## AGENDA FOR THE EDNAP MEETING

## **MADRID – 7 MAY 2019**

## ROOM: MEDICI II

Expected duration: 09.00 - 17.00

Coffee: 10.30-10.50 - Lunch: 12.30-13.30 - Coffee: 15.30-15.50

Host:	Victor José Esteban Ramos			
Chairman:	Niels Morling			
Welcome		Victor Esteban		
Update on	activities			
Methyla	ted DNA and age exercise	David Ballard		
Update	on exercise no. 2 on MPS mRNA typing	Cordula Haas		
mtDNA	quantification exercise	Arnoud Kal		
Updates fro	om other groups			
ENFSI		Sander Kneppers		
EMPOP	P (DNA.BASES)	Walther Parson		
A note of	Walther Parson			
STRidE	Walther Parson			
The VIS	Walther Parson			
ISFG		Walther Parson		
Final rej	port from the DNASEQEX project	Antonio Alonso		
Other activ	ities			
Investig	ative DNA profiling using Case Solver - illustration with a real	Peter Gill, Lourdes		
case of 2	>100 crime-stains	Prieto, Oyvind Bleka		
Future activ	vities			
Collabo	rative exercise on detection of mtDNA heteroplasmy by MPS	Walther Parson		
New col	llaborative exercise on MPS mRNA	Cordula Haas		
New as	sociated member: Institute of Legal Medicine and Forensic	Niels Morling		
Science	s, Charité Medical University, Berlin, Germany	C		
New col	laborative exercise on ancestry investigations? - Roewer	Niels Morling		
New col	laborative exercises on transfer of DNA? – Oorschot/Kokshoorn	Niels Morling		
Next EI	ONAP meeting: 23 October 2019 in Riga	Niels Morling		
Any other	business	Niels Morling		

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## EUROPEAN DNA PROFILING GROUP (EDNAP) MEETING

## Madrid, Spain

## 7 May 2019

Host: Victor Jose Esteban Ramos Chairman: Niels Morling

A list of participants is attached.

### Welcome

Victor Esteban welcomed members to Madrid.

## Update on exercises

Second exercise on methylated DNA and age David Ballard David Ballard presented an update of the results of the second collaborative EDNAP exercise on age estimation by means of measurements of methylation of selected DNA positions. A draft of a manuscript is expected to be circulated before the end of 2018 (presentation attached).

*Exercise no. 2 on mRNA typing with NGS* Cordula Haas/Guro Dörum Cordula Haas presented an update on the results of the second collaborative EDNAP exercise on discrimination between various tissues and body fluids as well as assignment to donors of the various tissue components with mRNA determined with MPS. A draft of a manuscript is expected to be circulated within the next weeks (presentation attached).

### mtDNA quantification exercise

Arnoud Kal updated about the planned collaborative exercise concerning quantification of mtDNA. Arnoud Kal on 29 Oct 2018 circulated the attached email. Please contact Arnoud Kal if you want to participate. The plan is to send out the exercise within the next few weeks (presentation attached).

## Updates from other groups

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

### EMPOP (DNA.BASES)

Walther Parson gave a short update of the activities of EMPOP (presentation attached).

### A note on mtDNA inheritance

Walther Parson presented a short overview on data that might suggest paternal inheritance of mtDNA. Walther Parson also presented mtDNA family data that suggested incorporation of mtDNA into autosomal chromosomes – nuclear mitochondrial DNA segment - NUMT (manuscript in preparation – no presentation attached).

### STRidER (DNA.BASES)

Walther Parson gave a short update on STRidER (presentation attached).

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Walther Parson

## Arnoud Kal

Walther Parson

Walther Parson

*The VISAGE project* Walther Parson Walther Parson gave an update on the VISAGE project (presentation attached).

ISFG

Walther Parson

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

Final report from the DNASEQEX projectAntonio AlonsoAntonio Alonso presented the final report of the DNASEQEX project (presentation attached).

## Other activities

Investigative DNA profiling using Case Solver - illustration with<br/>a real case of >100 crime-stainsPeter GillPeter Gill gave an overview on the free software Case Solver (presentation attached).

## **Future activities**

*Collaborative exercise on detection of mtDNA heteropasmy by MPS* Walther Parson Walther Parson summarized the purpose with the collaborative exercise on mtDNA heteroplasmy. The Innsbruck laboratory will send out the plan for the exercise and DNA extracted from individuals with point and/or length heteroplasmy before the end of June 2019. Laboratories with volunteers with heteroplasmia in the mtDNA are invited to submit 10 hair pieces that are at least 2 cm long and a buccal swab sample, e.g. on FTA-card, from each individual. Please contact Walther Parson. Laboratories that have not yet informed Walther Parson about their participation are kindly asked to do this as soon as possible. The laboratories should submit their results before the end of 2019 (no presentation enclosed, please see the presentation in the minutes from the previous EDNAP meeting).

*New collaborative exercise on MPS mRNA* Cordula Haas Depending on the success of the implementation of MPS analysis on TFS S5 that is performed by Jack Ballantyne and colleagues in Orlanda, USA, Cordula Haas and Jack Ballantyne may suggest a collaborative exercise on mRNA using the TFS S5 (see the presentation above).

*New collaborative exercise on ancestry investigations* Niels Morling The members were positive about the idea of participation in collaborative exercises on ancestry investigations under the umbrella of EDNAP as suggested by Lutz Roewer. The members asked the secretary to discuss with Lutz Roewer if a group including Chris Phillips, Lutz Roewer, and Walther Parson should discuss and - hopefully - take the idea forward?

*New collaborative exercises on transfer of DNA* Niels Morling Although very few details about Roland van Oorshot's and Bas Kokshoorn's ideas about collaborative exercises concerning transfer of DNA were available, the EDNAP members were positive about the idea. At least 15 laboratories expressed interest in participation. However, there is a need for information about the plan and discussions of the various aspects. Hopefully, Roland van Oorshot and Bas Kokshoorn will be able to circulate the ideas before the next EDNAP meeting 23 October 2019 in Riga.

### Next meetings

Niels Morling The next EDNAP meeting will take place 23 October 2019 in Riga, Latvia together with the meeting of the steering group of the ENFSI DNA Working Group.

### Any other business

There was no other business.

## Closing of the meeting

The meeting closed with sincere thanks to Victor Esteban and all colleagues, who helped organising the meeting.

## The minutes and attachments are found at the EDNAP website:

http://www.isfg.org/EDNAP/Meetings, including:

- Agenda
- List of participants
- Presentations
  - David Ballard: Update on methylated DNA and age determination
  - Cordula Haas: Update on the second collaborative exercise on mRNA NGS
  - Arnoud Kal: Update on the mtDNA quantification exercise
  - Sander Kneppers: Report from the ENFSI DNA Working Group
  - Walther Parson: EMPOP report (DNA.BASES)
  - Walther Parson: STRidER report (DNA.BASES)
  - Walther Parson: The VISAGE project
  - Walther Parson: ISFG report
  - Antonio Alonso: Final report from the DNASEQEX project
  - Peter Gill: Investigative DNA profiling using Case Solver.

Niels Morling

Niels Morling

# Methylated DNA & Age Exercise



EDNAP, Madrid 2019



#### Introduction

The development of methods that can accurately estimate an individual's chronological age from trace evidence is an ongoing quest in the field of forensic DNA intelligence. The retrieval of this information, as well as information regarding externally visible characteristics, like eye, hair or skin colour and hair morphology [1-4]. From DNA samples recovered from crime scenes, can significantly aid police investigations, especially in cases lacking eye witness testimonies and/or intact human remains.

While multiple biomarkers for chronological age have been suggested over the years [5-19], the quantification of DNA methylation, an epigenetic modification that mainly affects cytosines when these are followed by guanines in a 5'-3' direction and is a known modulator of genetic expression [20], has been the focus of recent research. The main reasons behind this choice are the strong and specific correlation of multiple methylation's biological stability over time [24-28].

Several different approaches have been established for the quantification of DNA methylation, with the four main ones being (i) massively parallel sequencing (MPS), (ii) pyrosequencing, (iii) methylation SNaPshot and (iv) MALDI-TOF mass spectrometry (EpiTYPER). Massively parallel sequencing offers high sensitivity as well as single-base resolution and is able to cope with large scale multiplexing, characteristics that place it to the top of the choices for DNA methylation guantification for forensic purposes. Furthermore, forensic laboratories worldwide are becoming increasing familiar with this technology as it has been applied to multiple aspects of forensic laboratories include the MiSeg (Illumina), MiSeg FGX (Verogen) and the ION Personal Genome Machine (ION PGM) and ION S5 systems (Life Technologies).

Recent publications reveal significant scientific leaps towards making age estimation through DNA methylation a reality for forensic casework, with the developed methods showcasing promising results in terms of accuracy, robustness and sensitivity [34-37]. However, even though few published methods have been successfully reproduced across the forensic community [38, 39] little research has been conducted on the transferability of the proposed methods between different laboratories as well as different instruments. While the stages of identification of promising markers and optimisation of the potential methods are vital to the development of new forensic tools, transferability between different forensic facilities is also an important factor that needs to be investigated, especially when the proposed methods involve high cost equipment like the MPS instrumentation.

In order to investigate further into this matter, this exercise focuses on the transferability of a previously described DNA methylation-based age prediction method originally developed on the MiSeg EGX platform [40]. The same protocol, with minor instrument-related alterations was performed in 14 different labs using different types of MPS technology including the MiSeg, MiSeg FGX, ION PGM and ION S5 systems and the results were compared both for standards of known methylation and real samples.

#### Materials and Methods

#### DNA methylation standards

For the first part of this study 7 pre-mixed methylation standards ranging from 0% to 100% methylation were purchased from EpigenDx (Massachusetts, USA) at a concentration of 50ng/µL. Standards were diluted and delivered to the participating laboratories at a final concentration of 2.5ng/µL.

#### Sample Collection

For the leading research group of this exercise in King's College London, sample collection for this study was performed under ethical approval granted by the Biomedical Sciences, Dentistry, Medicine and Natural & Mathematical Sciences Research Ethics Subcommittee (BDM/13/14-30). A total of 7 donors aged between 27.7 and 79.7 years were recruited for the collection of whole blood samples (samples A-G) via venepuncture following the acquisition of full informed consent. Samples were stored at 4°C.

#### Sample Shipping

Methylation standards were shipped to the participating laboratories in sealed 1.5mL snaptop containers, while samples A-G were shipped in the form of blood stain cards.

#### DNA Extraction and Quantification

Genomic DNA was extracted by different methods depending on the laboratory, with the BioRobot\*EZ1 automated purification instrument (Qiagen, Hilden, Germany) in combination with the EZ1 Blood and Investigator kits being the most popular choice. Other methods included the QIAamp DNA mini kit (Qiagen, Hilden, Germany), QIAamp DNA Investigator kit (Qiagen, Hilden, Germany), DNA IQ system (Promega Corporation, Wisconsin USA), Wizard\* Genomic DNA Purification kit (Promega Corporation, Wisconsin USA), PrepFiler Forensic DNA Extraction kit (Thermo Fisher Scientific, Massachusetts, USA), Chelex and organic extraction (Supplementary File 1a).

Similarly, several different methods were employed for the quantification of the DNA extracts with the most common ones being a fluorometric quantitation using Qubit for double stranded DNA high sensitivity (Thermo Fisher Scientific, Massachusetts, USA) and a real-time PCR quantitation with Quantifiler\* Trio DNA Quantification kit (Thermo Fisher Scientific, Massachusetts, USA) in full or half volumes. Additional methods included the Quantifiler\* Human DNA Quantification (Thermo Fisher Scientific, Massachusetts, USA), Quantifiler\* Duo (Thermo Fisher Scientific, Massachusetts, USA), Quantifiler\* Human Plus (Thermo Fisher Scientific, Massachusetts, USA), AluQuant\*\* Human DNA Quantitation (Promega Corporation, Wisconsin USA), Quantus\*\* Fluorometer in combination with the Quantificuor\*\* dsDNA Dyes (Promega Corporation, Wisconsin USA) and <u>NanoDrop</u> Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) (Supplementary File 1b).

A	В	C .	D	E
	Illumina			
7. NFI				
	KAPA Hyper Prep kit for Illumina®			
8. Oslo	Agencourt AMPure XP; Ion Plus Fragment Library Kit (Cat. no. 4471252); Ion PGM Hi-Q Chef Kit; Ion Xpress <sup>m</sup> Barcode Adapters 1–16 Kit; Ion 314 <sup>m</sup> Chip Kit v2 BC	ampliseg protocol?		
9. Zurich	IonXpress Plus gDNA Fragment Library Kit, Thermofisher	Ion PGM Hi-Q OT2 200 Kit, Ion PGM Hi-Q Sequencing Kit, Ion 318 Chip v2		
10.Florida	KAPA hyper prep kit for illumina; agencourt AMPpure XP beads; Roche SeqCap A/B adapters			
11. Singapore MiSeq	KAPA Hyper Prep for Illumina platforms, NimbleGen SeqCap Adapter Kit A and B Used 1.87 µl of 10µM of the index adapters	KAPA Hyper Prep for Illumina platforms, NimbleGen SeqCap Adapter Kit A and B Agencourt AMPure XP reagent, Beckman Coulter Genomics		
11. Singapore S5		Ion XpressPlus gDNA Fragment Library Preparation Ion Xpress Barcode Adapters Used 50ng PCR product for end-repair instead of 200ng, and performed library amp before library quan. Also have tried using 100ng without library amp following suggested PGM protocol, but 55 run failed. Agencourt AMPure XP reagent, Beckman Coulter Genomics		
12. Lyon	KAPA Hyper Prep kit for Illumina*, KAPABiosystems (cat.No.: KK8502, 48 reactions) Agencourt* AMPure* XP reagent, Beckman Coulter Genomics (cat.No.: A63881, 60 ml SeqCap Adapter Kit, Roche, Cat.No.: 07141530001 for set A07141548001 for set B			
13. NIST	KAPA Hyper Prep for Illumina, with adapters from Illumina TruSeq 96plex Adapter Plate			
14. Victoria				



cg04528819, cg22736354 and cg06493994.

In the first part of this collaborative exercise, the different laboratories were provided with the same pre-mixed primers as well as the same pre-mixed DNA methylation standards ranging from 0 to 100% methylation. All standards were analysed in duplicate by each participant laboratory and the detected methylation values were averaged across the labs using MiSeq technology and those using Ion PGM or Ion S5 sequencers (Fig.1). While results showed good correlation between the two different sequencing technologies for 7/12 markers, significant differences (p<0.05) were observed for

3.1 DNA standards of known methylation

3. Results

## Figure 1 – Laboratory prediction of samples A-F





# Manuscript

- Introduction
- Methods
- Results Phase 1
  - Methylation control reproducibility per lab and marker
    Methylation control differences MiSeq vs PGM/S5
- Results Phase 2
  - Age prediction reproducibility per lab
  - Blind prediction samples
- Discussion/conclusion

# Acknowledgments

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## DAVID BALLARD DNA ANALYSIS AT KING'S KING'S COLLEGE LONDON LONDON UK

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# EUROFORGEN / EDNAP mRNA NGS exercise 2 Assay for body fluid/tissue identification and assignment to donor(s)

Cordula Haas / Guro Dørum / Sabrina Ingold Erin Hanson / Jack Ballantyne

7. May 2019, Madrid







# 1. Collaborative exercise mRNA NGS part 2

- only MiSeq laboratories (1/2 library kit left from exercise 1)
- 2 separate assays:

   targeted mRNA NGS approach for the identification of blood, saliva, semen, vaginal secretion, menstrual blood, skin
   cSNPs assay to associate specific mRNA transcripts to an individual
- RNA extraction (manual or kit), DNase treatment, quantification
- Protocols and primerpools were provided
- Laboratories analyzed 16 samples provided by UZH
- Results (FASTQ files) were collected and evaluated by UZH



# 1. cSNPs = coding region SNPs

Body fluid / tissue specific SNPs

- In body fluid specific RNA we looked for SNPs that discriminate European individuals the most
- 34 off-the shelf cSNPs (MAF from 0.48 0.06)
- 3-11 markers per body fluid

cSNPs give a direct link between donors and body fluids

ightarrow Identification of individual and body fluid



## Targeted mRNA NGS approach for body fluid/tissue identification and assignment to donor(s)

Body fluid/tissue	Gene	mRNA 33plex	cSNPs 35plex
	ALAS2		-
	ANK1		4
Disad	SPTB		
Blood	CD3G		
	CD93		3
	AMICA1		2
	PRM1		
	PRM2		
6	TGM4		4
Semen	SEMG1		
	SEMG2		2
	KLK3		
	HTN3		
	HTN1		
	STATH		
Saliva	PRB3		
	PRB4		
	PRH2		
	MUC7		2
	CYP2B7P1		
	DKK4		
Vaginal	FAM83D		
	CYP2A6		
	CYP2A7		2
	MMP10		2
	LEFTY2		
Menstrual	MMP7		
	MMP11		
	SFRP4		
	LCE1C		2
	CCL27		
Skin	IL37		
SKIII	SERPINA12		
	KRT77		2
	COL17A1		3



# 1. Collaborative exercise mRNA NGS part 2

10 participating laboratories:  $\rightarrow$  1x no data

- Copenhagen, Denmark
- Innsbruck, Austria
- London, UK
- Lyon, France
- NFI, Netherlands
- NIST, USA
- Orlando, Florida, USA
- Rome, Italy
- Rotterdam, Netherlands
- Zurich, Switzerland



# **1. cSNP discussion**

- Analysis of RNA/cSNP in stains is challenging
  - RNA easily degrades some markers drop out
  - Residual DNA in RNA extract markers for body fluids not present "drop in"
  - Heterozygote read count ratio can deviate from an expected 1:1 ratio, due to stochastic amplification processes with low template targets
  - → Inconsistencies between reference profiles and stain/mixture profile
  - $\rightarrow$  Hindering for mixture deconvolution
- Combining evidence DNA, RNA and cSNP
- Need more suitable markers → Simultaneous identification of individual and body fluid



# 1. Manuscripts

- 1) cSNP proof of concept paper
  - 12 single source samples, 51 mixtures
  - STRs, mRNA, cSNPs •
  - **Statistics** ٠

2) Collaborative exercise mRNA NGS part 2  $\rightarrow$  ready to distribute

- 10 labs, 9 data sets •
- 16 samples ٠
- TOP6 and cSNP data •
- **Statistics** ٠

 $\rightarrow$  submitted to FSI Genetics



# 2. Thermofisher cSNP assay

- blood, semen, saliva, (vaginal, menstrual and skin)
- cSNP amplicons are useful for body fluid identification
- some marker overlap between the MiSeq cSNP assay and TF assay
- first trial experiment → genotypes can be distinguished for most of the body fluids, even amongst a small number of donors → good discrimination at the cSNP level
- $\rightarrow$  Possible collaborative exercise in autumn 2019

High degree of specificity (body fluid ID)

Unique RNA genotypes (body fluid to donor association)



cSNP	B7489	B7480	B7481	B7486	B7479	BL1-Z
1	AA	AA	AG	AG	GG	AA
2	CG	CG	CG	GG	GG	CG
3	CC	ст	ст	ст	ст	СТ
4	TT	ст	TT	TT	TT	TT
5	AG	GG	AG	GG	GG	GG
6	AG	AG	AG	AG	AG	AG
7	ст	ст	ст	ст	CC	СТ
8	AG	AG	AG	AG	AA	AG
9	ст	CC	СТ	СС	ст	СТ



## 3. FoRNAP - Forensic RNA Profiling Grüppli





# 3. FoRNAP - Forensic RNA Profiling Grüppli

- Exchange on RNA profiling applied to casework
- Methods, Validation, Interpretation, Cases, Recommendations, etc.
- 1. Meeting: 22./23. March 2018, Zurich
  2. Meeting: 22. Feb. 2019, Jena
  3. Meeting: 10. Sept. 2019, Prague
- 9 laboratories: Kiel, Ulm, Munich, Ljubljana, Zurich, Cologne, NFI, BKA, LKA Rheinland-Pfalz
- Online Platform to exchange / collect information
- Collaborative exercise 2019: 16 stains, use own RNA only or RNA/DNA co-analysis methods, CE/MPS

 $\rightarrow$  Laboratories who have applied RNA profiling in casework are welcome to participate

 $\rightarrow$  send an Email to: cordula.haas@irm.uzh.ch



# Thank you for your attention!





Netherlands Forensic Institute Ministry of Justice

# EDNAP Exercise mtDNA quantification

Kris van der Gaag Natalie Weiler Titia Sijen Arnoud Kal



# EDNAP exercise mtDNA quantification

- •Home made assay (cheap!)
- •Quantification of autosomal, Y and mtDNA
- •Establish if sufficient mtDNA is present in the sample
- •Optimize the input for your favourite typing method
  - Sanger (mini-mito)
  - MPS (equalize input for multiple samples in one run)



# Quantification Assay

Real time multiplex PCR assay:

- •42 cycles
- •Buffer system: TaqPath ProAmp Master Mix
- •Amplicons for autDNA, YDNA, long mtDNA and short mtDNA

DNA	Probe	Вр	Dye	Sensitivity
Total DNA	Alu Ya5	127 bp	VIC	0,5 pg/µl
Y DNA	DYZ5	137 bp	FAM	4 pg/µl
mtDNA	16533-180	217 bp	JUN	
mtDNA	2502-2571	70 bp	ABY	





# 20 Labs showed interest

NFI provides:

- •Primers and probes
- •Challenging samples
- •Protocols

Labs provide:

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



# Exercise sample shipping 1

- •Exercise samples and reagents shipped on 3 december 2018
- •Shipment to 20 labs
- •16 x Europe
- •4 x USA



# US Government shutdown

## •Samples stuck untill januari/februari 2019





# Exercise sample shipping 2

- •2 extra labs
- •3 labs asked for more samples/reagents

New samples prepared at NFI, allmost ready to be shipped


#### Data analysis

8 samples

Triplicate analysis undiluted
Triplicate analysis diluted
4 probes
21 labs
↓
6048 data points





#### Challenging Samples

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





#### To be continued

- 2nd shipment
- Wait for results
- Data analysis
- Draft publication
- Next update in Riga



Netherlands Forensic Institute Ministry of Justice

Update ENFSI DNA Expert Working Group activities

Alexander Kneppers Chair ENFSI DNA Expert Working Group

NFI Division Biological Traces

ENFSI update EDNAP Madrid May 2019

# **69 MEMBERS IN 37 COUNTRIES**





d May 2019

#### **EVERY MEMBER HAS TO:**

# ORIGINATE FROM A COUNCIL OF EUROPE MEMBER STATE

# **COVER 50 % OF THE WORKING GROUPS**

# **CREDIBLE STATUS IN THE COUNTRY**

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# **MORE THAN 25 REPORTING OFFICERS**

# ACCREDITATED AGAINST ISO17020 / ISO17025 OR ...

- Sanata and

# ... HAVE THREE YEARS TO ACHIEVE THIS



#### **SYNERGY IN NETWORKING**



> 1000 Forensic Experts

# **17 WORKING GROUPS**

# COMMUNICATION





#### Welcome to ENFSI!

The European Network of Forensic Science Institutes (ENFSI) was founded in 1995 with the purpose of improving the mutual exchange of information in the field of forensic science. This, as well as improving the quality of forensic science delivery in Europe have become the main issues of the network. Besides the general work in the fields of quality and competence management, research and development and education and training, different forensic expertizes are dealt with by 17 different Expert Working Groups. ENFSI therefore has been recognized as the monopoly organization in the field of forensic science by the European Commission.



## EXTERNAL: <u>WWW.ENFSI.EU</u>

### **COMMUNICATION**





EUROPOLEXPERTS CORPORATE EUROPEAN NETWORK OF FORENSIC SCIENCE INSTITUTES

EPE Home Private Messaging | F.A.Q. | Contact us

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ENFSI - European Network of Forensic Science Institutes	ENFSI - European Network of Forensic Science Institutes >				
Home	Welcome!	Contact			
Event Calendar		Property halo along contratity alter			
Documents •	15	administrators Elisabeth Dickersgill or Isabelle			
Finances	Casher	Jopp from the ENFSI Secretariat by sending			
Monopoly Programmes	VENFSI	an e-mail to secretariat@enfsi.eu			
Message Forum					
Blog	The ENFSI - European Network of Forensic Science Institutes is an interactive platform intended to bring	Q Q			
User Directory	together the forensic experts in Europe, allowing them to get involved with the content displayed. All users are				
ENFSI Public Site	advised to subscribe to every Blog and Forum in order to be timely notified on new posts/entries. It is possible to				
Animal, Plant and Soil Traces EWG	To get familiar with EPE please have a look here.				
Digital Imaging WG					
DNA EWG	Quick Navigation				
	ENFSI main site - Permanent Members	Quick Links			
Documents EWG	Standing Committees: Quality and Competence SC - Research & Development SC				
Drugs EWG	Working Groups: Animal, Plant and Soil Traces - Digital Imaging - DNA - Documents - Drugs -Evolosives - Fingergrint - Fire and Evolosions	Authorization process on ENESLEPE			
ENFSI - Permanent Members	Investigation - Firearms/GSR - Forensic Information Technology - Handwriting - Marks - Paint & Glass - Road Accident Analysis - Scene of Crime - Speech and Audio Analysis - Textile and Hair	Password & Policy tips     Summary on User rights			
Explosives EWG		ENFSI public website			
Eingenprint EWC	Message Boards				

#### **INTERNAL: EPE.EUROPOL.EUROPA.EU**





#### Strategic plan 2018 - 2019

- 1. Contribute to the establishment of a European Forensic Science Area 2020 through the implementation of the Action Plan.
  - 1. Facilitate the establishment and sharing of BPMs
  - 2. Facilitate the establishment and sharing of Forensic Databases.
  - 3. Facilitate the establishment of new Proficiency Tests and Collaborative Exercises
  - 4. Guide the coordinators of several actions of the EFSA2020 Action Plan



#### European Forensic Science Area 2020

- Work with the EC to obtain funding for creating new BPMs and updating existing BPMs, including their translation into EU languages.
- Ensure the data collected are harmonized and thus comparable. Make a clear separation between knowledge databases and case related databases.
- Work with Europol to achieve a solid platform and maintenance system for forensic databases.
- Work with the EC to provide funding for the development of new databases and their implementation into a common network.
- Work with the EC to provide funding for new Proficiency Tests and Collaborative Exercises.
- Explore the availability of ENFSI Member institutes and private service providers to produce the tests on a regular basis.



# STEFA Project Work Package 3



Collaborative exercise covering the forensic disciplines of DNA, document examination, fingerprint examination and handwriting examination



# Introduction



Historically Proficiency Tests (PT) and Collaborative Exercises (CE) have

- Been carried out within a single discipline
- Tended to only cover the examination and interpretation aspects of the individual forensic processes.

However the "real" world is normally more complex than single examination types, and in many instances forensic material must be examined for a number of different evidence types.



Participants



Two representatives from each of

- European Network of Forensic Handwriting Experts (ENFHEX),
- European Document Experts Working Group (EDEWG),
- European Fingerprint Working Group (EFP-WG) and
- ENFSI DNA working group (DNA-WG)



# Proposal



Within the four forensic discipline involved the project will:

- determine current availability and process for development of CEs
- determine the practicality of developing a multi-discipline CE
- prepare guidelines for the development of a multi-discipline CE
- develop, run and evaluate a multi-discipline CE covering the four areas of forensic science and the laboratory management processes



# Benefits



The benefits of the project are:

- The CE will act as a benchmarking exercise between laboratories (not between different forensic disciplines)
  - The CE will assess the movement of exhibits through various forensic areas/activities in the laboratory
- The CE will develop a process for running multi-discipline Ces
- The CE may identify best practice in examining certain types of material
- FREE TO USER



#### Horizon 2018 funding opportunities

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





#### Strategic plan 2018 - 2019

- 2. Strengthen the capability of the ENFSI organization as a resource to support the forensic science community.
  - 1. Strengthen the ENFSI Board
  - 2. Organize ENFSI Forensic Governance Forum
  - 3. Re-activate the "One Day One Topic Seminars (OOS)"
  - 4. Facilitate and stimulate the use of the new intranet platform
    - 1. Evaluation Report, Training for EPE Managers at Europol
  - 5. Improving external communication
    - 1. An ENFSI Newsletter shall be created. The newsletter shall be provided to Members and relevant strategic partners each quarter.
  - 6. Development of ENFSI Annual Meetings





#### Strategic plan 2018 - 2019

- 3. Consolidate the interaction with the stakeholders and partners
  - 1. Involve Strategic Liaison Officers of ENFSI Stakeholders such as EA, ILAC, ICC, CEN/ISO, NIST/OSAC
  - 2. Establish an ENFSI Advisory Committee.
  - 3. Appoint liaison officers for the communication with partner organizations





#### ENFSI DNA Working group Steering Committee

Chair	Sander Kneppers NFI, the Netherlands
Vice chair	Livia Zatkalikova, Ministry of Interior, Slovakia
Secretary	Astrid Quak, NFI, the Netherlands
Treasurer	Ingo Bastisch, BKA, Germany
QCLG	June Guiness, FSR Home Office UK
R&D	Shazia Khan, MP UK
E&T	Paula di Simone, Italian National Police
Webmaster	Fabrice Noel, NICC Belgium
EDNAP	Niels Morling, Univ. Copenhagen, Denmark
	Peter Schneider, ILM, Univ. Cologne, Germany

5 subgroups with subgroup chairs





# DNA working group

- Annual meeting of the working group in association with
  - the European CODIS meeting
  - EDNAP meeting (European DNA Profiling group)
- Annual ENFSI board meeting with workgroup chairs
- Two steering committee meetings DNA working group

Group A: Quality AssuranceGroup B: DNA Analysis Methods & InterpretationGroup C: DNA Database and LegislationGroup D: Automation & Expert Systems

Group E: Forensic Biology and casework





#### (Associate) Members DNA working group

- Currently 75 labs are participating in the DNA working group
  - 46 member laboratories
  - 29 associate member laboratories

#### 4 task forces





Task force	Project title	Project description	Task Force member	Task Force leader	
1	BPM DNA pattern Revising of the existing BPM ecognition and		Ricky Ansell	Ela Zaimi	
	comparison		Peter GIII Walther Parson		
2	BPM Human DNA Analysis	Writing of BPM to cover the general accepted	Ricky Ansell	Stavroulla	
		procedures and workflows for the processing of human DNA: from the collection of evidence to the	June Guiness (editorial)	Xenophontos	
		reporting of findings	Ate Kloosterman		
			Astrid Quak		
			Tacha Hicks Paula di Simone		
			Ela Zaimi		
			Tom Heylen		
			Aikaterini Kondili		
3A	Guideline: Training of	A: Update the Guideline for the training of staff	Heli Autere	Tom Heylen	
	DNA staff	working in the forensic DNA laboratory	Paola Di Simone		
			June Guiness		
3B	Guideline: ENFSI Quality	B: Update the Guideline regarding the ENFSI	June Guiness		
	Assurance Program for	ce Program for Quality Assurance Program for DNA Laboratories	Elisabetta Mei		
	DNA Laboratories		Maria Vouropoulou		
			Task Group 3a or 3B up to task force leader:		
			Kua Guo Wei		
			Coro Fernández Oliva		
4	Guideline: 'Minimum	Update the Guideline regarding the minimum	Christina Forsberg	Annick Delaire	
	Criteria for the Validation	criteria for the validation of various aspects of the	Kon Gainy Aikaterini Kondili		
	DNA Profiling Process'	Diva profiling process	Wong Hang Yee		





### Madrid 7<sup>th</sup> – 10<sup>th</sup> May 2019 Annual meeting ENFSI DNA Expert Working Group

Host Criminalistic Service Guardia Civil

135 persons attending
103 participants
32 company
representatives
12 companies
31 countries represented
>60 speakers







#### QA-subgroup co-chairs Annick Delaire (France), Tom Heylen (Belgium)

o Quality documents

Guideline: Training of DNA staff **Guideline: 'Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process'** Guideline: ENFSI Quality Assurance Program for DNA Laboratories





#### Methods analysis and interpretation sub group co-chairs Antonio Alonso (Spain) and Walther Parson (Austria)

- Software analysis tools
  - Case Solver
  - Dna.bases/EMPOP/STRidER)
  - DNAxs
  - EuroForMix with link to STRidER
- MPS updates
  - Verogen/Qiagen/Promega
  - Nomenclature (STRAND meeting)
  - Casework





#### DNA database and legislation subgroup co-chairs Dyan Daly (Ireland) and Izanda Puncule (Latvia)

- DNA Database Management Document
  - Feedback on audit
- Updates
  - Databases
  - Legislation across Europe
  - Statistics
  - Interpol
- Discussion on EU Data protection directive/CODIS update





#### Automation and LIMS subgroup Co-Chairs Christina Forsberg & Shazia Khan

- Automated workflows for NGS
- Rapid DNA analysis
- Laboratory automation
- LIMS





Forensic Biology and Casework subgroup co-chairs Arnoud Kal (Netherlands), Ricky Ansell (Sweden) and Livia Zatkalikova (Slovakia)

- Routine casework examples in and beyond DNA testing
- Possible solutions for forensic labs to cope with high demands/backlogs
- Investigative genealogy vs familial searching



#### Contact ENFSI DNA Working group

Chair Sander Kneppers NFI, the Netherlands <u>s.kneppers@nfi.nl</u>

Vice chair Livia Zatkalikova Ministry of Interior, Slovakia, <u>livia.zatkalikova@minv.sk</u>

Secretary Astrid Quak NFI, the Netherlands, <u>a.quak@nfi.nl</u>



EDNAP Meeting, Madrid, Spain, May 07 2019



**MEMP** 

# **EMPOP Update**

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

# EMPOP v4 is fully phylogenetic





Alignment-Free Query

Röck et al 2011 **FSIG** Huber et al 2018 **FSIG** 

#### **Phylogenetic Alignment**

Parson et al 2014 **FSIG** Dür et al in prep

#### Haplogrouping

Röck et al 2013 **FSIG** Dür et al in prep



# **EMPOP 4 workflow**

#### Query

Query	Result	Details	Neighbors	Alignment	Haplogrouping
Sample ID					
Ranges	16024	-576			
Profile	16183	- 16217C 16	5519C 263G 3	15+C 499A	11.

#### Result of database search

Query	Result	Details	Neighbors	Alignment	Haplogrouping		
Sample ID	ID (none specified)						
Ranges	16024-	16024-576					
Profile	16183- 16217C 16519C 263G 315+C 499A						
Entire Data	base			Frequency	Clopper Pearson Cl		
-	-	_	2/334	47 5.9796e-5	[7.2416e-6, 2.1599e-4]		

#### Phylogenetic alignment

Query	Result	Details	Neight	oors	Alignment	Haplogro	uping					
Phylog	genetic	alignm	nent									
Input P	rofile		263G	315.1	C 499A	16183-					16217C	16519C
Phyloge	enetic alig	nment	263G	315.1	C 499A		16183C	16188T	16189C	16193-	16217C	16519C

#### Haplogrouping result

Query	Result Details Neighbors	Alignment	Haplogrouping	
Haplo	grouping			
Rank 1: M	/RCA: <b>B4b</b> 1.88			
Source	Haplogroup	Range	Diagnostic Mutations	Cost
PT17	B2 B2b B2b3 B2c B2c1 B2c1a B2c1b B2c1c B2e B2f B2i B2n B2p B2q B2r B4b	16024-576	16183M 16189C 16217C 16519Y 73G 263G 315.1C 499A	1.88
PT17	B2b4	16024-576	16183M 16189C 16217C 16239Y 16353Y 16519Y 73G 159Y 195Y 263G 315.1C 499A	1.88
Rank 2: I	MRCA: <b>B2b+152</b> 2.26			
Source	Haplogroup	Range	Diagnostic Mutations	Cost
PT17	B2b+152	16024-576	16183M 16189C 16217C 16519Y 73G 152C 263G 315.1C 499A	2.26

#### **EMPOP workshops**



# mtDNA/EMPOP publications

May 2018 - April 2019

Simao et al (2018) Forensic Sci Int Genet **34**: 97-104 Parson et al (2018) Forensic Sci Int Genet **36**: 148-151 Huber et al (2018) Forensic Sci Int Genet **37**: 204-214 Simao et al (2019) Forensic Sci Int Genet **39**: 66-72 Lutz-Bonengel and Parson (2019) Proc Natl Acad Sci USA doi: 10.1073/pnas.1820533116

Scientific Working Group on DNA Analysis Methods

Interpretation Guidelines for Mitochondrial DNA Analysis by Forensic DNA Testing Laboratories



approved April 23 2019 alignment and nomenclature based on phylogenetic considerations in EMPOP

https://www.swgdam.org/publications



SWGDAM



#### Metapopulation Distribution



# **EMPOP Release 12**

ORIGIN						
Continent	Country	# samples				
Africa	Guinea-Bissau	80				
Anica	Angola	5				
	Argentina	899				
	United States of America	871				
America	Bolivia	720				
	Brazil	511				
	Paraguay	123				
	Pakistan	636				
	South Korea	515				
Acia	Timor-Leste	323				
ASId	United Arab Emirates	168				
	China	107				
	Indonesia	1				
	Netherlands	664				
	Spain	656				
	Romania	563				
	Bulgaria	<mark>3</mark> 13				
	Slovakia	291				
	Portugal	256				
Europe	Hungary	164				
	Greece	150				
	Serbia	136				
	Ukraine	57				
	Norway	16				
	Kosovo	1				
	Montenegro	1				
	SUM 8227					


## XI. Haploid Marker Meeting "Inferring Ancestry from DNA"

Bydgoszcz, May 17-19 2018



<u>Congress chair</u>: Tomasz Grzybowski/Urszula Rogalla-Ladniak 190 participants from 49 countries, 40 talks and 62 poster presentations <u>Keynotes</u>: Chris Phillips, Mark Jobling, Chris Tyler-Smith



#### **EMPOP trainings** May 2018 - April 2019

EMPOP meeting at SWGDAM, Woodbridge, VG, USA, Jul 07 2018 EMPOP workshop at ISFG Summer School, Catanzaro, ITA, Sep 03 2018 EMPOP workshop at GHEP, Araraquara, BRA, Sep 13 2018 EMPOP workshop at ISFG ESWG Meeting, St. Petersburg, RUS, Sep 13 2018 NGS workshop at ISHI, Phoenix, AZ, USA, Sep 24 2018 Targeting Mitochondria, Berlin, GER, Oct 24 2018 EMPOP Meeting at SAGF, Buenos Aires, ARG, Nov 21 2018 EMPOP workshop at GEDNAP, Jena, GER, Feb 21 2019



#### **EMPOP training** Araraquara, BRA, 13 Sep 2018





### **EMPOP training**

Buenos Aires, ARG, 21-22 Nov 2018





## Acknowledgements

#### Development, code programming, testing

Nicole Huber, Arne Dür (Innsbruck)

#### IT (Innsbruck)

Stefan Troger, Martin Pircher, vxweb

#### **EMPOP 4 testers (international)**

Lara Adams, Kimberly Andreaggi, Laura Catelli, Constance Fisher, Mario Gysi, Douglas Hares, Jodi Irwin, Rebecca Just, Hwan-Young Lee, Sabine Lutz-Bonengel, Charla Marshall, Dixie Peters, Dirk Sauer, John Tonkyn

#### **EMPOP** analysts (Innsbruck)

Christiane Bauer, Cordula Berger, Martin Bodner, Mayra Eduardoff, Liane Fendt, Theresa Harm, Antonia Heidegger, Gabriela Huber, Anita Kloss-Brandstätter, Anna König, Simone Nagl, Harald Niederstätter, Daniela Niederwieser, Jannika Oeke, Alexander Röck, Lisa Schnaller, Filipa Simao, Christina Strobl, Catarina Xavier, Bettina Zimmermann

**Richard Scheithauer (Director)** 

Big THANK YOU to all EMPOP contributors worldwide

EU 779485 — STEFA — ISFP-2016-AG-IBA-ENFSI







Security Fund of the European Union

# MONOPOLY 2016 - STEFA - WP G7

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

#### STRIDER & EmPOP

Jan 2018 - Dec 2019

**STRidER** 

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



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## dna.bases EMPOP

EDNAP Meeting, Madrid, Spain, May 07 2019



Martin Bodner<sup>1</sup>, Walther Parson<sup>1,2</sup>

<sup>1</sup> Institute of Legal Medicine, Medical University of Innsbruck, Austria <sup>2</sup> Forensic Science Program, Penn State University, PA, USA



## **STRidER**



STRidER in the field of forensic STR typing (from Bodner et al. 2016)



## STRICER STRs for identity ENFSI Reference database, v2



https://strider.online/

HOME QUERY

BATCH QUERY ABOUT

FREQUENCIES FORMULAE

STR SEQUENCE NOMENCLATURE CONTACT TERMS OF USE

#### Frequencies

These tables include allele frequencies and number of samples (n) from the most recent database release sorted by marker and country. In these tables, "1" represents all rare alleles shorter than the accepted allele categories. The value "99" represents all rare alleles longer than the accepted categories.

This data can be downloaded as 🔂 XML file.

#### VWA

Allele	AUSTRIA	BELGIUM	BOSNIA AND HERZEGOWINA	CZECH REPUBLIC	DENMARK	FINLAND	FRANCE	GERMANY	GREECE	HUNGARY	IRELAND	MONTENEGRO	NORWAY	POLAND	SLOVAKIA	SLOVENIA	SPAIN	SWEDEN
	222	206	171	200	200	230	208	662	208	224	304	200	202	206	247	207	449	424
11								7.5529e-4										
12									4.8077e-3									
13			1.1696e-2					2.2659e-3	2.4038e-3	2.2321e-3			2.4753e-3		2.0243e-3	2.4155e-3	6.6815e-3	1.1792e-3
14	1.0586e-1	1.0680e-1	1.1111e-1	1.0000e-1	7.0000e-2	1.3043e-1	8.6539e-2	9.7432e-2	9.3750e-2	1.1161e-1	1.1349e-1	1.4500e-1	8.6634e-2	7.7670e-2	1.1943e-1	1.0145e-1	1.1024e-1	9.4340e-2
15	9.2342e-2	1.2136e-1	1.2573e-1	9.7500e-2	9.7500e-2	5.2174e-2	1.2740e-1	1.0347e-1	7.9327e-2	1.1384e-1	1.0197e-1	9.0000e-2	9.9010e-2	8.4951e-2	1.1943e-1	1.2077e-1	1.2361e-1	8.9623e-2
16	1.7568e-1	1.9903e-1	2.0468e-1	1.7500e-1	2.6000e-1	1.7609e-1	2.4038e-1	2.2130e-1	1.6827e-1	2.0536e-1	2.1875e-1	1.7500e-1	2.2277e-1	2.2330e-1	1.9231e-1	1.8599e-1	2.4276e-1	2.0991e-1
17	2.8604e-1	2.7185e-1	2.3977e-1	3.1250e-1	2.3000e-1	2.7174e-1	2.3317e-1	2.5453e-1	3.1731e-1	3.0134e-1	2.7138e-1	2.8750e-1	2.8960e-1	2.7670e-1	2.7530e-1	2.8985e-1	2.7171e-1	2.6533e-1
18	2.5901e-1	2.0146e-1	2.1053e-1	2.2750e-1	2.4000e-1	2.0435e-1	2.1154e-1	2.2054e-1	2.4279e-1	1.7634e-1	1.9243e-1	2.1250e-1	1.9802e-1	2.4757e-1	2.0445e-1	2.1739e-1	1.7038e-1	2.4174e-1
19	7.2072e-2	8.0097e-2	9.0643e-2	7.2500e-2	8.2500e-2	1.3696e-1	8.6539e-2	8.6103e-2	7.4519e-2	7.1429e-2	9.3750e-2	7.2500e-2	8.6634e-2	8.0097e-2	7.6923e-2	5.5556e-2	6.1247e-2	7.9009e-2
20	9.0090e-3	1.9418e-2	5.8480e-3	1.5000e-2	1.7500e-2	2.1739e-2	1.4423e-2	1.2840e-2	1.4423e-2	1.5625e-2	8.2237e-3	1.7500e-2	1.4852e-2	9.7087e-3	1.0122e-2	2.1739e-2	1.3363e-2	1.6509e-2
21					2.5000e-3	6.5217e-3		7.5529e-4	2.4038e-3	2.2321e-3						4.8309e-3		2.3585e-3
'H01																		
		RELCIUM	BOSNIA AND	CZECH			EDANCE	GERMANY	CREECE			MONTENEGRO	NORWAY				CDAIN	

Allele	AUSTRIA	BELGIUM	BOSNIA AND HERZEGOWINA	CZECH REPUBLIC	DENMARK	FINLAND	FRANCE	GERMANY	GREECE	HUNGARY	IRELAND	MONTENEGRO	NORWAY	POLAND	SLOVAKIA	SLOVENIA	SPAIN	SWEDEN	
	222	206	171	200	200	230	208	662	208	224	304	200	202	206	247	207	454	425	

STRIDER STRs for identity ENFSI Reference database, v2



# May 06, 2019

STRidER QC stage (since Aug 2017)	# of datasets
Successfully passed QC	39
Withdrawn by submitter	35
Rejected by STRidER	40
Waiting for authors reply	25
Handled by GMI	23
Total	162

thdrawn



	Successfully passed QC	
	Withdrawn by submitter	
	Rejected by STRidER	
	Waiting for authors reply	
	Handled by GMI	
	Total	
can can can sum y an	34% accepted, 66% rejected/w	i

Data kindly provided by M. Bodner, GMI



**MPS STR data** 

submitted CE datasets since Aug 2017

## dna.bases

**CE STR data** 

sample number

## **MPS Nomenclature**

# STRAND working group

David Ballard Martin Bodner Lisa Borsuk Katherine Gettings Jonathan King Walther Parson Christopher Phillips



#### STR Nomenclature Meeting April 11-12, 2019 London





#### 5' to 3':

Walther Parson, Lisa Borsuk, Peter Schneider, Brian Young, Rebecca Just, Jodi Irwin, David Ballard, Sascha Willuweit, Cydne Holt, Chris Phillips, Jonathan King, Tunde Huszar, Peter Gill, Christian Sell, Kris Van der Gaag, Laurence Devesse, Claus Borsting, Doug Hares, Katherine Gettings, Rob Lagace, Jerry Hoogenboom, Martin Bodner, Peter deKnijff, Sebastian Ganschow, Pedro Barrio, Teresa Gross



# STRAND Meeting, London, UK April 11-12 2019

Participants provided updates on their work on MPS STRs

- Applied presentations
- Alignment concepts
- Software solutions at different stages of development
- Discussion on minimum requirements
- Feedback from industry
- Feedback from database curator

Positive and constructive discussions STRAND group will write up proceedings of the meetings





Security Fund of the European Union

# MONOPOLY 2016 - STEFA - WP G7

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

#### STRIDER & EmPOP

Jan 2018 - Dec 2019

**STRidER** 

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



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## dna.bases EMPOP

EDNAP Meeting, Madrid, Spain, May 07 2019





## Update VISAGE

Catarina Xavier<sup>1</sup>, Walther Parson<sup>1,2</sup>

<sup>1</sup> Institute of Legal Medicine, Medical University of Innsbruck, Austria <sup>2</sup> Forensic Science Program, Penn State University, PA, USA



## VISAGE - Visual Attributes Through Genomics



Manfred Kayser (Coordinator) Rotterdam, NED Wojciech Branicki Krakow, POL Chris Phillips, Angel Carracedo S. de Compostela, ESP Walther Parson Innsbruck, AUT Michael Nothnagel Cologne, GER Barbara Prainsack Vienna, AT Peter M. Schneider Cologne, GER Ingo Bastisch Wiesbaden, GER François-Xavier Laurent Lyon, FRA Titia Sijen The Hague, NED Johannes Hedman Linkoping, SWE Shazia Khan London, UK Magdalena Spólnicka Warsaw, POL www.visage-h2020.eu



# VISAGE OBJECTIVES



The **VISAGE** - Consortium is developing genotyping and statistical prototype tools, forensically validate and implement them into forensic practice for predicting **appearance**, **age**, and **ancestry** from DNA traces and study its ethical, societal & regulatory dimensions (period: 05/2017-04/2021).

Tool I: Appearance & Ancestry (SNP multiplex)

Tool 2: Age (quantitative methylation)







## VISAGE PROGRESS

- WP1 MANAGEMENT (EMC)
- WP2 MARKER DISCOVERY (EMC, JU, USC)
- WP3 PROTOTYPE ANALYSIS TOOL DEVELOPMENT AND VALIDATION (MUI)
- WP4 STATISTICAL PREDICTION MODELLING AND SOFTWARE DEVELOPMENT (UoK)
- WP5 ETHICAL, SOCIETAL AND REGULATORY DIMENSION MAPPING (KCL)
- WP6 IMPLEMENTATION OF PROTOTYPE TOOLS IN RELEVANT ENVIRONMENT (BKA)
- WP7 EDUCATION AND TRAINING (UKK)



## WP2 MARKER DISCOVERY

Started in M1 (May 2017) - EMC

D2.1 (M3) Report on previously validated markers for predicting **basic** appearance, age, ancestry from DNA

D2.2 (M24) Report on new markers for predicting **enhanced** appearance, age, ancestry from DNA





## WP3 PROTOTYPE TOOL DEVELOPMENT

- Started in M1 (May 2017) MUI
- D3.1 (M12) Report on new MPS prototype tool(s) for constructing **basic** composite sketches from DNA
- D3.2 (M24) Report on forensic developmental validation of new MPS prototype
- tool(s) for constructing **basic** composite sketches from DNA
- D3.3 (M24) Report on new MPS prototype tool(s) for constructing **enhanced** composite sketches from DNA



#### VISAGE Basic Tool

Tool 1: Appearance & Ancestry (SNP multiplex)

\*

#### Design, develop and validate prototype tools





# Age estimation by quantitative methylation



https://www.gatc-biotech.com





## WP4 STATISTICAL MODELLING

Started in M1 (May 2017) - UoK

D4.1 (M24) Report on new integrative statistical framework for combined appearance, age, and ancestry prediction from DNA

D4.2 Develop prototype software for constructing composite sketches from DNA (M36)



## WP5 ETHICS

Started in M1 (May 2017) - KCL

- D5.1 (M12) Report on current and expected legal and regulatory landscape at
- (a) EU level and
- (b) specific for each participant country

D5.2 (M24) Report on challenges to the implementation of FDP in an ethical and societally responsible manner, with special emphasis on privacy and data protection



## WP6 IMPEMENTATION

Starting in M25 (May 2019) - BKA

D6.1 Implementation of MPS prototype tools for constructing basic composite sketches from DNA (M36)



## WP7 EDUCATION AND TRAINING

- Started in M19 (Nov 2018) UKK
- D7.1 (M24) Report on European-wide inquiry on training needs among stake holders and end users
- D7.2 Develop training curricula tailor-made for the different target groups on technical, societal, regulatory challenges of constructing composite sketches from DNA in forensic practice (M36)



## VISAGE - Consortium Meeting Lyon, France, 10-11 Sep 2018









QIAGEN



## Acknowledgements



Catarina Xavier Antonia Heidegger Harald Niederstätter Maria de la Puente Walther Parson





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

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THE 28<sup>th</sup> CONGRESS OF THE INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

PRAGUE, 9–14<sup>TH</sup> SEPTEMBER 2019 CZECH REPUBLIC, PRAGUE CONGRESS CENTRE

## Congress Travel Bursaries Purpose: To support young scientists presenting at an ISFG congress



Travel bursaries will be made available again for the congress in Prague 2019

## ➢ For current Terms of Reference, see

<u>https://www.isfg.org/files/ISFG\_Bursaries\_Nov2016.pdf</u>



# Short-term fellowships

- Purpose: To support transnational exchange visits between collaborating research groups for specific projects related to forensic genetics
- For Terms of Reference, see
  - https://www.isfg.org/files/ISFG\_Fellowships\_Nov2016.pdf
- Financial support for travel and accommodations for up to 1000 euros (within continent) and 2000 euros (between continents)
- Application rounds: (1) April 2017, (2) October 2017, (3) April 2018, (4) October 2018, (5) April 2019 see <a href="https://www.isfg.org/Members+Area/Short+Term+Fellowships">https://www.isfg.org/Members+Area/Short+Term+Fellowships</a>
- Selection committee included the Working Group chairs and was chaired by John Butler from the ISFG Executive Board




Tunesien

**ISFG** 

# ISFG Summer School Program

- Paternity and kinship testing including X-chromosomal markers
  - Thore Egeland, Daniel Kling (2 days)
- DNA interpretation in criminal casework using probabilistic software (LRmix Studio and Euroformix)
  - Peter Gill, Lourdes Prieto, Corina Benschop, Oyvind Bleka (2 days)
- Mitochondrial DNA analysis and interpretation using EMPOP
  - Walther Parson (0.5 days)
- Next generation sequencing and population studies using Snipper and Structure analysis
  - Christopher Phillips, Leonor Gusmao (1 day)
- ISO17025 accreditation procedures and DNA database management
  - Renato Biondo (0.5 days)



### MAKING SENSE OF FORENSIC GENETICS

What can DNA tell you about a crime?

Published in 2017

### German

Italian

Polish

Portuguese

Spanish (published)

Hungarian





DNA WG MEETING Madrid, 07-10 May 2019

# **DNASEQEX** update

Presented by Antonio Alonso on behalf of the DNASEQEX Consortium









Background image: Sequence strings of STRs Download at: STRICER https://strider.online



1. Identification of interest and the main challenges for MPS implementation by the European forensic DNA laboratories.



Participation: 54% of all ENFSI DNA Labs

Lack of nomenclature and reporting standards National DNA Databases cannot accommodate... Not sufficient population data There is a lack of an adequate national... No specific validation guidelines available No specific proficiency tests available Not demonstrated to be reliable Others Number of labs in December 2016







European survey on forensic applications of massively parallel sequencing Antonio Alonso, Petra Müller, Lutz Roewer, Sascha Willuweity, Bruce Budowle, and Walther Parson

2. Inter-laboratory validation data have been provided for three different MPS-STR kits





Inter-laboratory validation study of the ForenSeq<sup>™</sup> DNA Signature Prep Kit Steffi Köcher<sup>a,\*,1</sup>, Petra Müller<sup>b,1</sup>, Burkhard Berger<sup>b</sup>, Martin Bodner<sup>b</sup>, Walther Parson<sup>b,c</sup>, Lutz Roewer<sup>a</sup>, Sascha Willuweit<sup>a</sup>, The DNASeqEx Consortium



Systematic evaluation of the early access applied biosystems precision ID Globalfiler mixture ID and Globalfiler NGS STR panels for the ion S5 system

Petra Müller<sup>a</sup>, Antonio Alonso<sup>b</sup>, Pedro A. Barrio<sup>b</sup>, Burkhard Berger<sup>a</sup>, Martin Bodner<sup>a</sup>, Pablo Martin<sup>b</sup>, Walther Parson<sup>a,c,\*</sup>, The DNASEQEX Consortium

#### 3. Population data were obtained in three European populations

PowerSeq Auto / Y System (Austria and Germany) and Precision ID Globalfiler NGS STR V2.0 (Spain)



GENETICS

#### Massively parallel sequence data of 31 autosomal STR loci from 498 Spanish individuals revealed concordance with CE-STR technology and enhanced discrimination power

Pedro A. Barrio, Pablo Martín, Antonio Alonso, Petra Müller, Martin Bodner, Burkhard Berger, Walther Parson, Bruce Budowle, The DNASEQEX Consortium

#### Under review

4. A Web Self-Maintaining NOMenclature AUThority (NOMAUT) software has been proposed <a href="https://nomaut.org/">https://nomaut.org/</a>





Review

Current state-of-art of STR sequencing in forensic genetics

Antonio Alonso 🔯 , Pedro Alberto Barrio, Petra Müller, Steffi Köcher, Burkhard Berger, Pablo Martin, Martin Bodner, Sascha Willuweit, Walther Parson, Lutz Roewer, Bruce Budowle ... See fewer authors 🔺

5. Feedback on the performance of the analytical workflow as well as on the MPS data analysis software requirements has been provided to the MPS-STR manufacturing companies



- ISFG Nomenclature
- Flanking region sequence information
- CODIS output files
- STRIDER output files for population data

6. Dissemination of the ISFG recommendations on minimal nomenclature requirements and quality control measures of autosomal STR allele frequency databasing.



# More than 30 presentations

in international meetings, workshops, and courses

- ENFSI DNA WG (Warsaw, Vilnius, Rome, and Madrid)
- EDNAP (Vilnius, Rome, and Madrid)
- ISFG 27th Congress (Seoul)
- 10th International Haploid Markers Meeting (Berlin)
- HIDS (Vienna, Rome)
- Illumina Forensic Workshops
- Investigator Forum (Berlin)
- GHEP-ISFG MPS Workshop (Coimbra)









#### 500 donors



**17** Autonomous Communities



enght Repeat Region Sequence Flanking Region Sequ



Massively parallel sequence data of 31 autosomal STR loci from 498 Spanish individuals revealed concordance with CE-STR technology and enhanced discrimination power

Pedro A. Barrio, Pablo Martín, Antonio Alonso, Petra Müller, Martin Bodner, Burkhard Berger, Walther Parson, Bruce Budowle, The DNASEQEX Consortium









\*CMP: Combined Match Probability



Massively parallel sequence data of 31 autosomal STR loci from 498 Spanish individuals revealed concordance with CE-STR technology and enhanced discrimination power

Pedro A. Barrio, Pablo Martín, Antonio Alonso, Petra Müller, Martin Bodner, Burkhard Berger, Walther Parson, Bruce Budowle, The DNASEQEX Consortium



### Plataforma en Red Banco Nacional de ADN Carlos III

### **CE-STR and MPS-STR discordant results**





### CE-STR and MPS-STR discordant results: PENTA D

#### GeneMaper IDX

400

9 7515

2.2

8698

#### Converge 2.1

Penta C

Penta C

#### ge 2.1 STRaitRazor\_v3



IGV

#### Manual alignments using Forensic STR Sequence Guide



5' anchor sequence (CONVERGE) aligns on the deleted 13 bp region





### CE-STR and MPS-STR discordant results: D19S433



#### Manual alignments using Forensic STR Sequence Guide



The allele 13.2 is really an allele 14 with the 2 bp deletion rs745607776





### CE-STR and MPS-STR discordant results: D2S441



#### Manual alignments using Forensic STR Sequence Guide

D25441	Instant rolding	rs74640515																			1			2			3			4			5			6			7			8			9			10			11			12			13		14	1																
Reference sequence	X G	G	in 6	X.	F G	T	G	GI	Ŧ	κ.	10	F C	T	ń.	T G	25	à.	6 8	x	T	T	6 3	A	T	6 1	1	T	T.	T	T	T.	Т	Λ T	5	Ŧ	T	0	T	N T	T	T	T	10	T	T	C	T	T	R.	T I	T	T	T	T	1 1	T A		1				- 13	T A	Т	E I	T	10	10	0. W	4	-	-C.	A. C	GI	C	6 1
Flanking SNP IUPAC codes		R																			т	C T	r A	T	C 1	r A	т	с	TA	Т	c	T	A T	с	T	A T	c	т	A T	с	TA	T	c	TA	T	с	TA	A T	с	т /	A T	c	TA	т	C 1	TA																				
GRCh38 coordinates	68011920	68011922	68011923 68011924	68011925	65011927	68011928	68011929	68011930	68011932	68011933	68011934	96611089	68011937	68011938	00011089	19611089	68011942	68011943	68011945	68011946	68011947	68011948	05611089	15611089	68011952 68011053	68011954	68011955	68011956	68011957	65611089	68011960	68011961	68011963	68011964	68011965	00611080	68011968	69611089	68011970	68011972	68011973	57011975	68011976	68011977	62611089	68011980	18611089	68011983	68011984	68011985	68011987	68011988	68011989	16611089	68011992 con11002	68011994						20011002	68011996	26611089	68011998	68012000	68012001	68012002	68012003 68012004	68012005	68012006	68012008	68012009	68012010 68012011	68012012	68012013 68012014
GRCh37 coordinates	25095239	68239054	68239055	25065289	68239059	68239060	68239051	68239062	68239064	68239065	68239066	10055250	69062330663	68239070	17095259	68239073	68239074	68239075	68239077	68239078	68239079	68239080	682339082	68239083	68239084	68239086	68239087	68233088	68033090	1605239	68239092	68239093	68239095	68239096	68239097	680523038	68239100	10165289	68239102	68239104	68239105	10162289	68239108	68239109	68239111	68239112	68239113	68239115	68239116	68239117	68239119	68239120	68239121	68239123	68239124	68239126						201000000	68239128	68239129	68239130	68239132	68239133	68239134	68239135	68239137	68239138	68239140	1919539141	68239142 68239143	68239144	68239145
Distance from repeat region	27	52	24	33	2 8	19	18	17	12	2	1	1 1	9	m 1	- 10	un	in .	4 M	-					-	-				-	-	-			-		-	-		~	-			-		-			-		-			-	-								,		m	4 1	n ve	~	90	n 9	1	12	1 1	5 3	16	12	S 12
13 14	4	G G	л н А. И	a a	F G	n T	er G	G I	T T	90 10		F F	9 7	A	F G	ii K	1		14 M	T I	Ť Ť	2 3	~ ~	Ŧ	1		1 7	1. 1.	т. d	Э Т	18. x	ά Ŧ	a T A T	14 H	T T	T T		Ť Ť	а 1 1 1 7	1. C	Ť .	T T	т т	Ý J	Ť Ŧ	10 L	T T	T T	4 1	Ť Ŧ	с 7 5 7	T	T a	X T	· 1	T of	Ψ. 6 Ŧ. 1	T T	A T	10	Ŧ	A	T A	T T	R 7	r T	11 11	10 - 14		8		1	a e	6 F		<u>.</u>
13.1	1	6	<u>л л</u>	Ŀ	T. G	τ	6	<b>6</b>	Ţ	¢.	A .	r c	т	A	T C	A			c	т		1	A	т		T A	T	T	T. P	τ	5	т	n I	1	т	n T	x	Ţ	<u>л</u> т	T.	T 1	T	1	T /	Ţ	1	τ, 1	T	x	T	л т	T	T A	Ţ	T	T M	T. I	τ	<b>A</b> 1	T	τ	7	T	т		л т	٨						×	6 X	E	A
14	1	G		L	Ť G	T	6	G (	T	C	A	T C	Ŧ	A	t e	Å			14	T	Ŧ	c 1	1		0 1	A	T	X	ŤÅ	Ţ	2	Т	4 1	¢	Ĩ	å T	L	T	A T	¥.	T	T	£	T	T	¢	Ţ	K T	L	Ť	4 T	¢	T 4	T	τ	T A	T Z	T	<u>k</u> 1	L	Ť	4	Ť Å	Ť		A T	A						4 4	<mark>8</mark> 1	£	

Alignment by Converge exclude Adenine from the repeat region

### **CE-MPS Concordance study highlights**

- Deletions in the flanking region (13 bp deletion in Penta D associated to CE allele 2.2) may cause "**bioinformatic null alleles**" if the selected region matches the anchor region of sequence recognition by the software.
- Deletions and insertions in the flanking regions also generated discrepancies between the CE micro-variant alleles and MPS data for Penta D and D19S433. The knowledge of the MPS sequence demonstrated that these microvariants were erroneously called by CE, as they were alleles with complete repeat units plus insertions or deletions in the flanking region.
- The identification and classification of these small discrepancies in the different STR markers will allow software improvement and the development of **better comparison tools between CE and MPS data**.





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UNT HEALTH Center for Human Identification at the University of North Texas Health Science Center. EEUU



CIENCIAS FOREN

National of Toxicology and Forensic Sciences.Madrid Department. Spain

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# CaseSolver: An investigative open source expert system based on EuroForMix

**Oyvind Bleka** 

Lourdes Prieto

Peter Gill

Forensic Science International: Genetics 41 (2019) 83-92





Forensic Science International: Genetics

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

# CaseSolver: An investigative open source expert system based on EuroForMix

#### Øyvind Bleka<sup>a,\*</sup>, Lourdes Prieto<sup>b</sup>, Peter Gill<sup>a,c</sup>

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<sup>b</sup> Instituto de Ciencias Forenses, Grupo de Medicina Xenómica, Universidade de Santiago de Compostela, Santiago de Compostela, Spain <sup>c</sup> Institute of Clinical Medicine, University of Oslo, Oslo, Norway



GENETICS





- Bug when performing DC with locus drop-out: Fixed by changing line 843 in gui().

#Changes version 1.0.1

- The bug "setupAdvanced could not be found", after saving "Advanced Setting", has been fixed.

#### File attachments:

casesolver\_1.4.0.zip
Tutorial for CaseSolver v1.0
Tutorial data for CaseSolver
casesolver\_1.3.7.zip
casesolver\_1.1.1.zip
casesolver\_1.0.1.zip
casesolver\_1.0.0.zip

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# Background

- CaseSolver is a digital solution which supports forensic experts to quickly visualise profiles as EPGs and to separate easy and difficult profiles
- It provides a dynamic framework to analyse profiles: e.g. deconvolution, conditioning on known/unknown contributors
- Fast comparison between reference and evidential profiles and reference to references
- Users can select profiles to analyse further with EuroForMix
- Scope: STR or SNP MPS data.

## Complex case review

- For very serious crimes, reporting scientists often have to contend with complex cases where literally hundreds of items are submitted by investigators for analysis.
- In order to efficiently expedite the challenge of comparing reference profiles to evidence profiles, many of which are mixtures, we have developed an investigative open source expert system CaseSolver.
- Example (real) case shown where CaseSolver was used to analyse 119 evidence profiles and three reference profiles
- Power of the system illustrated by searching a fictive database of 1m individuals

## Case circumstances

- Following a report from a concerned relative whose sister (REF1) had been missing for several days, the police forcibly entered her residence to find her deceased along with her son (REF2). There had clearly been a violent struggle where the victims had been stabbed multiple times.
- Initially there was no suspect hence it was decided to carry out comprehensive sampling of the crime scene
- Total of 119 crime stains from 46 different items
  - 68 were single contributor profiles and 44 were mixtures
- Majority were blood stains, but contact traces were taken from cigarette butts, mobile telephones, handles of drawers, knife handles and blades.
- Analysis with GlobalFiler

# Investigative vs evaluative analysis

- Before defendants are identified, the process is investigative
  - Propositions are not yet formulated until suspects are found
- When defendants are identified, the process is evaluative
  - Propositions are formulated and fixed for court-going purposes
- Investigation highlights a potential suspect a reference sample was taken (REF3).

# Plan of crime-scene



	Crime Scene - # samples														
Hall	Kitchen	Bathroom	Living room	Main bedroom	Second bedroom										
30	15	11	43	3	9										

Autopsy - # samples											
Female	Male										
4	4										

Reference samples													
Female	Male	Suspect											
2	2	2											

# Organisation of data into a single file directly from GeneMapper

Data	Match matrix	Match list (Qual LR)	Match list (Quan LR)	Mixtures	Deconvoluted							
Sel	ect Case ID											
C	hange view	RealCase1	~ Import	Sort Evid	ls by #	Sort Refs by #	~					

#### -Evidence profile(s) and Reference profile(s)

ID	5	SampleName	MatchStatus	D3S1358	VWA	D16S539	CSF1PO	TPOX	YINDEL	AMEL	D8S1179 ^
#1	19.01		mixture	15						X/Y	12/13/14
#2	40.03		mixture						2		13/14
#3	08.01		mixture	14/17	15/16				2	X/Y	11/12/13
#4	13.02		mixture	14/15/17	15/16	11/12	9/10		2	х	12/13/14
#5	20.01		mixture	14/15/17	14/18				2	X/Y	12/13/14
#6	30.01		mixture	15/17	15/16	11/12	9/10	7		Х	12/13/14
#7	37.01		mixture	15/17	15/16	11/12	9/10	7		Х	12/14
#8	38.15		mixture	15/17	15/16	11/12	9/10	7		Х	12/14
#9	02.01		REF3	14/15	14/18	8/12	9/11	7	2	X/Y	13/14
#10	07.01		REF3	14/15	14/18	8/12	9/11	7	2	X/Y	13/14 🗸
<											>
ID		Sampl	eName	D3S1358	VWA	D16S539	CSF1PO	TPOX	YINDEL	AMEL	D8S1179
#1	REF1			15/17	15/16	11/12	9/10	7/7		X/X	12/14
#2	REF2			14/17	15/16	11/12	10/11	7/7	2	X/Y	12/12
#3	REF3			14/15	14/18	8/12	9/11	7/7	2	X/Y	13/14
<											>

#### Functionalities

Compare

## Automatic identification by case-import

- Every reference is compared to evidence profiles
- Matching refs identified and labelled in MatchStatus.
- Supports missing loci

# Investigative propositions

- $H_p$ : "Person of interest (POI) and K 1 unknowns (unrelated) are contributors to evidence profile E"
- $H_d$ : "K unknowns (unrelated) are contributors to evidence profile E"
- The number of contributors is calculated automatically using the *allele count method* (the maximum number of alleles at a locus divided by two and rounded up)

# Steps

- Matching Allele Count (MAC): Simple allele comparison is the proportion of alleles in the evidence that 'match' a crime-stain profile
  - MAC set to default 0.8
- Qualitative program based on LRmix Studio is used to assign a LR to all surviving candidates. We used LR>1000 to select candidates
- A LR threshold is selected by the user for the third step which is assigning a **quantitative** LR based on **Euroformix**
- Unknown contributors can be deconvolved and used as reference samples (MAJ)

# A small part of the output following three step strategy

Sample Name	#contr (by vlew)	References	MixProp (%)	MAC (%)	Qual. LR (log10)	Quan. LR (log10)
01.01	2 (1?)	MAJ	91	100	16.5	29.4
03.01	2	REF2/REF1	72/28	100/100	18.5/18.8	29.4/18.7
03.02	2	REF2/REF1	79/21	100/98	17.3/17.2	29.5/13.0
04.01	3 (4)	REF1/REF2/REF3/MAJ	÷	100/100/100/98	10.3/9.9/7.6/8.3	28.3/16.2/13.5/11.6
(maxK = 3, 4)	4 (4)	REF1/REF2/REF3/MAJ	47/23/21/8.7	100/100/100/98	10.3/9.9/7.6/8.3	28.1/16.2/13.4/10.8
05.01	2 (1)	REF3	87	100	16.1	20.7
08.01	2	REF2	79	100	8.6	13.6
13.01	2	REF1/REF3	85/15	100/98	17.1/11.9	30.9/19.9
13.02	2(1)	REF1	89	100	14.6	21.6
14.01	2 (1)	REF3	98	100	16.2	26.2
16.01	2 (1)	REF3	95	100	15.8	26.2
16.02	2	REF3/REF1	88/12	100/83	14.7/6.0	26.1/11.7
16.03.R	2 (1)	REF3	98	100	16.2	26.2
16.09	2	REF3	84	100	15.6	25.9
16.11	2	REF1/REF3	43/57	100/100	17.0/12.6	18.2/15.3
16.11.R	3	REF3/REF1/ REF2	45/37/17	100/100/100	10.2/13.3/12.6	13.5/18.7/12.7
16.12	2(1)	REF1	88	100	18.7	27.6
17.01	2	REF3	83	100	15.4	22.4
20.01	2 (1)	REF3	93	0.97	11.8	20.0

# **Graphical representation**

- Orange nodes are mixtures with 2 contributors
- Red nodes are mixtures with more than 2 contributors
- Cyan nodes are single source profiles
- Green nodes are references



# Time taken to do the analysis

- 2.8 hours for all three steps if limited to four contributors maximum
- 10 minutes for all three steps if limited to three contributors maximum

# Comparison with manual inspection

- A rough approximation on the time taken was 8 hours
- The time taken for the comparison depended on the complexity of the evidence profiles. In general, when these are SS profiles with good quality, the comparison is easy.
- Comparison with higher order mixtures took much longer time and sometimes there were no clear conclusions.
- In addition, as some profiles come from related individuals (two of the references shared many alleles), the probability of making a mistake was high.
# Searching a database – if there are no suspects identified in the initial investigation

- One million randomly generated reference profiles were simulated
- The reference samples were added to this database
- CaseSolver was used to search the database

### Analysis time to search 1m references against 44 mixtures and 75 single source profiles

- Time to analyse 44 classified mixtures against 1m reference samples
  - Time to simulate, import and visualise data = 14min
  - Time taken to compare 44 mixtures with 1m references with MAC method = 34min. Generates a huge table of 12.5GB
    - A total of 56,521 comparisons are carried through to step 2
  - Step 2: Time = 8h.
    - A total of 302 comparisons satisfy criterion LR>1000
  - Step 3: Time= 4h (where max no of contributors=4)
    - False positive rate 7 (three persons) and 24 (four persons)
    - Largest false positive log10LR=6.1 (MAC=0.94) This was the only false positive comparison using the threshold LR>1m, hence all of the reference samples were recovered
  - Total Time for analysis is about 12 hours
- A limitation is the quality of the profile partials will match more often

### Validation study

- Carried out on 25 mixtures and 14 reference samples where ground truth was known
- Various settings tried out. The format x/y/z means number of false pos/negs after applying the three steps: (MAC/Qual/Quan) methods

MAC	Qual.LR	Quan.LR (maxK)	Time	#Comp. qual/quan	False Positives	False Negatives
0.8	1000	_	2 min	82/-	17/0/-	5/8/-
0.8	1	_	2 min	82/-	17/0/-	5/5/-
0.8	1	1000 (3)	26 min	82/65	17/0/0	5/5/10
0.8	1	1000 (4)	12.5 h	82/65	17/0/0	5/5/6
0.8	1	1 (3)	26 min	82/65	17/0/0	5/5/9
0.8	1	1 (4)	12.5 h	82/65	17/0/0	5/5/6
0.7	1000	_	2 min	124/-	54/0/-	0/7/-
0.7	1	_	2 min	124/-	54/0/-	0/1/-
0.7	1	1000 (3)	26 min	124/69	54/0/0	0/1/6
0.7	1	1000 (4)	12.5 h	124/69	54/0/0	0/1/2
0.7	1	1 (3)	26 min	124/69	54/0/0	0/1/5
0.7	1	1 (4)	12.5 h	124/69	54/0/0	0/1/2

# False positive rate is related to the size of the LR itself

- How often a LR=x or more occurs is accommodated by p(LR>x|Hd)<1/x, so we never expect more than N/x chance matches where N is the size of the database ref:Gill et al (2014) FSI:Gen 13. 167-175
- There is an inherent limitation with poor quality, low level LRs since there will be numerous false positives observed.

### Application

- CaseSolver is integrated with LIMS system to enable easy management
- Allows routine comparison with staff and police elimination databases for contamination checks.
- Can be used to link cases together
- Very flexible tool which allows operator input. Allows relatedness testing

#### Moving from investigation to evaluation

- Prioritising police resources when there is limited information in the case.
  - When the profile is a complex mixture of three or more persons then it is inevitable that there are many false positive matches in the candidate list. In order to carry out an investigation, this list is ranked according to the likelihood ratio, and the police will investigate the individuals in this ranked order.