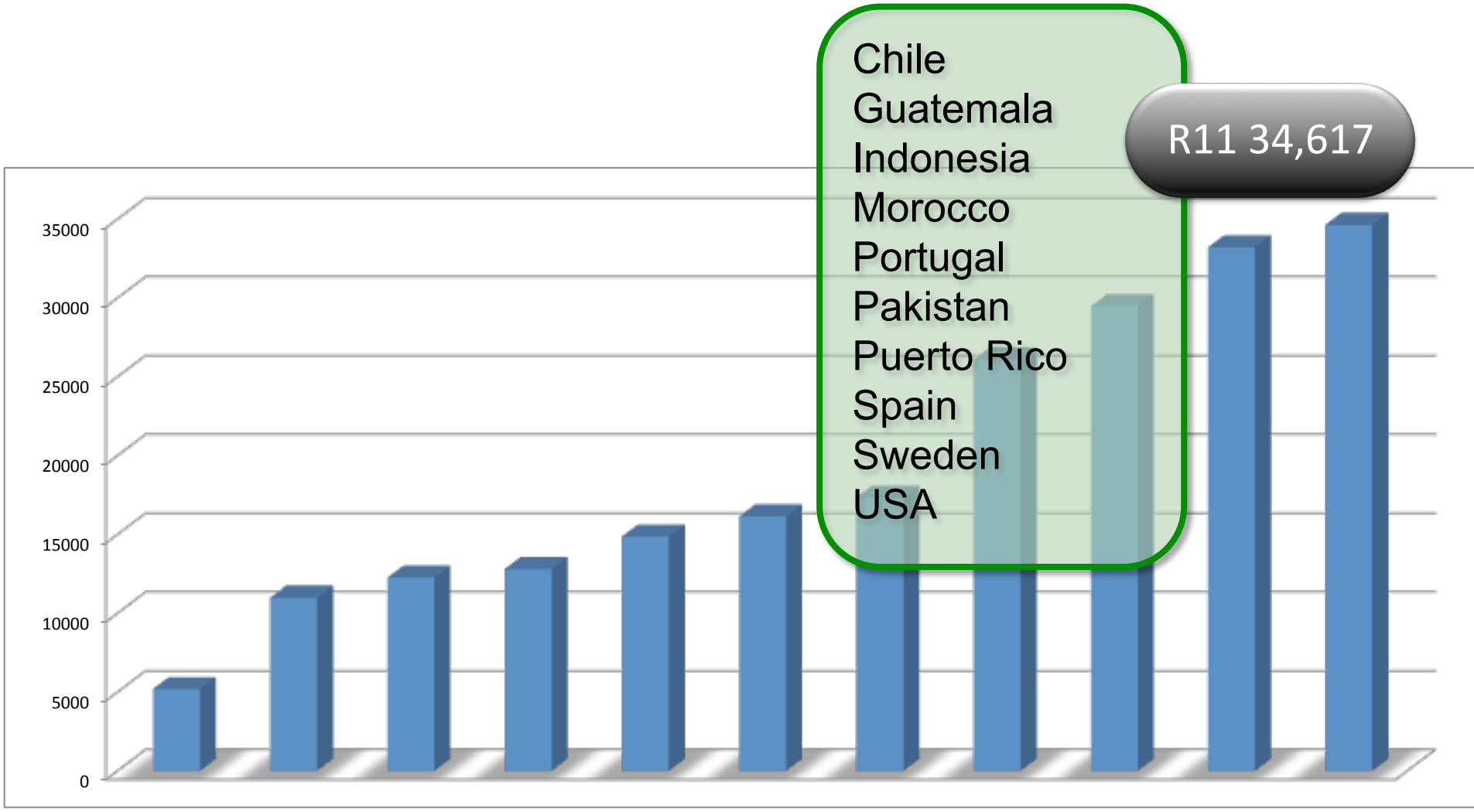
LT User Meeting, Florence, Italy (Sep 13, 2013)

APJ Genetic Analysis Summit, Bali (Sep 29-Oct 02, 2013)

FSS Conference, Manchester, UK (Nov 06-08, 2013)

5th AFSN Meeting, Singapore (Nov 12-13, 2013)

4. EMPOP Releases 10 and 11



Upcoming meetings

Bundeskriminalamt

Interpol update

Ingo Bastisch

Fast-ID

- New software available soon
- Based on new Interpol forms
- DNA match module not intergrated (although often announced)
- Shall be used for DVI and MP/UB
- New decision by SG Noble to use „Bonaparte" that was offered for no costs by NFI/dutch university Trigger was „Maria's case"!!!

Currently in preparation

- Best practice principles (Draft)
  Recommendations for establishment of a DNA database
- Best practice principles (Draft)
  Recommendations for international database searching and exchanges
- Recommendations for identification of MP/UB (pre-Draft)

INTERPOL

INTERPOL DNA Monitoring Expert Group

Best Practice Principles:

Recommendations for Establishment of a DNA Database

This document is designed to convey key considerations and recommendations for INTERPOL member countries wishing to establish a national DNA database.

The intended audience for this document is:

- Forensic Laboratory Heads
- Law Enforcement Agency Heads
- Legal and Policy Agencies
- Other Criminal Justice System Agencies

Summary

The following document provides an overview of considerations to be addressed when establishing a national DNA database capability. It is important to consider what criminal justice outcomes the country is trying to achieve; what types of DNA profiles will be included in the database, how the information contained on the database will be used and by whom; and a mechanism to inform these decisions.

INTERPOL

INTERPOL DNA Monitoring Expert Group

Best Practice Principles:

Recommendations for international DNA database searching and exchanges

This document is designed to convey key considerations and recommendations for INTERPOL member countries wishing to engage in international DNA database searching and exchanges.

The intended audience for this document is:

- Forensic Laboratory Heads
- Law Enforcement Agency Heads
- Managers of national DNA databases
- Legal and Policy Agencies
- Other Criminal Justice System Agencies

Summary

The following document provides an overview of considerations to be addressed by Interpol member countries wishing to engage in international DNA database searching and exchanges. The topics covered are categorised into three broad sections addressing the INTERPOL DNA Database, legislation and policy considerations and mechanisms for exchange.

New tool:
Bilateral matcher

Interpol DNA Monitoring expert group

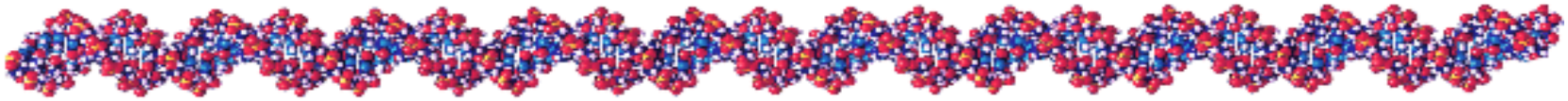
- **Recommendations on the use of DNA for the identification of Missing Persons and unidentified bodies**
  - **Global importance**
  - **Mainly use same standards as in DVI**
  - **Use informed consent that allows for intl. comparison**
  - **Cooperate with NGOs and intl. organisations**
  - **Set up framework and database**

Handling and analysis of samples

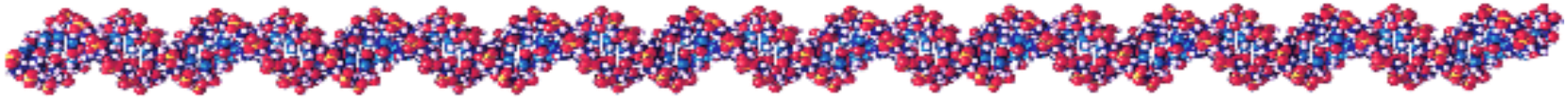
- **DNA analysis should include STR typing with ALL markers used in the different regions worldwide.**
  ->combination of the European Standard Set of Loci (ESS) and the U.S. Combined DNA Index System (CODIS)
- Samples should be retained to allow for additional testing (e.g. mtDNA, Y-STR).

Data handling and matching

- Electronic data processing should ideally be based international standards
- A matching database should be established that a allows for both direct and familial matching.
- The possibility to compare PM data to known offenders or crime stains should be evaluated.



NIST Update



Peter Vallone, Michael Coble, Katherine Gettings, Becky Hill, Erica Butts, Kevin Kiesler, Margaret Kline

Applied Genetics Group

U.S. National Institute of Standards and Technology

EDNAP Meeting

November 2013

Athens, Greece

NIST Human Identity Project Team

within the Applied Genetics Group

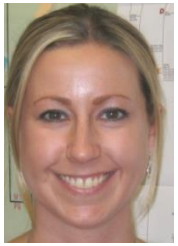
Forensic DNA Team

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

Katherine Gettings

NRC Postdoc

Data Analysis Support

Dave Duewer

Sources of external funding

NIJ National Institute of Justice

Department of Justice Federal Bureau of Investigation

U.S. Department of Homeland Security

Topics

- Status of SRM 2372
- Rapid DNA instrumentation
- Casework expert systems
- New Y STR loci
- Next-generation sequencing
- Update on new STR loci and typing kits
- Sequencing of variant alleles (SRM 2391c)
- Completion of PLEX-ID mass spectrometry work
- Assessing DNA extraction efficiency

Status of SRM 2372

- NIST SRM 2372 Human DNA Quantitation Standard – **returned to sale** (as of January 8, 2013)

- Certified based on absorbance value

Why Was SRM 2372 Taken Off the Market?

- During measurement of the DNA samples to verify stability of certified values we observed **that the UV absorbance values for the samples had changed significantly**
  - Not due to degradation of the DNA but rather unraveling or opening up of the DNA strands in the TE^{-4} buffer (**single-stranded DNA absorbs more UV light than double-stranded DNA**)
  - SRM 2372 is certified for UV absorbance
- **The sample changes over time that impact UV absorbance do not appear to affect qPCR sample performance**

How did we re-certify SRM 2372?

- **Force the material to an all ssDNA conformation**
- Measurements were made using a modification of ISO 21571 Annex B "Methods for the quantitation of the extracted DNA"
  - Combine equal volumes of the DNA extract and freshly prepared 0.4 mol/L NaOH
  - Measure against a reference of equal volumes of TE^{-4} buffer and the 0.4 mol/L NaOH
    - Microvolume spectrometers may have issues with NaOH solutions
- Apparent Absorbance is $D_{10\ (260\ nm)} - D_{10\ (320\ nm)}$

Component A	Component B	Component C
0.777 (0.725 – 0.829)	**0.821** (0.739 – 0.903)	**0.804** (0.753 – 0.855)

Convert Apparent Absorbance to ng/µL

- Conventional concentration values are derived from the assertion that a solution of ssDNA with an absorbance of 1.0 at 260 nm and a pathlength of 1.0 cm has a DNA mass concentration of 37 µg/mL (37 ng/µL)

Parameter	A	B	C
2012 DNA Mass Concentration	**57**	**61**	**59**
2007 DNA Mass Concentration	52.4	53.6	54.3
Theoretical difference, %	**9 %**	**14 %**	**9 %**
Theoretical difference, Ct	**0.12 cycle**	**0.19 cycle**	**0.12 cycle**

Difference between the original and re-certified values is within the noise of the assay

Digital PCR is Planned as the Next Certification Method

- The next generation of SRM 2372 will be certified for "copy/target number" not UV absorbance
  - dPCR assays require optimization to improve measurement accuracy and reproducibility

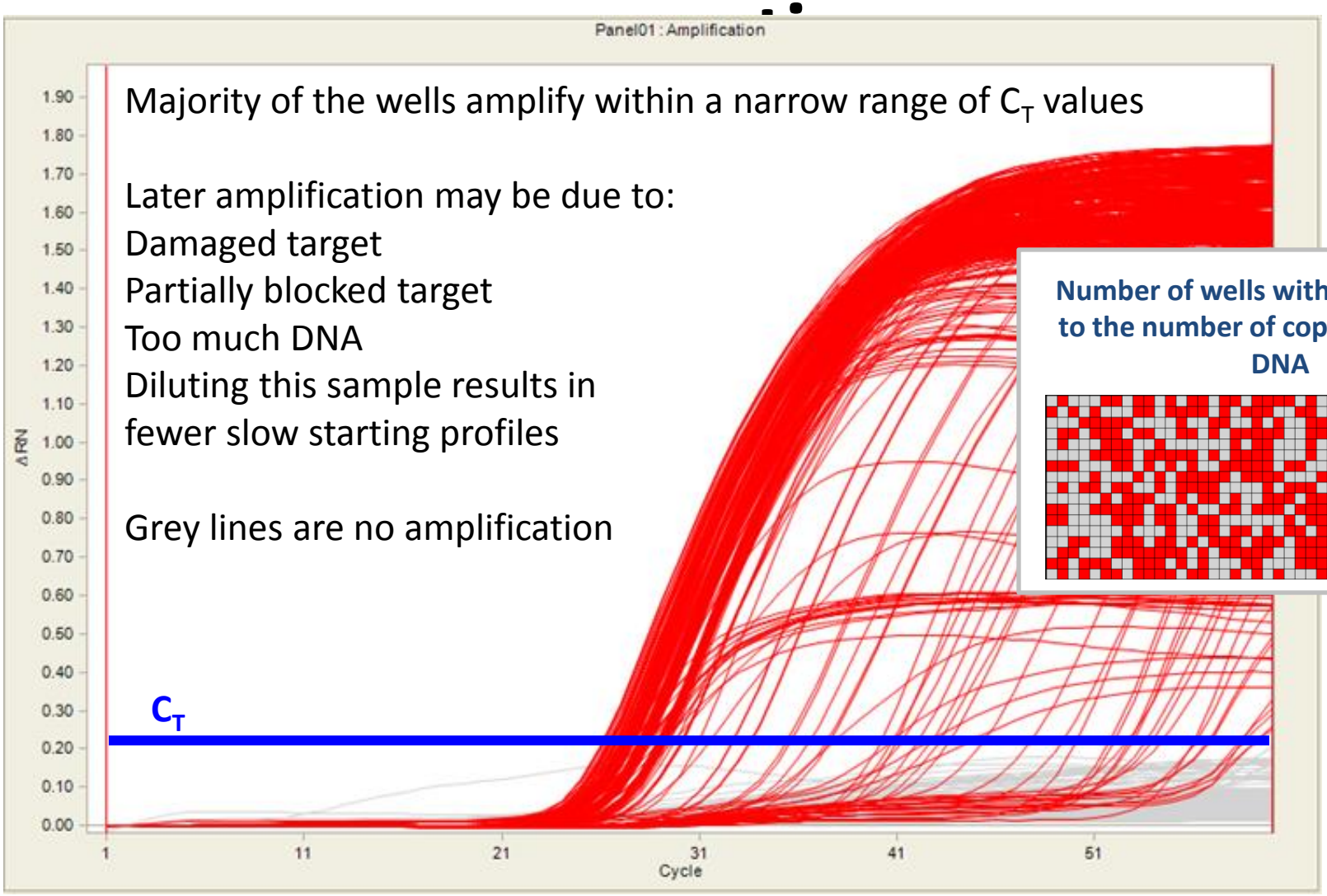
- It is important to realize that there is no one human genomic material that will have the same "target number" for all assays; **lots of variability is being discovered at the genome level in terms of copy number variants and chromosomal rearrangements**

Digital PCR (dPCR) Overview

- Estimates the number of *accessible amplifiable* targets without an external calibrant
- Samples are split into 100s to 1000s of reaction chambers
  - Fluidigm 12.765 Digital Array
  - 765 chambers × 12 panels = **9180 dPCR reactions**
- The count of the number of chambers containing at least 1 target can be used to estimate the total number of targets in a sample

BioRad QX100 Droplet Digital PCR System
≈20,000 PCR droplets

Fluorescent signal as a function of amplification cycle in **765 dPCR**



dPCR Copy Number Estimates

Table 6: CN_{assay} and CN_{genome} Estimates, dsDNA Templates/µL

Unique Target Assay	Component A		Component B		Component C	
Measurand	CN	*u*(CN)	CN	*u*(CN)	CN	*u*(CN)
$[DNA]_{D6S474}$	19100	800	19600	600	16000	800
$[DNA]_{D9S2157}$	21100	1700	20500	800	10400	800
$[DNA]_{D14S1434}$	23200	1200	22400	500	24900	1800
$[DNA]_{Quantifiler}$	18500	1300	18600	200	19200	1100
$[DNA]_{Genome}$	20500	1000	20300	700	17600	3000

Use of multiple target assays for dPCR copy number estimates
Each assay may perform differently

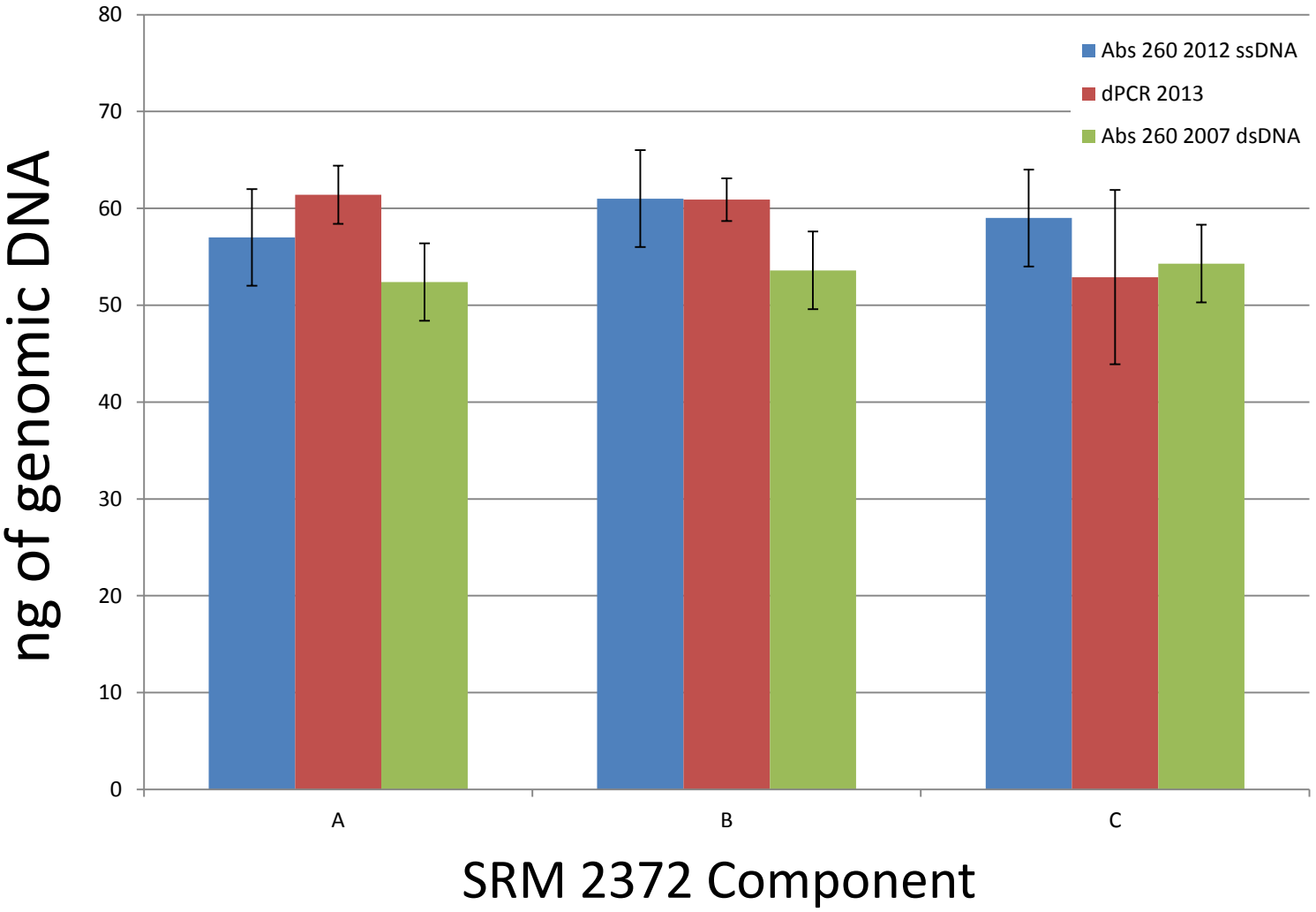
dPCR DNA Concentration Estimates

Table 5: $[DNA]_{assay}$ and $[DNA]_{genome}$ Estimates, ng/µL

Unique Target Assay	Component A			Component B			Component C		
Measurand	$\bar{X}$	$u(\bar{X})$	P	$\bar{X}$	$u(\bar{X})$	P	$\bar{X}$	$u(\bar{X})$	P
$[DNA]_{D6S474}$	57.2	2.3		58.9	1.7		48.1	2.4	
$[DNA]_{D9S2157}$	63.2	5.0		61.6	2.5		31.2	2.5	
$[DNA]_{D14S1434}$	69.7	3.7		67.2	1.4		74.7	5.5	
$[DNA]_{Quantifiler}$	55.6	3.9		55.9	0.5		57.7	3.3	
$[DNA]_{Genome}$	61.4	3.0	0.13	60.9	2.2	0.02	52.9	9.0	0

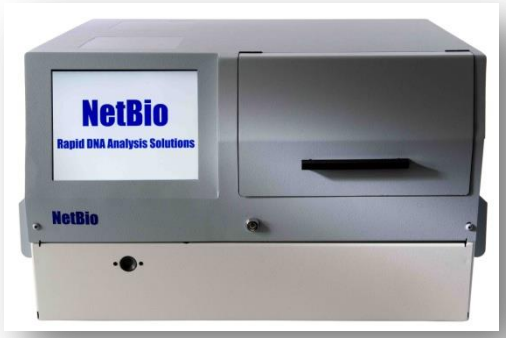
	Comp A	Comp B	Comp C
2012 DNA Mass Concentration (ng/µL)	57	61	59

dPCR DNA Concentration Estimates Comparison to Absorbance



Rapid DNA Prototype Assessment

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

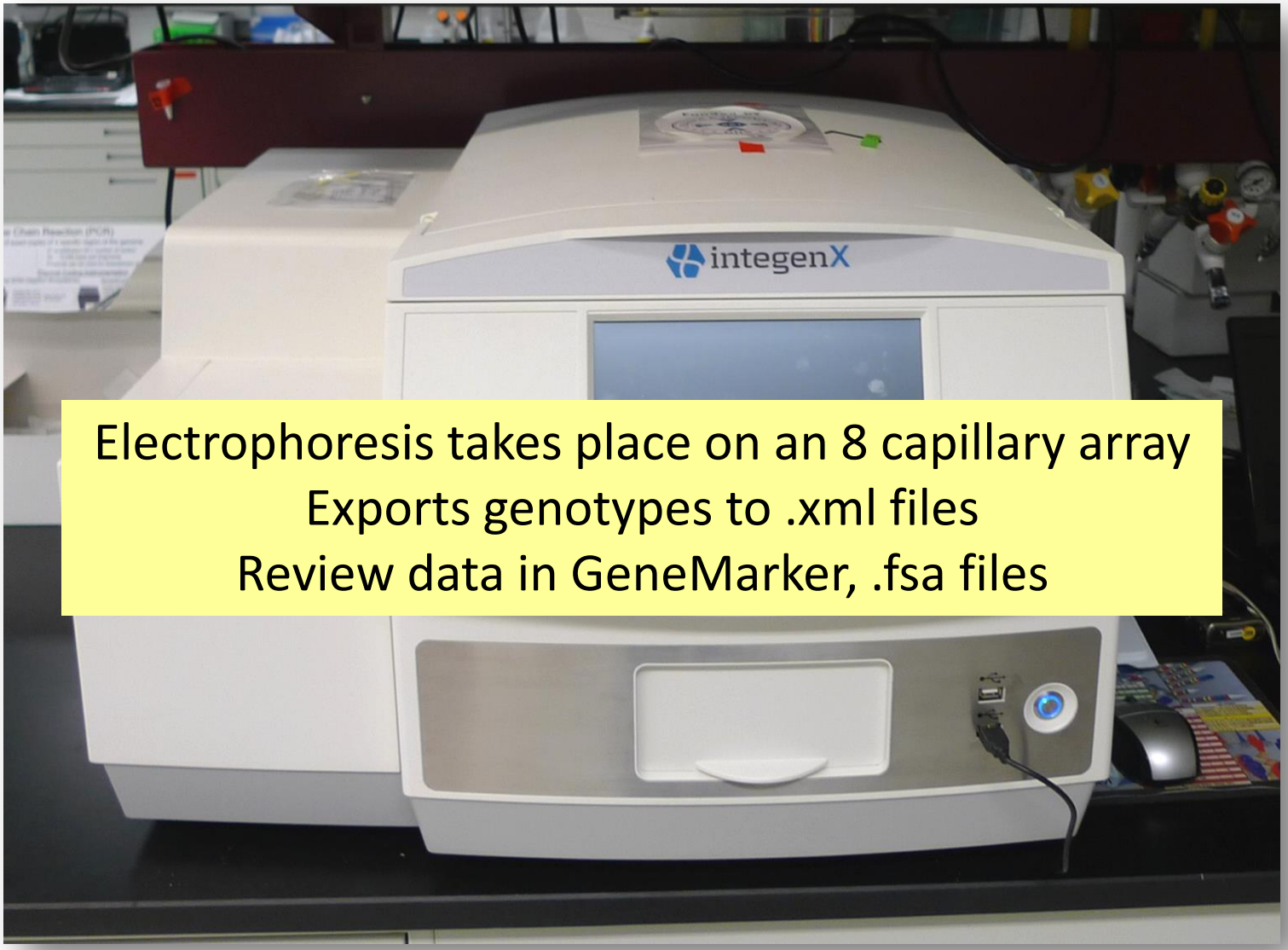


- Over 250 samples (buccal cells on swabs) have been run on each platform

- 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

RapidHit200 IntegenX



Electrophoresis takes place on an 8 capillary array
Exports genotypes to .xml files
Review data in GeneMarker, .fsa files

ANDE NetBio

Electrophoresis takes place on chip
Exports genotypes to .xml files
View egram as bitmap
.fsa files are exported (can be opened in GeneMapper/Genemarker)

Defining Success

- A complete and correct CODIS core 13 STR profile (as called by the expert system software)
  - If any of the 13 loci allele calls were incorrect or absent this was deemed a lane failure
  - Comparing correct genotypes (lab generated) to the types exported to cmf

- Note: we are not including chips that failed due to hardware issues in success calculations

NIST Rapid-DNA Interlaboratory Study

Peter Vallone (NIST) presentation at the BCC Rapid DNA Session, September 18, 2013,Tampa, FL

Purpose:

To disseminate the results of the NIST interlaboratory study for two Rapid DNA (R-DNA) instruments

Indicate where we are as a community with R-DNA

- **NIST R-DNA interlaboratory study design**
- Anonymous buccal collection of 50 samples
- 5 replicates of 10 unique individuals
- Swabs were collected 15 months prior to testing
- Schematic of runs below (10 chips)

	Chip									
Lane	1	2	3	4	5	6	7	8	9	10
1	A	F	J	E	A	C	F	H	D	I
2	B	G	I	D	B	E	F	J	E	J
3	C	H	H	C	B	D	G	J	A	F
4	D	I	G	B	A	D	G	I	B	G
5	E	J	F	A	C	E	H	I	C	H
Ladder										

Defining Success

- A complete and correct CODIS core 13 STR profile (as called by the expert system software)
  - –If any of the 13 loci allele calls were incorrect or absent this was deemed a lane failure
  - –Comparing correct genotypes (lab generated) to the types exported to cmf

R-DNA Instruments

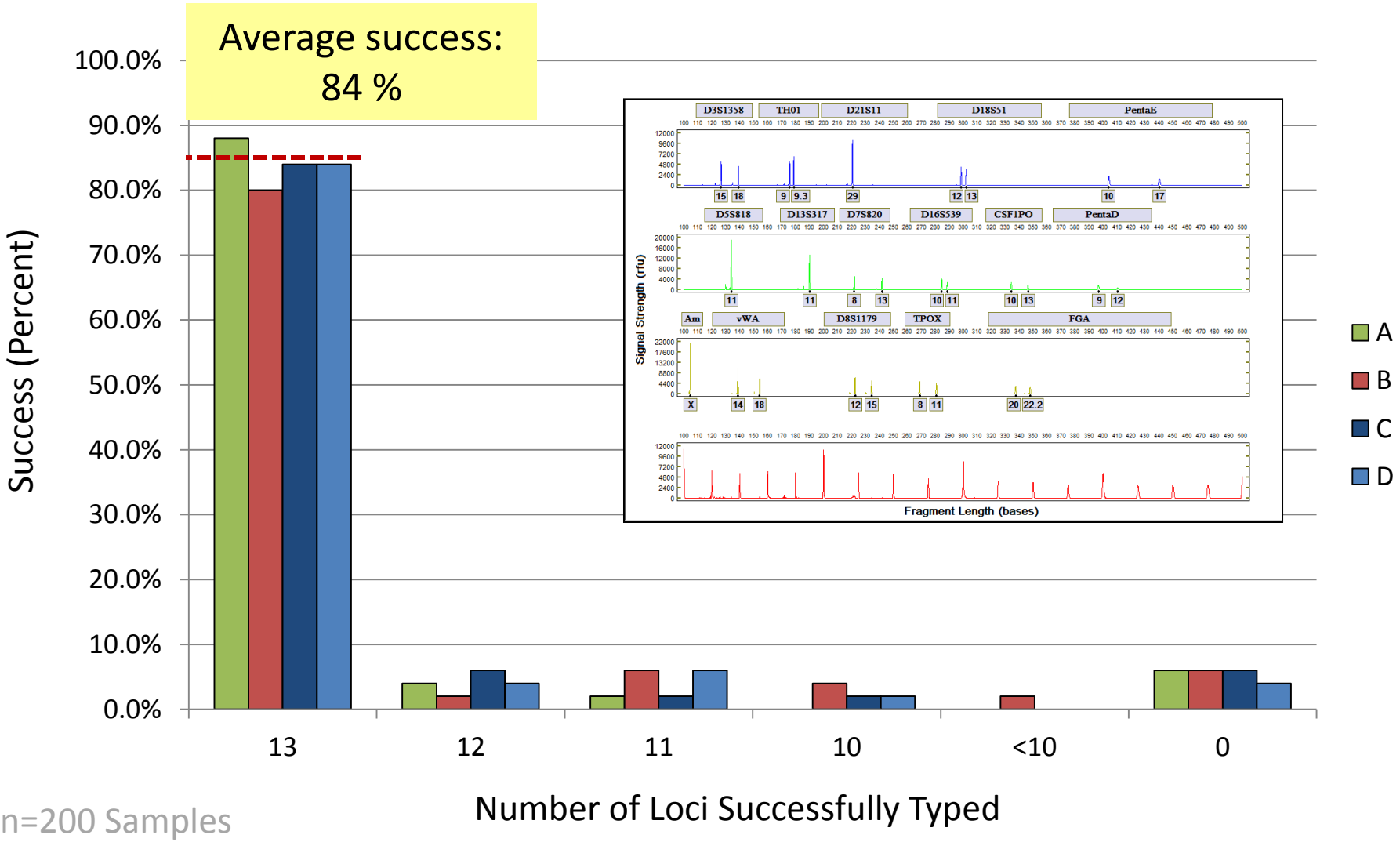
NIST	FBI	USACIL DFSC
Erica Butts Testing on behalf of DHS (Chris Miles)	Lilliana Moreno	Karen Olson Brigid O'Brien
Instrument A	Instrument A	Instrument A Instrument A
Instrument B	Instrument B	Instrument B

All instruments were tested with the same version of scripts and software

Instrument A
Software: 1.0.0.0007
Scripts: JUL 22 2013 17:47:070000

Instrument B
Software: 1.000.0711.1
Scripts: 20130611.1

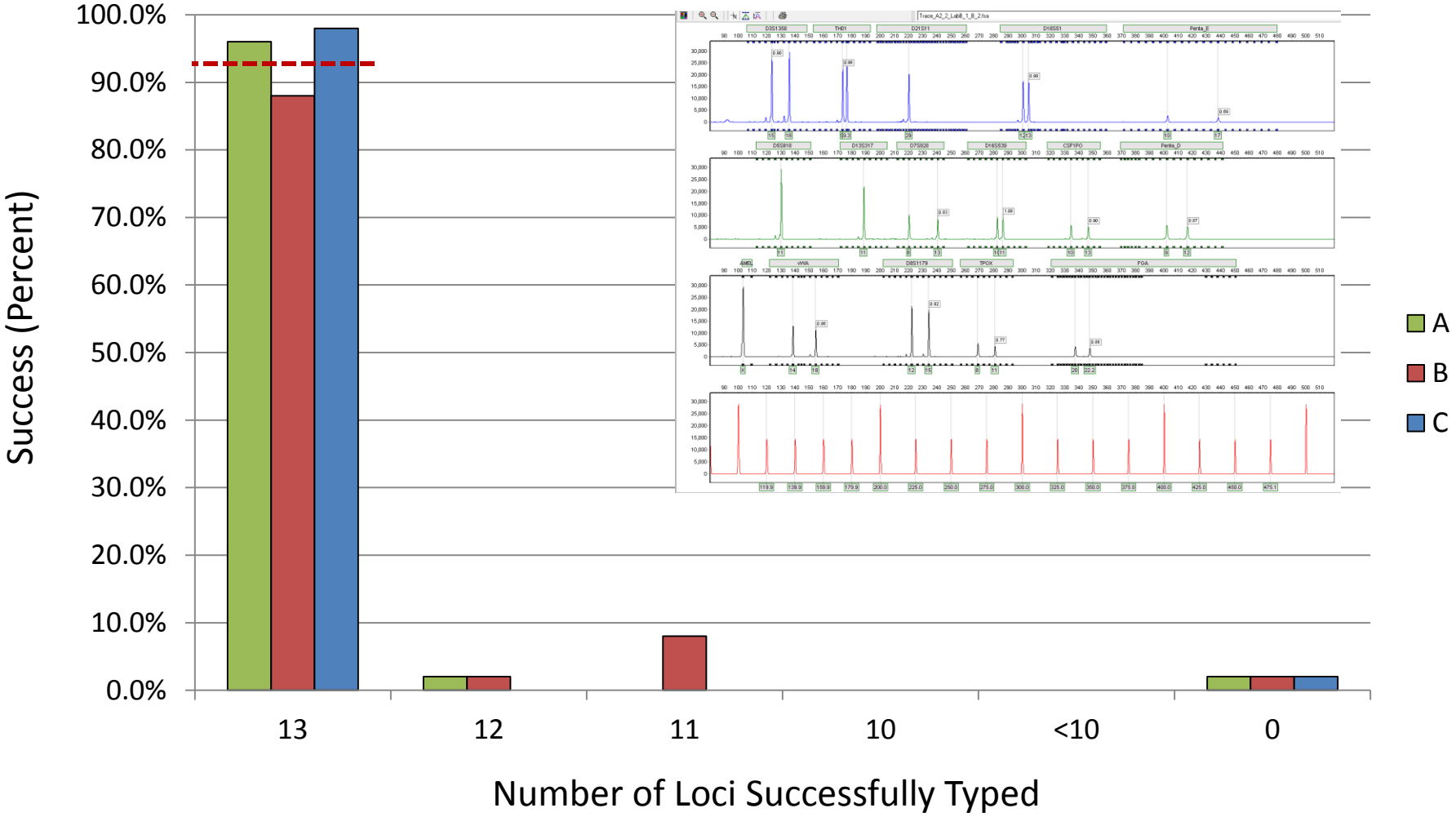
Successful 13 CODIS locus profile Instrument A



n=200 Samples
(50 Samples; 4 Instruments)

Successful 13 locus CODIS profile Instrument B

Average success:
92 %



Summary

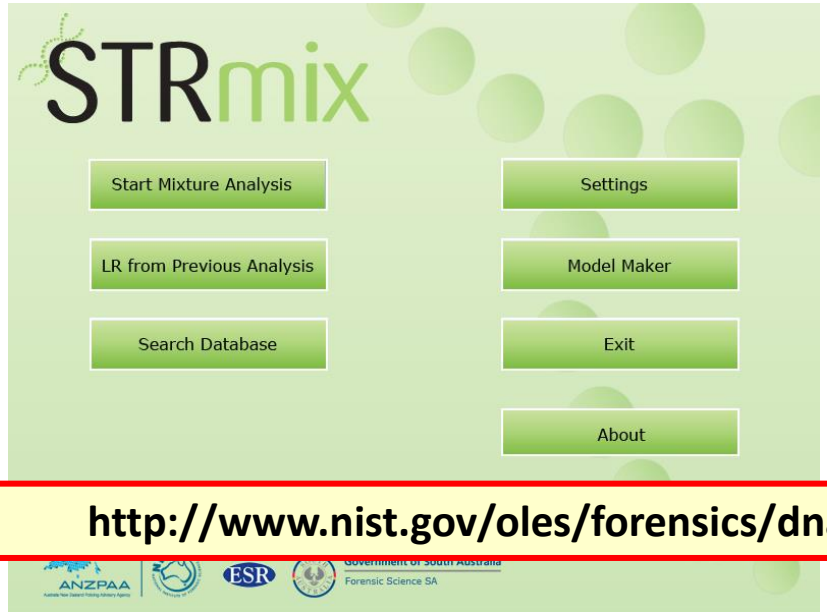
- Where are we at with R-DNA?
  - Fully integrated instruments capable of generating a DNA profile in less than 2 hours
  - Substantial improvements have been observed over the last 12 months

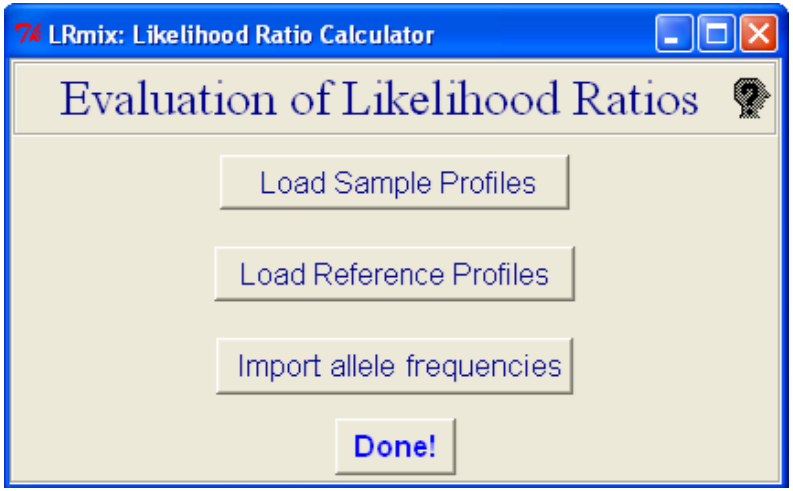
- Combined R-DNA Interlaboratory Results: **88% Success**
  - 309/350 Samples correctly typed for CODIS 13 loci

- **If sustained**, success levels are high enough to perform developmental validation studies once hardware and software versions are finalized

DNA Mixture Interpretation

- **Continued exploration** of the various mixture software programs that use probabilistic approaches to interpretation.
- An oral presentation of this work was presented at the ISFG in September.
- **A manuscript is in progress**.





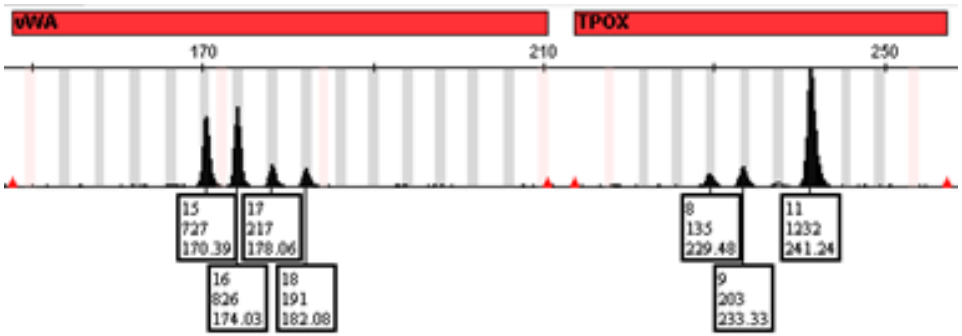
http://www.nist.gov/oles/forensics/dna-analyst-training-on-mixture-interpretation.cfm

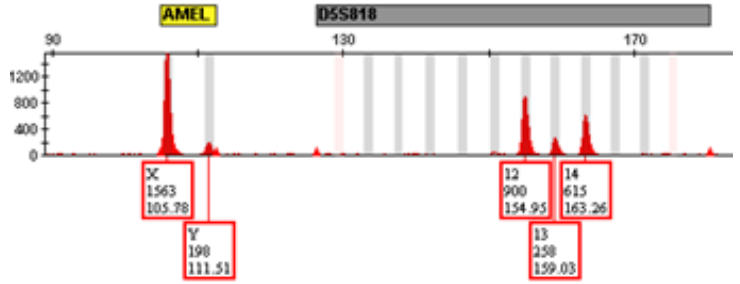
Lab Retriever

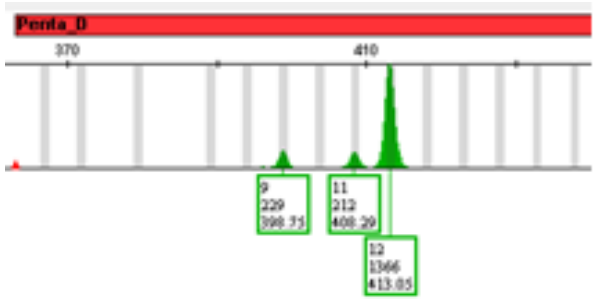
Software >
likeLTD (likelihoods for low-template DNA profiles)

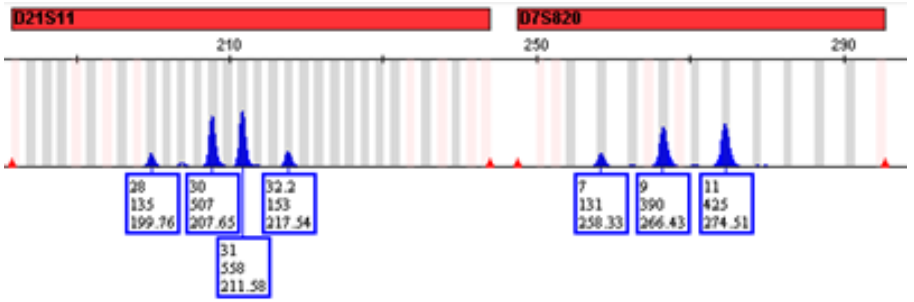
MIX13 – Inter-Laboratory Study

- An inter-laboratory study (MIX13) with several mixture examples has been completed for the US – 101 labs have returned their reports.
- This is to assess how well labs are applying the 2010 SWGDAM recommendations.
- **This week, all 170 DNA Technical Leaders will meeting during the CODIS conference to get a first look at the results.**
- Analysis of the data is ongoing with a target for publication in 2014.



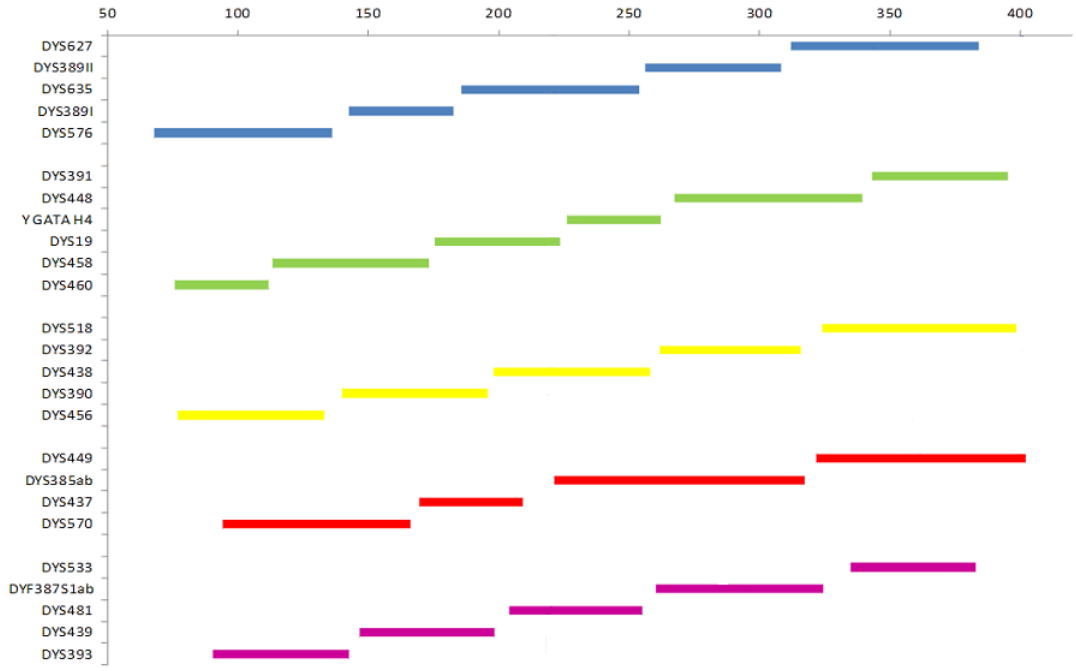






Y STRs

- Currently working on a MTA to test the new 27 Y-STR multiplex kit from Life Technologies (Yfiler Plus). The kit uses 6 fluorescent dye technology.
- Includes all 17 Yfiler loci, 7 Rapidly Mutating (RM) loci, and 3 single copy Y-STR loci.
- Mutation rate information will be needed to assist in interpreting differences among close male relatives.

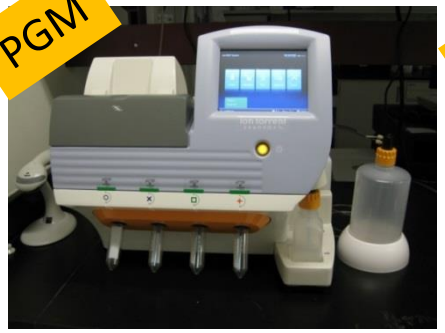


N = 948 males	PowerPlex Y	Yfiler	PowerPlex Y23	Yfiler Plus*
# haplotypes	**816**	**930**	**945**	**946**
discrimination capacity	0.8608	0.9810	0.9968	0.9979
# times haplotype observed	PPY (12 loci)	Yfiler (17 loci)	PPY23 (23 loci)	Yfiler Plus (27 loci)
1	751	916	942	944
2	42	11	3	2
3	12	2	.	.
4	4	1	.	.
5	2	.	.	.
6	2	.	.	.
7	.	.	.	.
8	1	.	.	.
9	.	.	.	.
10	.	.	.	.
11	1	.	.	.
12	.	.	.	.
13	.	.	.	.
14	.	.	.	.
15	.	.	.	.
16	.	.	.	.
17	.	.	.	.
18	.	.	.	.
19	.	.	.	.
20	1	.	.	.

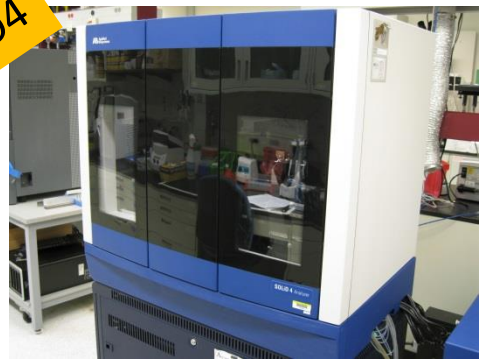
Next-Generation Sequencing

- 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



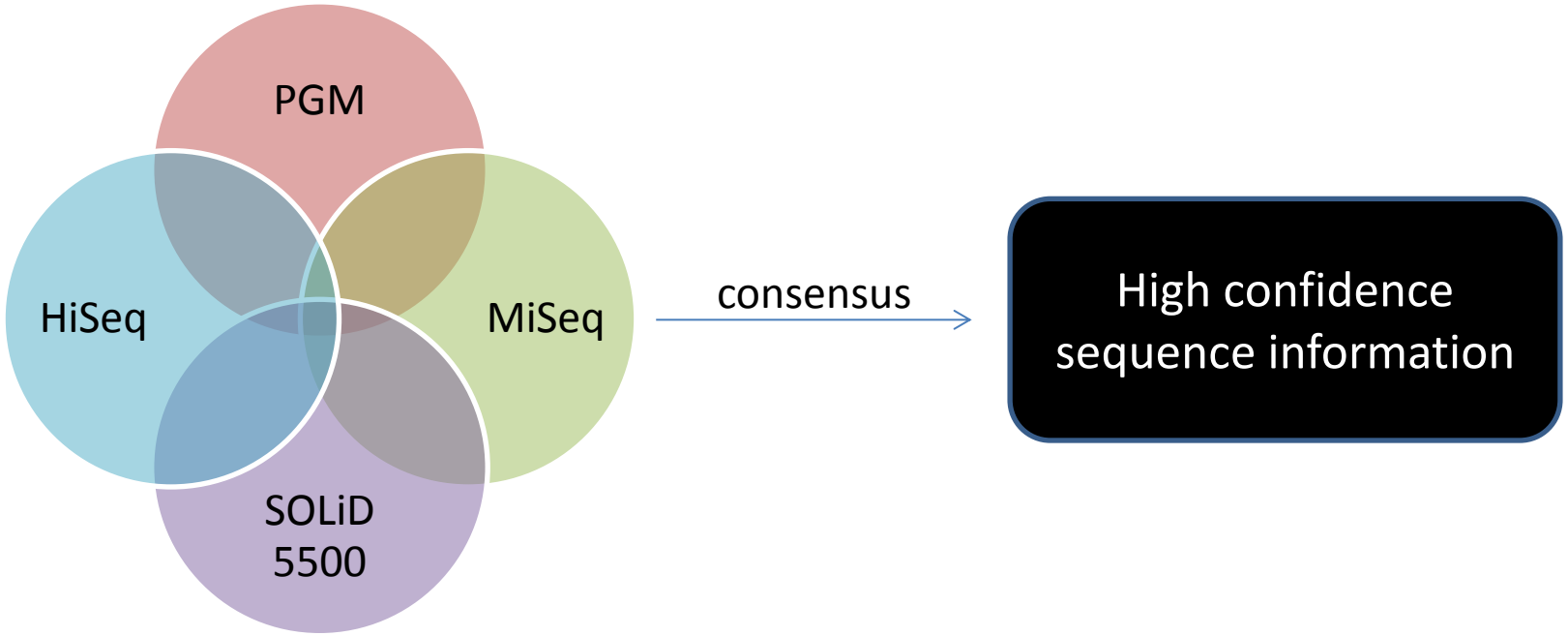
SOLiD4



SRM

Multiple NGS Platforms

- Use of multiple platforms to obtain a consensus sequence for the SRMs
  - Identify and reduce the false positives and negatives
  - Identify and control for bias in a specific chemistry and/or informatics pipeline



NIST SRM 2392 & 2392-I

- Mitochondrial DNA sequencing Standard Reference Materials
  - Characterized for mtDNA genome sequence composition
  - Reference used to validate measurement techniques
  - Recommended by FBI as positive control for sequencing labs

- SRM 2392
  - Contains 3 components (extracted DNA)
    - 2392 A – From cell line CHR
    - 2392 B – From cell line 9947A
    - 2392 C – Cloned region of heteroplasmy

- SRM 2392–I
    - From cell line HL-60

False Positives and False Negatives

Using platform specific informatics pipeline

		PGM 1	PGM 2	PGM 3	HiSeq	MiSeq	5500
9947A	FP	1	5	3	21	9	11
	FN	3	4	3	3	3	3
CHR	FP	2	6	10	21	9	10
	FN	3	5	4	3	3	4
HL-60	FP	1	8	8	20	9	8
	FN	1	2	1	1	1	1
Avg Coverage		280	6,500	9,000	49,000	41,000	29,000

Calls made to the rCRS

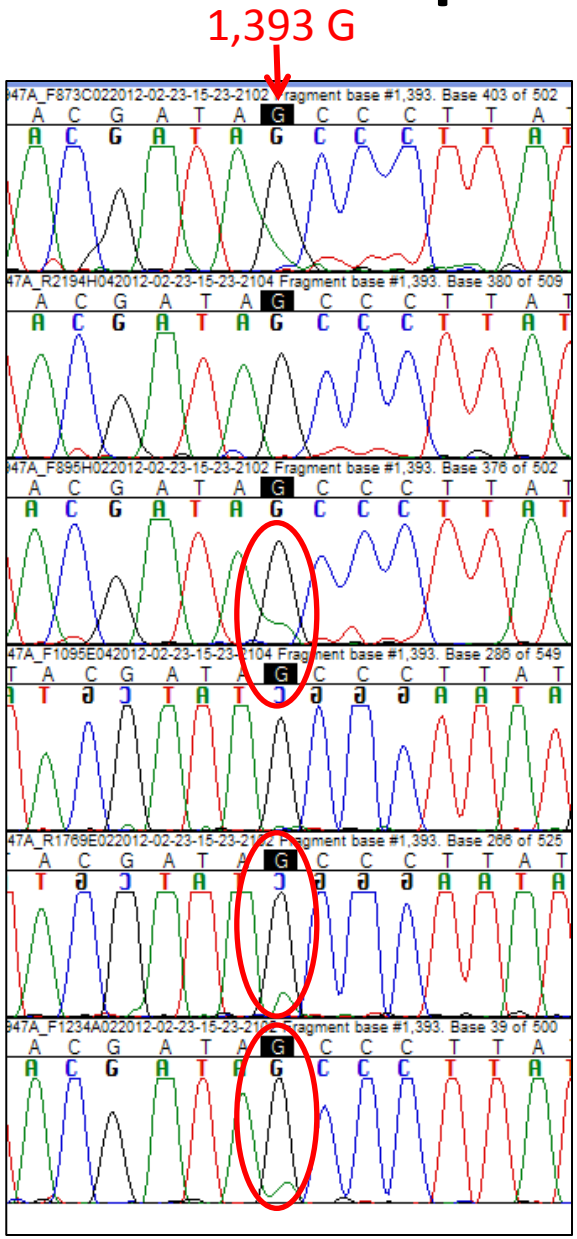
On average 99.94 % agreement with Sanger sequencing

Heteroplasmy at Position 1,393

SRM 2392 Component B (9947A)

Nucleotide Position	rCRS Reference Sequence	SRM 2392 Component B Sanger Call	EdgeBio PGM	NIST PGM run 1	NIST PGM run 2	EdgeBio Illumina MiSeq	Beckman Genomics Illumina HiSeq	NIST SOLiD
93	A	G	G	G	G	G	G	G
195	T	C	C	C	C	C	C	C
214	A	G	G	G	G	G	G	G
263	A	G	G	G	G	G	G	G
309.1	:	C						
309.2	:	C						
315.1	:	C						
750	A	G	G	G	G	G	G	G
1393	G	G	G/A	G/A	G/A	G/A	G/A	G/A
1438	A	G	G	G	G	G	G	G
4135	T	C	C	C	C	C	C	C
4769	A	G	G	G	G	G	G	G
7645	T	C	C	C	C	C	C	C
7861	T	C	C	C	C	C	C	C
8448	T	C	C	C	C	C	C	C
8860	A	G	G	G	G	G	G	G
9315	T	C	C	C	C	C	C	C
13572	T	C	C	C	C	C	C	C
13759	G	A	A		A	A	A	A
15326	A	G	G	G	G	G	G	G
16311	T	C	C	C	C	C	C	C
16519	T	C	C	C	C	C	C	C

Heteroplasmy at 1,393?



- 6x coverage by Sanger
- 3/6 of reads indicate low-level heteroplasmy
  - Red circles
- Not reproducible in all reads
  - Not always detected by Sanger sequencing

Heteroplasmy detected by NGS at Site 1,393

- Agreement across platforms (high confidence)

≈ 17.6% (± 2.6%) minor component "A"

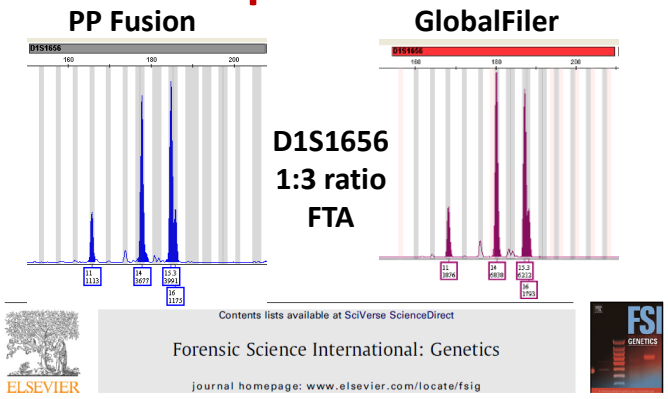
Experiment	Reference "G"	Variant "A"	Coverage
EdgeBio PGM	77.3%	22.7%	97 x
NIST PGM Run 1	82.5%	17.5%	2940 x
NIST PGM Run 2	83.4%	16.6%	3275 x
Illumina MiSeq	83.7%	16.3%	26,234 x
Illumina HiSeq	84.4%	15.6%	62,186 x
NIST SOLiD	82.5%	16.9%	24,226 x

Site 1,393 also confirmed by Niels Morling's lab using 454 technology (Martin Mikkelsen)

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)
- **We are working closely with the FBI in a consortium validation project to determine how well these new loci perform in the new multiplex kits**

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



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

Letter to the Editor

U.S. population data for 29 autosomal STR loci

Dear Editor,

We determined the genotypes and allele frequencies for a total of 1036 unrelated U.S. population samples using 29 autosomal short tandem repeat (STR) loci that are available in commercial STR multiplex kits including D1S1656, D2S441, D2S1338, D3S1358, D5S818, D6S1043, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, F13A01, F13B, FESFPS, FGA, LPL, Penta C, Penta D, Penta E, SE33, TH01, TPOX, and vWA.

run and population statistics were confirmed using the PowerMarker v3.25 statistics program [10].

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2012.12.004.

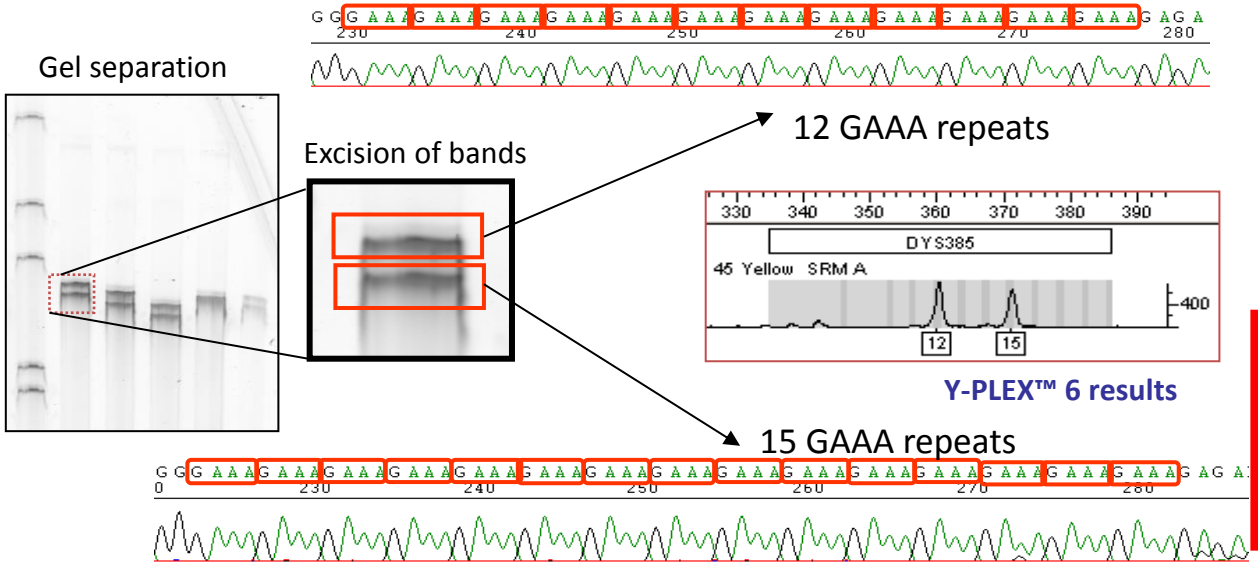
There were 14 instances where statistically significant deviations from Hardy–Weinberg expectations based on the exact test were observed ($p < 0.05$); however, after using the Bonferroni's correction [11] with 116 comparisons ($p < 0.00043$) there are only two statistically significant deviations from HWE in this data set (D13S317 and F13B in the combined population data set). SE33 has the highest H_{obs} (0.9353) and PPE values (0.8880) and lowest P_i value (0.0066), making it the most variable locus when compared

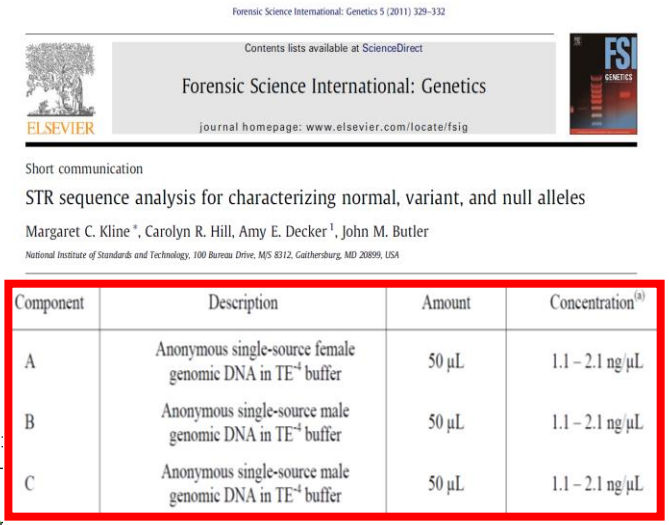
Sequencing Variant Alleles and SRM 2391c

- Sequencing of “off-ladder” variant alleles, null alleles or any other “odd” result seen in datasets is a free service funded by the NIJ
- Results are provided to the customer and listed on STRBase:

  http://www.cstl.nist.gov/biotech/strbase/STRseq.htm

- NIST DNA sequencing procedures and all sequencing primers were published in FSI: Genetics in 2011
- **The purpose of sequencing of SRM 2391c, Components A-C, is to further characterize and determine interesting genomic characteristics within STR fragments - this work will support Next Generation Sequencing of Components A-C**





Forensic Science International: Genetics 5 (2011) 329–332

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Short communication

STR sequence analysis for characterizing normal, variant, and null alleles

Margaret C. Kline *, Carolyn R. Hill, Amy E. Decker [1], John M. Butler

National Institute of Standards and Technology, 100 Bureau Drive, M/S 8312, Gaithersburg, MD 20899, USA

Component	Description	Amount	Concentration[a]
A	Anonymous single-source female genomic DNA in TE^{-4} buffer	50 µL	1.1 – 2.1 ng/µL
B	Anonymous single-source male genomic DNA in TE^{-4} buffer	50 µL	1.1 – 2.1 ng/µL
C	Anonymous single-source male genomic DNA in TE^{-4} buffer	50 µL	1.1 – 2.1 ng/µL

Sequencing Results

- All sequencing results of Components A-C for 41 STR markers, including repeat structures of individual alleles, can be found on the following poster:

http://www.cstl.nist.gov/biotech/strbase/pub_pres/Hill-ISFG2013-SRM2391c.pdf

Marker	Component	Allele	Allele Repeat Structure
D8S1179	C	17	$[TCTA]_2$ TCTG $[TCTA]_{14}$
D12S391	A	22	$[AGAT]_{13}$ $[AGAC]_8$ AGAT
D12S391	C	19	$[AGAT]_{13}$ $[AGAC]_5$ AGAT
D12S391	C	23	$[AGAT]_{12}$ $[AGAC]_{10}$ AGAT
D21S11	B	32	$[TCTA]_4$ $[TCTG]_6$ {$[TCTA]_3$ TA $[TCTA]_3$ TCA $[TCTA]_2$ TCCATA} $[TCTA]_{14}$
SE33	C	31.2	$[AAAG]_2$ AG $[AAAG]_3$ AG $[AAAG]_9$ AAAAAG $[AAAG]_{21}$ G AAGG$[AAAG]_2$ AG
DYS389II	B	31	$[TCTG]_6$ $[TCTA]_{12}$ $[TCTG]_3$ $[TCTA]_{10}$
DYS458	B	17.2	$[GAAA]_{15}$ AA $[GAAA]_2$
DYS635	B	20	$[TCTA]_4$ $[TGTA]_2$ $[TCTA]_2$ $[TGTA]_2$ $[TCTA]_{10}$
DYS635	C	21	$[TCTA]_4$ $[TGTA]_2$ $[TCTA]_2$ $[TGTA]_2$ $[TCTA]_{11}$

Novel repeat motifs that were not listed in Butler J.M. (2012) or STRBase fact sheets

SNPs Found in Repeat Flanking Regions

- Multiple SNPs were found in the DNA sequence in the repeat flanking regions. Primers that bind on SNPs can result in null alleles when STR typing.
- Note that the variants characterized in this work are constrained by the size of the original PCR amplicon generated (Kline et al. 2011).

Marker	Component	Allele	Flanking Region Variants
D5S818	A	12	T→C 13 bp us of the repeat
D5S818	B	13	T→C 13 bp us of the repeat
D5S818	B	13	G→T 4 bp ds of the repeat
D5S818	C	10	T→C 13 bp us of the repeat
D5S818	C	11	T→C 13 bp us of the repeat
D7S820	C	10	T→G 65 bp ds of the repeat
D13S317	C	11	A→C 115 bp ds of the repeat
D16S539	A	10	A→C 16 bp ds of the repeat
D16S539	A	10	C→A 95 bp us of the repeat
D16S539	A	11	C→A 95 bp us of the repeat
D16S539	B	10	C→A 95 bp us of the repeat
D16S539	C	10	C→A 95 bp us of the repeat
Penta E	A	10	G→A 123 bp us of the repeat
Penta E	A	10	A→G 268 bp us of the repeat
Penta E	A	10	A→C 280 bp us of the repeat
Penta E	B	7	G→A 123 bp us of the repeat
Penta E	B	7	A→G 268 bp us of the repeat
Penta E	B	7	A→C 280 bp us of the repeat
Penta E	B	15	G→A 123 bp us of the repeat
Penta E	B	15	A→G 268 bp us of the repeat
Penta E	B	15	A→C 280 bp us of the repeat
TPOX	A	8	T→G 148 bp ds of the repeat
TPOX	B	8	T→G 148 bp ds of the repeat

Abbreviations: bp = base pairs, us = upstream, ds = downstream

Completion of PLEX-ID Work

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)

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

Comparison of Base Composition Analysis and Sanger Sequencing of Mitochondrial DNA for 4 U.S. Populations

Kevin M. Kiesler, Michael D. Coble, Thomas Hall, Peter M. Vallone

Abstract

A set of 711 samples from four U.S. population groups was analyzed using a novel mass spectrometry

In Press - FSI Genetics

FORENSIC SCIENCE

Croat Med J. 2013;54:225-31
doi: 10.3325/cmj.2013.54.225

CMJ

225

Croat Med J 2013, 54: 225-31

Allele frequencies for 40 autosomal SNP loci typed for US population samples using electrospray ionization mass spectrometry

Kevin M. Kiesler, Peter M. Vallone

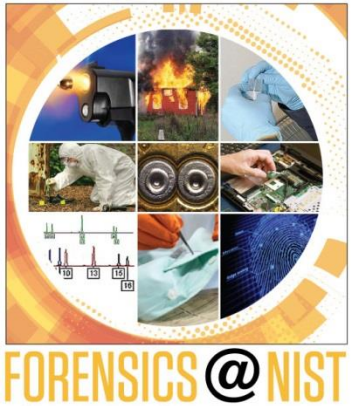
Biomolecular Measurement Division, National Institute of Standards and Technology, Gaithersburg, MD, USA

Assessing DNA Extraction Efficiency

- Absolute extraction efficiency is the ratio of the amount of DNA recovered (quantitated) to the original amount of DNA (known) after extraction
  - Knowing the original starting amount of DNA allows for the ability to evaluate the absolute efficiency of the extraction process
- Currently examining the efficiency of three extraction methods: **Organic**, **Chelex**, and **Qiagen EZ1 Advanced XL** robotic platform.
- DNA from three sources tested in varying amounts: **purified DNA**, **human cell lines**, **whole blood**

Erica Butts presentation at the American Academy of Forensic Sciences meeting (Washington, D.C.), February 21, 2013, "Evaluation of DNA Extraction Efficiency"

AMERICAN ACADEMY of FORENSIC SCIENCES



Current testing shows a **loss of 70-80% of the initial sample** during the extraction process. The loss is independent of extraction method and source of DNA (i.e. purified DNA, human cells, blood, etc)

Coming Up

- AAFS (Feb 17 – 22, 2013, Seattle Washington)
  - Talks: **Characterization of DNA-Based Certified Reference Materials with New and Emerging Technologies (Peter Vallone)**
  - **NIST Inter-Laboratory Studies for DNA Mixture Interpretation (Mike Coble)**

NIST/NRC Postdoc Program

Working in the Applied Genetics Group at NIST

- Current stipend (2013) is **$65,600 per year**
  - Currently a limit of 120 slots per year
  - Congressionally-mandated program for NIST
  - Maximum 2-year appointments
- Awardees **must be U.S. citizens**
- Awardees are chosen through a **national competition** administered by the National Research Council of the National Academy of Sciences.
- Two competitions per year
  - **deadlines of February 1 and August 1**
- **Contact either Dr. Peter Vallone (peter.vallone@nist.gov) or or Dr. Michael Coble (michael.coble@nist.gov)**

Selected Topics
Rapid DNA Typing
DNA Mixture Analysis
Forensic Applications of Next-Gen Sequencing
DNA Extraction efficiency
Forensic SNPs
Y-STRs
Open to suggested topics/projects

http://www.nist.gov/iaao/postdoc.cfm
http://nrc58.nas.edu/RAPLab10/Opportunity/Program.aspx?LabCode=50

Thanks for your attention!

Questions?

Peter.Vallone@nist.gov

301-975-4872

Outside funding agencies:

FBI - Evaluation of Forensic DNA Typing as a Biometric Tool

NIJ – Interagency Agreement with the Office of Law Enforcement Standards

DHS – Rapid DNA for Kinship Analysis

The European Forensic Genetics Network of Excellence – Update on Activities (Nov. 2013)

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 research projects
- **WP4**
  - Ethical and legal aspects, and the societal dimension of forensic genetics
- **WP5**
  - Education, Training and Career Development

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WP1+2: Newsletter published

ISSUE 1/2013

NEWSLETTER

European Forensic Genetics Network of Excellence

WELCOME

Dear colleagues – we want to keep you up-to-date with the activities of the EUROFORGEN Network of Excellence: Our consortium aims to improve the exchange of information about new research developments, funding opportunities, training resources and educational activities, and to explore the impact of modern forensic genetics on the society.

The EUROFORGEN Network of Excellence has now almost completed the second year of the five year funding period 2012-2016. During the first year, numerous activities have been initiated to establish the ground for better networking structures in the field of forensic genetics. So in this first newsletter we will provide you with information about

- first results compiled from **European-wide surveys** among forensic laboratories to inquire about the situation both in routine work and research,

Furthermore, a total of 179 laboratories across Europe were identified as active in the field, and have accepted to contribute to the activities of EUROFORGEN-NoE. Significant information was derived concerning the number and type of labs in the different European countries, the level of standardization, the educational needs, the research activities and the challenges in the field.

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WP2 – Integrating research and networking

- **Deliverable "Directory of Forensic Genetic Research Laboratories in Europe" published online**
  - Available from EUROFORGEN website as PDF document
  - All participants listed in online directory
  - Directory will be continuously updated
- **Short Term Fellowship program**
  - Approx. 10 fellowships have been awarded
  - Money for 10 more fellowships is still available
  - Open for all European applicants

WP2 – Integrating research and networking

- **European Virtual Centre of Forensic Genetic Research**
  - Implementation via website by introducing new pages
  - More interactive features with personal access
  - Call for research proposals in mid 2014
- **Public Relations Conference in Brussels 2014**
  - Aimed at high level politicians and administrators, as well as the press
  - To demonstrate the achievements of forensic genetics
  - To adress the lack of adequate funding for European research in forensic genetics (especially in Horizon 2020)
  - To be held after the May elections of the European Parliament

WP3 – Exemplar Research Projects

EP1: Crime scene investigation and human DNA discovery

- **Interlaboratory exercise on mRNA detection and interpretation completed**
  - Presented at ISFG 2013
  - Manuscript in preparation
- **A protocol that describes best practice at crime scene (D3.12)**
  - Contact/feedback planned from European labs
  - Interaction with ENFSI DNA WG desirable
    - "Best practice manuals"

WP3 – Exemplar Research Projects

EP2: Guiding investigations by genetic analysis of physical traits and tailored multiplex developments

- **Interlaboratory exercise on AIM SNP and InDel multiplexes**
  - Completed, data presented at ISFG 2013
  - EDNAP exercise planned (USC)
- **New ancestry multiplexes under evaluation**
  - Global AIM set
  - Eurasian sub-continental AIM set
- **Study on male baldness pattern**
  - Sample collection and evaluation ongoing

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WP3 – Exemplar Research Projects

EP3: Bioinformatics, in silico modeling and statistics

- **LRmix**
  - LR-based mixture interpretation following ISFG recommendations
  - Collaboration with H. Haned to add more features
- **euroMix**
  - R package with functions for simulating mixtures with relatives for both linked and unlinked markers, and for computing likelihood ratios conditioned on pedigrees.
- **STR Validator**
  - https://sites.google.com/site/forensicapps/strvalidator
  - open source R-package for internal validation of forensic STR kits
  - analysis modules for stutter, balance and dropout

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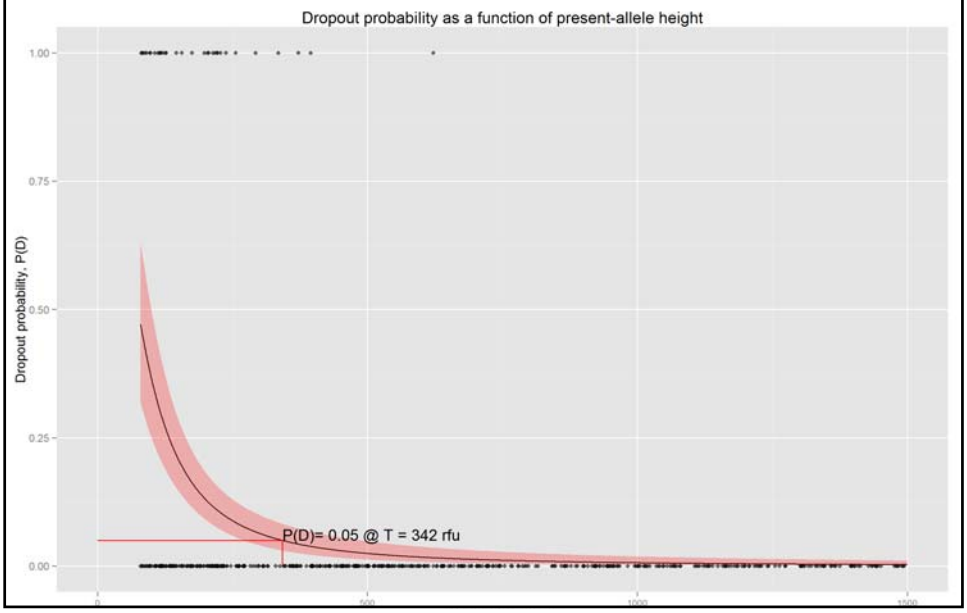
STR Validator



WP 4 – Ethical and legal aspects, and societal dimension

- **Systematic review of the literature on ethical, legal & social issues (UNN)**
  - Deliverable finalized, to be published early next year
- **Report on research undertaken into public concerns**
  - Work in progress, to be completed next summer
- **Report on legal audit of EU legislation on forensic DNA profiling and databasing**
  - Delayed due to transition of partner institution, expected next year
- **Collaboration planned with**

sense about science
equipping people to make sense of science and evidence

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Sense About Genetic Ancestry Testing

There are now many companies which offer to tell you about your ancestors from a DNA test. You send off a sample of your DNA and £100–£200 ($150–300), and in return you receive a report. The results of these tests may find a connection with a well-known historical figure. They might tell you whether you are descended from groups such as Vikings or Zulus, where your ancient relatives came from or when they migrated.

Adverts for these tests give the impression that your results are unique and that the tests will tell you about your specific personal history. But the very same history that you receive could equally be given to thousands of other people. Conversely, the results from your DNA tests could be matched with all sorts of different stories to the one you are given.

It is well known that horoscopes use vague statements which recipients think are more tailored than they really are (referred to as the 'Forer effect'). Genetic ancestry tests do a similar thing, and many exaggerate far beyond the available evidence about human origins. You cannot look at DNA and read it like a book or a map of a journey. For the most part these tests cannot tell you the things they claim to – they are little more than genetic astrology.

… drafted in February 2013 by Professor David Balding, Professor Mark Thomas and Tabitha Innocent, Sense About Science; with assistance from Dr Turi King, Dr Lounès Chikhi, Dr Rosalind Harding, Professor Mark Jobling & Professor Guido Barbujani.

sense about science

equipping people to make sense of science and evidence

- **Making Sense of Uncertainty**
  - Why uncertainty is part of science
    - The way scientists use uncertainty to express how confident they are about results.
    - That uncertainty can be abused to undermine evidence or to suggest anything could be true.
- **Making Sense of Statistics**
  - Like words, numbers and statistics mean different things in different contexts
  - Just because something is statistically significant it doesn't mean it is practically significant or of importance to society
- **http://www.senseaboutscience.org/**

WP5 – Education, Training and Career Development

- **White Book on Education published**
  - Available from EUROFORGEN website as PDF document
  - Searchable list of courses available online
- **CEPOL course Madrid, June 4-7, 2013**
  - "Mixtures, complex DNA profiles, and familial testing: interpretation workshop schedule"
  - New course planned for 2014 (not yet approved)
- **First "Train the Trainers" Workshop**
  - Copenhagen, Oct. 7-10, 2013
  - Participants have been asked to develop plans for dissemination at the national/local level
  - Next workshop in May/June 2014

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WP5 – Education, Training and Career Development

- **Introduction of an Educational Board for academic career development**
  - To develop recommendations for the academic education of forensic geneticists
- **Long term perspectives**
  - Introduction of online learning tools:
    - Self education about basic topics
    - Online lectures about advanced topics
  - Implementation via "Virtual Center" website, or in association with established online learning institutions, such as e.g.
    - Udacity
    - Coursera

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About EUROFORGEN-NoE
The Group
The Project
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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.
About EUROFORGEN-NoE
The main objective of the European Forensic Genetics Network of Excellence – EUROFORGEN-NoE – is the creation of a European Virtual Centre of Forensic Genetic Research.
Forensic genetics is a highly innovative field of applied science with a strong impact on the security of citizens. The project, funded by the SECURITY programme of the European Commission under the 7th Framework Programme, started in January 2012 and will run for 5 years. It is coordinated by Prof. Peter Schneider of the Institute of Legal Medicine at the University Hospital of Cologne, Germany.
The network includes 12 partners from 8 countries including leading groups in European forensic genetic research. It aims to create a close integration of existing collaborations as well as to establish new interactions in this highly specialized field of security. Therefore, all key players such as scientists, stakeholders and end-users (e.g. police institutions and the justice system), educational centres and scientific societies will be integrated into these activities. Furthermore, the societal dimension of applying forensic genetics in criminal investigations, potentially affecting privacy rights and minority protection, will be addressed. Finally, training and educational activities will be implemented to establish common European-wide standards for generating and interpreting genetic data related to biological evidence.
With the establishment of a long-term collaborative network, it will become possible to effectively connect all scientific groups active in the field of forensic genetics and to initiate a sustained effort covering all aspects of research within the field. If you are working in the field, and interested to contribute to our network, please → contact us.
Quicklinks
Newsletter 1/20013
The European landscape in forensic genetics
Geographical display and contact data
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RECENT POSTS
Peter Schneider
Dear friends, we are all very concerned about the fate of the people affected by typhoon Hayan in the Phillipines. Our colleague Dr. Cora De Ungria from the DNA Analysis Center in Manila recommends these two organizations for donations.
http://www.redcross.org.ph/
and
About 76 members
Open Group
The EUROFORGEN-NoE proposal aims to develop a network of excellence for the creation of a E... See More
76 members (1 new) · Invite by Email
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Tiziana Melis