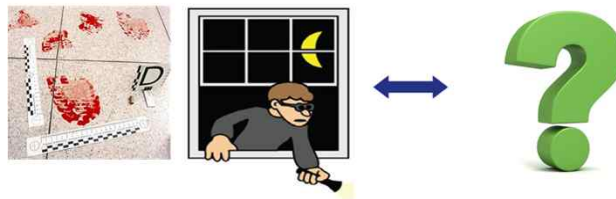


## Forensic DNA Phenotyping for Investigative Leads



## Conventional Forensic DNA Typing

- ❑ Forensic cases -- **matching suspect with evidence**
- ❑ Paternity testing -- **identifying father**



*Involves generation of DNA profiles usually with the same genetic markers  
and then **MATCHING TO REFERENCE SAMPLE***

Picture from [www.cstl.nist.gov/strbase/NISTpub.htm](http://www.cstl.nist.gov/strbase/NISTpub.htm)

## Advanced Forensic DNA analysis

- Human identification testing
- DNA database search
- DNA mass screening



**Identification markers**

### Forensic DNA phenotyping (FDP)

- Ancestry inference
- Externally visible characteristics
- Forensic age estimation



**Intelligence markers**

Slide from the ISFG 2015 workshop presentation by W. Branicki

## Forensic Human Identification

- Autosomal STRs:** multiplex HID kits available
- Y-STRs:** HID kits available
- X-STR:** HID kits available
- mtDNA:** published protocols for CR and complete genomes
- SNPs, INDELS:** published protocols

**STRbase** ENFSI DNA WG  
STR Population Database, v2

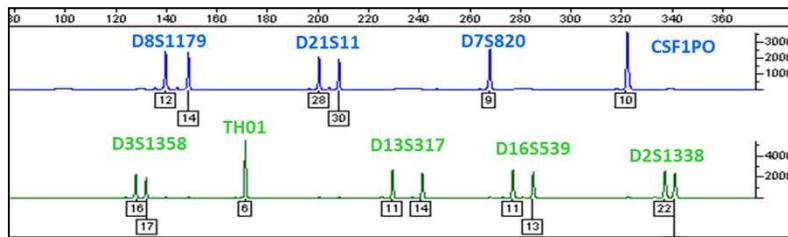
**EMPOP** mtDNA database, v3/R11

**YHRD** Search the Database Tools Resources

**ChrX-STR.org 2.0**  
This database covers many issues concerning the usage of X-chromosomal markers for forensic purpose

Slide from the ISFG 2015 workshop presentation by W. Branicki

# Human Identification Testing



- ❑ Matching DNA profiles – evidence in favor of hypothesis that two samples come from the same individual
- ❑ Reference DNA samples needed to perform human ID testing
- ❑ In many cases suspect is unknown to the investigation

Slide from the ISFG 2015 workshop presentation by W. Branicki

# DNA Database Search

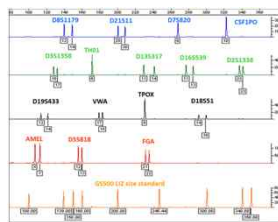


DNA profiling

12,14  
28, 30  
9  
10  
16, 17  
6  
11, 14  
11, 13  
22, 23  
X, Y



Evidence DNA profiles



Matching DNA profiles



12,14  
28, 30  
9  
10  
16, 17  
6  
11, 14  
11, 13  
22, 23  
X, Y



Reference DNA profiles

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# DNA Mass Screening



Source: Internet



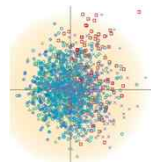
- ❑ ENFSI report: 439 finished mass screens – 315 successful
- ❑ 1998 Germany: rape and murder – 11200 males investigated
- ❑ 2001 Poland: 14 rapes and murder – 421 males investigated

Slide from the ISFG 2015 workshop presentation by W. Branicki

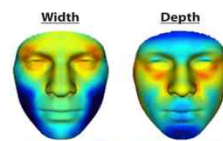
# Intelligence Markers



DNA test



Legend:  
 Africa  
 Americas  
 Europe  
 East Asia  
 Oceania  
 South Asia



Narrower than average jaw and wider eyes; less protruding chin and more prominent cheekbones.

## Predicted (■) & Excluded (⊗) Phenotypes



Picture from snapshot.parabon-nanolabs.com

# Ancestry Inference



<https://www.sott.net/>

- Lineage markers (mtDNA and Y-STR)
- Ancestry Informative Markers (AIMs)
  - Single Nucleotide Polymorphisms, INDELS
  - Several to several hundreds markers analyzed
  - Usually "continental" resolution (East Asia – Europe – Africa)

# Madrid Bomber: 34Plex AIM-SNP Assay

## Ancestry Analysis in the 11-M Madrid Bomb Attack Investigation

Christopher Phillips<sup>1,2\*</sup>, Lourdes Prieto<sup>3</sup>, Manuel Fondevila<sup>1</sup>, Antonio Salas<sup>1</sup>, Antonio Gómez-Tato<sup>4</sup>, José Alvarez-Dios<sup>5</sup>, Antonio Alonso<sup>6</sup>, Alejandro Blanco-Verea<sup>1</sup>, María Brión<sup>2</sup>, Marta Montesino<sup>3</sup>, Ángel Carracedo<sup>1,2</sup>, María Victoria Lareu<sup>1</sup>

<sup>1</sup> Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain, <sup>2</sup> Genomic Medicine Group, CIBERER, University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain, <sup>3</sup> University Institute of Research Police Sciences (UIRCP), DNA Laboratory, Comisaría general de Policía Científica, Madrid, Spain, <sup>4</sup> Faculty of Mathematics, University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain, <sup>5</sup> Instituto Nacional de Toxicología y Ciencias Forenses, Delegación de Madrid, Spain



Bomber from Morocco (2004)

Table 3. Ancestry assignment probabilities from AIM-SNP analysis using a Bayes classifier (output: -log likelihoods), derived likelihood ratios (LR) and ancestries reported to the investigation.

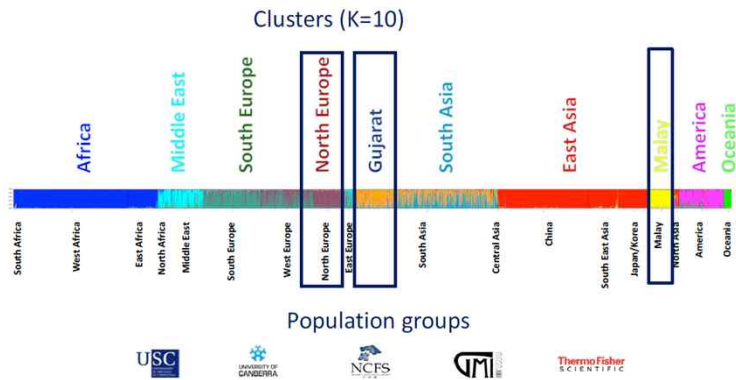
case sample	-log likelihood: African	-log likelihood: European	LR North African	LR European	Ancestry assignment AIM-SNPs	Assessment of ancestry Ymt loci	Ancestry assignment reported
Rabat	9.694E-15	2.5873E-16	36.08*	-	-	-	-
Handprint on bag	2.4805E-18	4.259E-15	-	1,717	European	European	European
Toothbrush	1.6811E-14	2.9613E-23	567,680,319	-	N African	European	N African
Blanket	9.0947E-17	1.509E-18	60.27*	-	-	N African	-
Hat	2.4254E-15	5.2773E-23	45,969,585	-	N African	N African	N African
Scarf profile 1	2.2546E-20	2.4632E-21	9.15*	-	-	NT	-
Scarf profile 2	1.3096E-19	1.0111E-27	129,513,382	-	N African	NT	N African

# Global AIMs panel v2

STRUCTURE v2.3.4

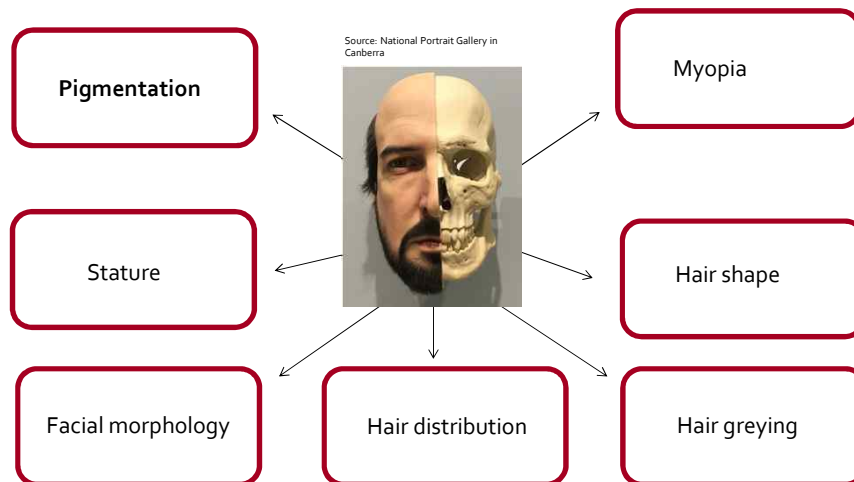
No. of individuals: 2,653

No. of markers: 214 SNPs (144 single site SNPs + 19 Microhaplotype markers)



Slide from the ISFG 2017 presentation by D. Runa

# Externally Visible Characteristics



Slide from the ICGSK 2016 presentation by W. Branicki


# Eye Color DNA Phenotyping

Single Nucleotide Polymorphism (SNP)

Ind1: CATTTCGCGAT  
Ind2: CATTTTGCGAT

TT  
TC  
CC

**The IrisPlex System**




Gene	SNP	Allele	No. of Alleles
HERC2	rs12913832	T	0 1 2 NA
OCA2	rs1800407	A	0 1 2 NA
SLC24A4	rs12896399	T	0 1 2 NA
SLC45A2	rs16891982	C	0 1 2 NA
TYR	rs1393350	T	0 1 2 NA
IRF4	rs12203592	T	0 1 2 NA

Display Predicted Phenotype | Download Predicted Phenotype

Predicted phenotype		
	p-value	AUC Loss
blue eye	0.976	0.002
intermediate eye	0.021	0.003
brown eye	0.003	0.001

**The IrisPlex System**



Gene	SNP	Allele	No. of Alleles
HERC2	rs12913832	T	0 1 2 NA
OCA2	rs1800407	A	0 1 2 NA
SLC24A4	rs12896399	T	0 1 2 NA
SLC45A2	rs16891982	C	0 1 2 NA
TYR	rs1393350	T	0 1 2 NA
IRF4	rs12203592	T	0 1 2 NA


Display Predicted Phenotype | Download Predicted Phenotype

Predicted phenotype		
	p-value	AUC Loss
blue eye	0	0.002
intermediate eye	0.024	0.003
brown eye	0.976	0.001

<http://www.erasmusmc.nl/47743/3604975/HIris?lang=en>

# Eye and Hair Color DNA Phenotyping

**The HirisPlex System**



Gene	SNP	Allele	No. of Alleles
MC1R	rs96insA	A	0 1 2 NA
MC1R	rs11547464	A	0 1 2 NA
MC1R	rs985479	T	0 1 2 NA
MC1R	rs1805006	T	0 1 2 NA
MC1R	rs1805005	T	0 1 2 NA
MC1R	rs1805006	A	0 1 2 NA
MC1R	rs1805007	T	0 1 2 NA
MC1R	rs1805009	C	0 1 2 NA
MC1R	Y152C>G	A	0 1 2 NA
MC1R	rs2228479	A	0 1 2 NA
MC1R	rs1110400	C	0 1 2 NA
SLC45A2	rs28777	C	0 1 2 NA
SLC45A2	rs16891982	C	0 1 2 NA
KITLG	rs12821256	G	0 1 2 NA
EXOC2	rs4959270	A	0 1 2 NA
IRF4	rs12203592	T	0 1 2 NA
TYR	rs1042602	T	0 1 2 NA
OCA2	rs1800407	A	0 1 2 NA
SLC24A4	rs2402130	G	0 1 2 NA
HERC2	rs12913832	T	0 1 2 NA
ASIP	rs2378249	C	0 1 2 NA
SLC24A4	rs12896399	T	0 1 2 NA
TYR	rs1393350	T	0 1 2 NA
TYRP1	rs683	G	0 1 2 NA

Display Predicted Phenotype | Download Predicted Phenotype

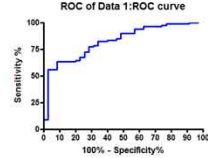
Predicted phenotype		
	p-value	AUC Loss
blue eye	0.972	0
intermediate eye	0.025	0
brown eye	0.002	0
blond hair	0	0.003
brown hair	0	0.003
red hair	1	0.01
black hair	0	0.002
light hair	0.989	0.002
dark hair	0.011	0.002

Current Database AUC values at a population level	
Blue eye colour	0.94
Intermediate eye colour	0.74
Brown eye colour	0.95
Blond hair colour	0.81
Brown hair colour	0.75
Red hair colour	0.92
Black hair colour	0.85
Hair colour shade	0.90

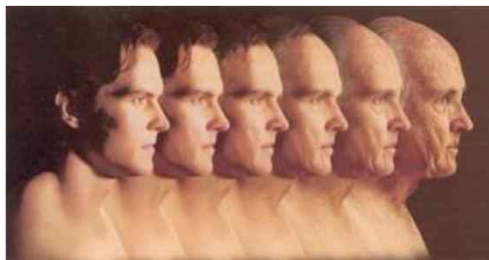
**ROC of Data 1: ROC curve**



<http://www.erasmusmc.nl/47743/3604975/HIris?lang=en>

# Forensic Age Estimation

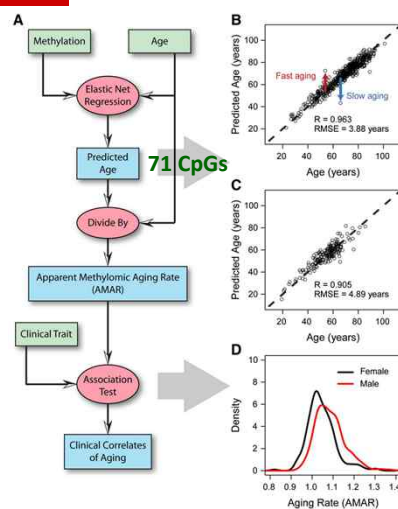
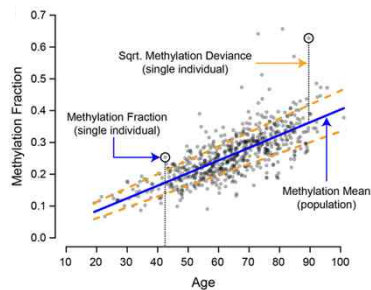
- Information about chronological age of an offender
  - Predicted age can be used to narrow down the search range
- Information about biological age of an offender
  - Predicted age can improve forensic DNA phenotyping



<https://internetmedicine.com/aging-and-nanomedicine/>

Slide from the ISFG 2015 workshop presentation by W. Branicki

# Age Prediction with DNA Methylation

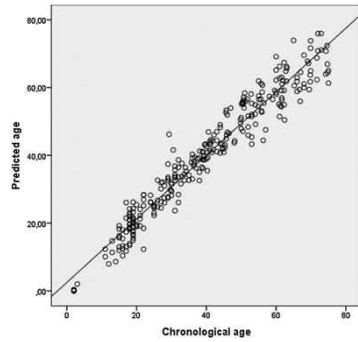


Hannum et al. Mol Cell (2013)



# Age Prediction in Blood

- 5 CpGs in the genes ELOVL2, C1orf132, TRIM59, KLF14 and FHL2

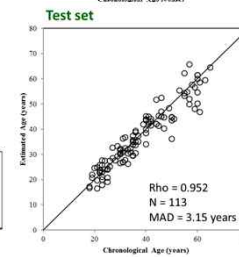
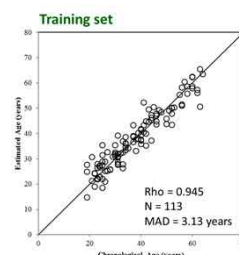
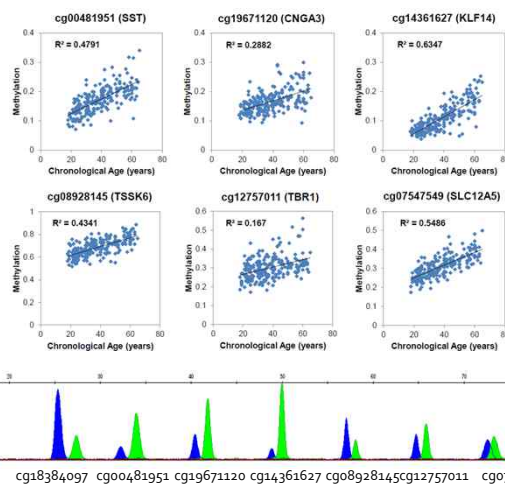


MAD (Mean Absolute Deviation) from chronological age = 3.9 years

$$\text{Regression function: Age} = a + b \times \text{CpG}_1 + c \times \text{CpG}_2 + d \times \text{CpG}_3 + \dots$$

Zbieć-Piekarska et al. Forensic Sci Int Genet (2015)

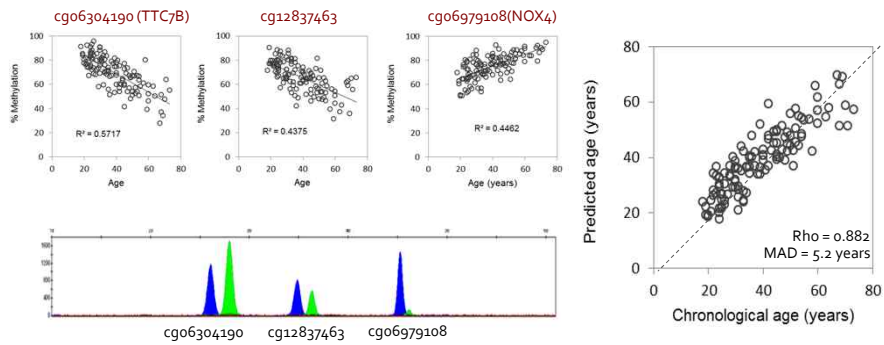
# Age Prediction in Saliva



Hong et al. Forensic Sci Int Genet (2017)

## Age Prediction in Semen

- Age correlation of the 3 CpGs and predicted versus chronological ages of 125 semen samples



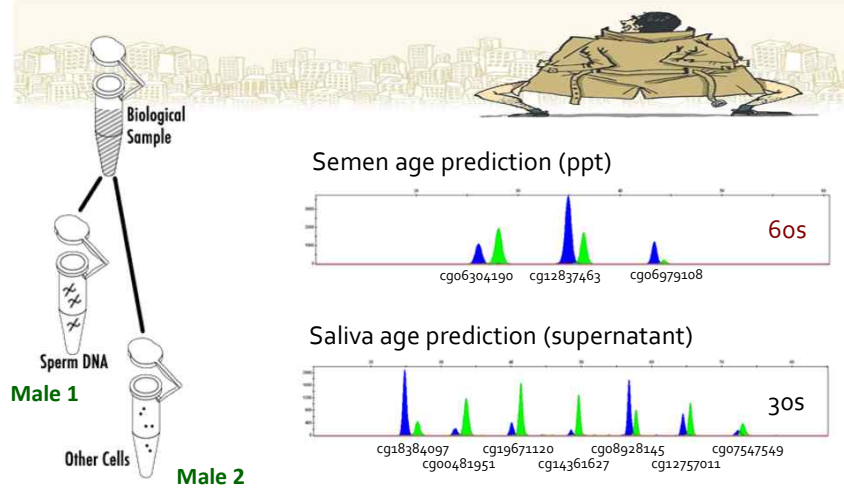
Lee et al. Forensic Sci Int Genet (2015)

## Analysis of a Casework Example



- A stain preliminary positive for semen and saliva
- Two men's mixed STR profile

## Analysis of a Casework Example



## Ethical and Legal Issues in FDP

- FDP is prediction of appearance from forensic samples
- FDP is developed to guide police investigations in cases without known suspects
- FDP includes **forensic use of DNA for investigation, not in the courtroom**
- Benefit and risk analysis** will be needed
- Law: Netherlands, Texas..



**Thank you for your attention!**

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<http://forensic.yonsei.ac.kr>

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Picture from internet