NGS assays in forensic genetic case work:

Past experiences and what is next.

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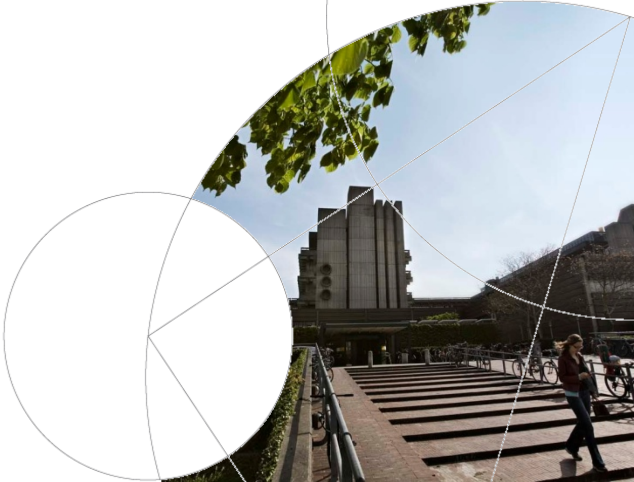


NGS assays in forensic genetic case work

SNP typing in relationship case work

- Precision ID Identity Panel

Ancestry inference in crime case work

- Precision ID Ancestry Panel

mtDNA typing in case work

- Precision ID Whole mtDNA Genome Panel

SNP typing in relationship case work

- 2006: SNP*for*ID PCR-SBE-CE assay published[a]
- 2007: Evaluation of the SNP*for*ID PCR-SBE-CE assay[b]
  Validation according to ISO17025 standard[c]
- 2014: Evaluation of the Precision ID Identity Panel/Ion PGM[d]
- 2015: Validation according to ISO17025 standard[e]
- 2016: Automation of library building[f]
- 2017: Pilot study on non-invasive prenatal testing[g]
- 2018: Ion S5 replaced Ion PGM

[a] Sanchez *et al*., Electrophoresis (2006) 27, 1713-24.
[b] Børsting *et al*., FSI genet. (2008) 2, 292-300.
[c] Børsting *et al*., FSI genet. (2009) 4, 34-42.
[d] Børsting *et al*., FSI genet. (2014) 12, 144-154.
[e] Buchard *et al*., Electrophoresis (2016) 37, 2822-2831.
[f] van der Heijden *et al*., FSI genet. (2017) 31, 118-125
[g] Christiansen *et al*., HIDS poster (2018)

SNP typing in relationship case work

Precision ID Identity Panel

- 90 autosomal human identification SNPs
- 34 upper clade Y-SNPs

Conditions

- Input amount: >500 pg gDNA or 1.2 mm FTA-card punches
- Automated library building using half-volume reactions*

Used as supplementary investigation in relationship case work

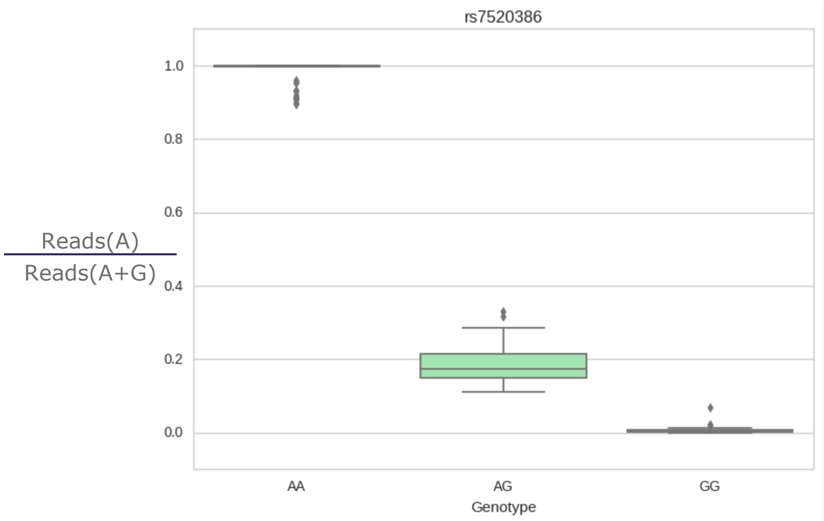
- Trio cases with one or two genetic inconsistencies in STR loci
- Used in all duo cases
- Reported in 229 cases (November 2015 – April 2018)

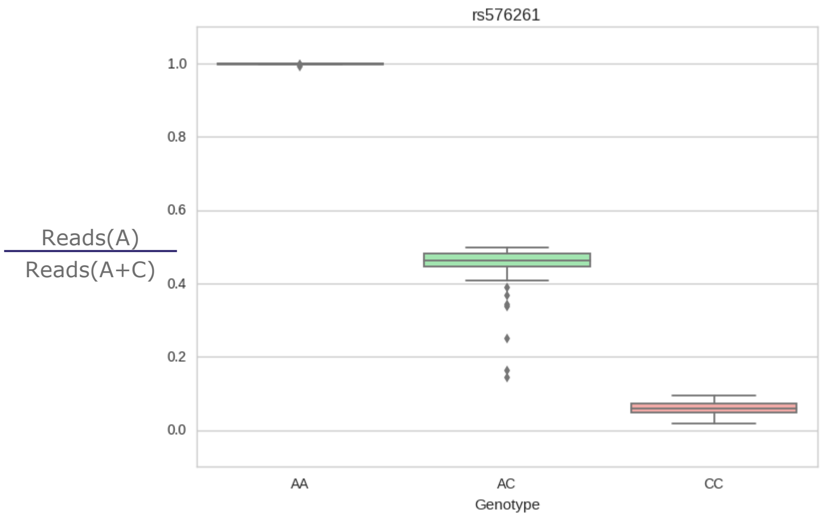
*van der Heijden *et al.*, FSI genet. (2017) 31, 118-125.

SNP typing in relationship case work

Validation study*

- Two autosomal SNPs excluded (rs7520386 and rs576261)
- Do not report Y-SNP haplotype
- Report 88 autosomal SNPs (and 16 autosomal STRs)





*Buchard *et al*., Electrophoresis (2016) 37, 2822-2831.

SNP typing in relationship case work

Analysis criteria*:

- Locus read depth: ≥100 reads
- Heterozygote balance: 0.33≤ Hb ≥3 (typically Hb≈1)
- Noise reads: <3% (typically <1%)

	Number of SNPs with warnings for	
Sample	Hb<0.33 or 3<Hb	Noise >3%
1 to 1 mixture	13	1
1 to 3 mixture	21	1.5
1 to 6 mixture	5.5	20
1 to 12 mixture	0.25	23.5
1 to 24 mixture	0.25	8.75
1 to 48 mixture	0	3
Single source	0.85	1.3

*Buchard *et al.*, Electrophoresis (2016) 37, 2822-2831.

SNP typing in relationship case work

SNPonPGM Python script/data analysis

- Assists the data analyst by highlighting genotypes that do not fulfill the analysis criteria

SNPonPGM Python script/reporting

- Compare profiles from duplicate typing
- Generate consensus profiles from duplicate typing
- Collect approved SNP genotypes for all individuals in a case
- Export the SNP genotypes of all individuals in the case to a text file that may be used for likelihood calculations and for the final report

SNP typing in relationship case work

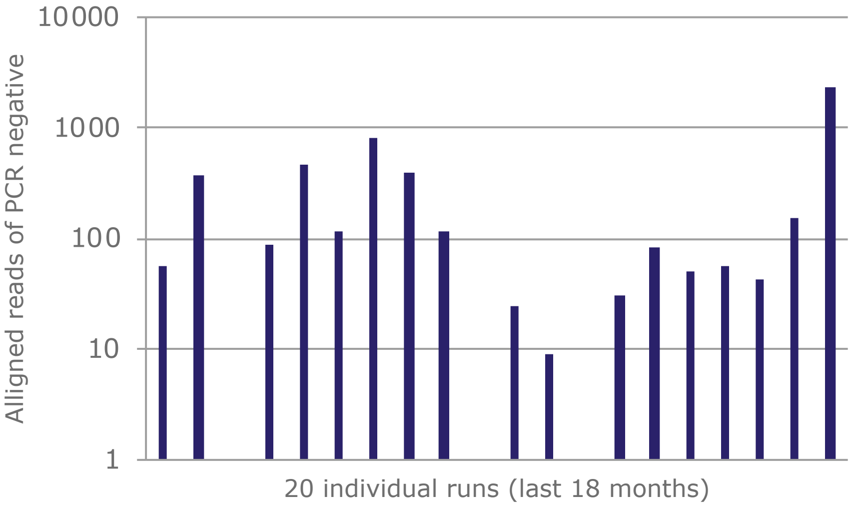
Always use two negative controls

- PCR negative (water instead of DNA)
- Library negative (water instead of PCR products)

Observe alligned reads in PCR negative (0.1% of samples)

- No apparent increase over time

No alligned reads in library negative



Ancestry inference in crime case work

- 2009: The Seldin AIMs panel published[a]
- 2014: The Kidd AIMs panel published[b]
- 2015: Greenlandic reference population[c]
- 2017: Evaluation of the Precision ID Ancestry Panel[d]
  Development of outlier test[e]
  Development of the GenoGeographer software[f]
- 2018: Report ancestry inference in crime case work

[a] Nassir *et al.*, BMC genet. (2009) 10, 39.
[b] Kidd *et al.*, FSI genet. (2014) 10, 23-32.
[c] Themudo *et al.*, FSI genet. (2016) 24, 60-64.
[d] Pereira *et al.*, FSI genet. (2017) 28, 138-145.
[e] Tvedebrink *et al.*, Theor. Popul. Biol. (2018) 120, 1-10.
[f] Tvedebrink *et al.*, FSI genet. suppl. (2017) 6, e463-465.

Ancestry inference in crime case work

Precision ID Ancestry Panel

- 165 autosomal ancestry informative SNPs from two selection panels (Seldin and Kidd)

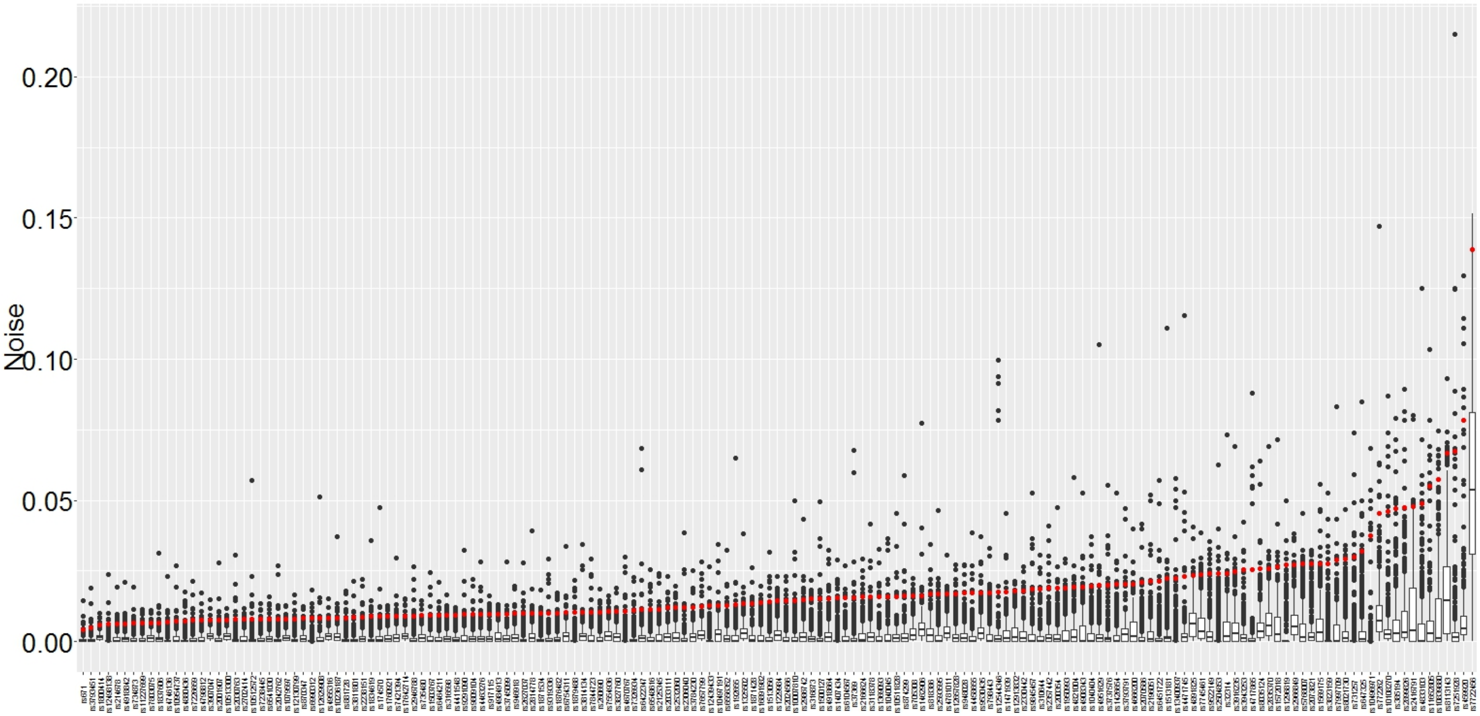
Used as supplementary investigation in crime case work

- Requested by the police
- Only single source samples are used
- Input amount: minimum 500 pg gDNA

Ancestry inference in crime case work

Analysis criteria:

- Locus read depth: ≥100 reads
- Heterozygote balance: 0.33≤ Hb ≥3 (typically Hb≈1)
- Noise reads: <3% (typically <1%)



Ancestry inference in crime case work

Report ancestry inference with a likelihood ratio

- LR = P(Genotype | Population A)/P(Genotype | Population B)

LR may be misleading

- Without an appropiate reference population in the database
- If the most likely population group is not selected as population A or B

Outlier test* for concordance between the AIM profile and a reference population (z-score)

- H_0: AIM profile belong to population
- z-score <1.64: Cannot reject H_0
- z-score >1.64: Reject H_0
- z-score >1.64 for all reference populations: Inconclusive result

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*Tvedebrink *et al*., Theor. Popul. Biol. (2018) 120, 1-10.

Ancestry inference in crime case work

Case example:

- Murder case from 2012
- Single source sample from the possible offender
- STR profile without match in the police STR database

Full AIM profile was obtained with the Precision ID Ancestry Panel

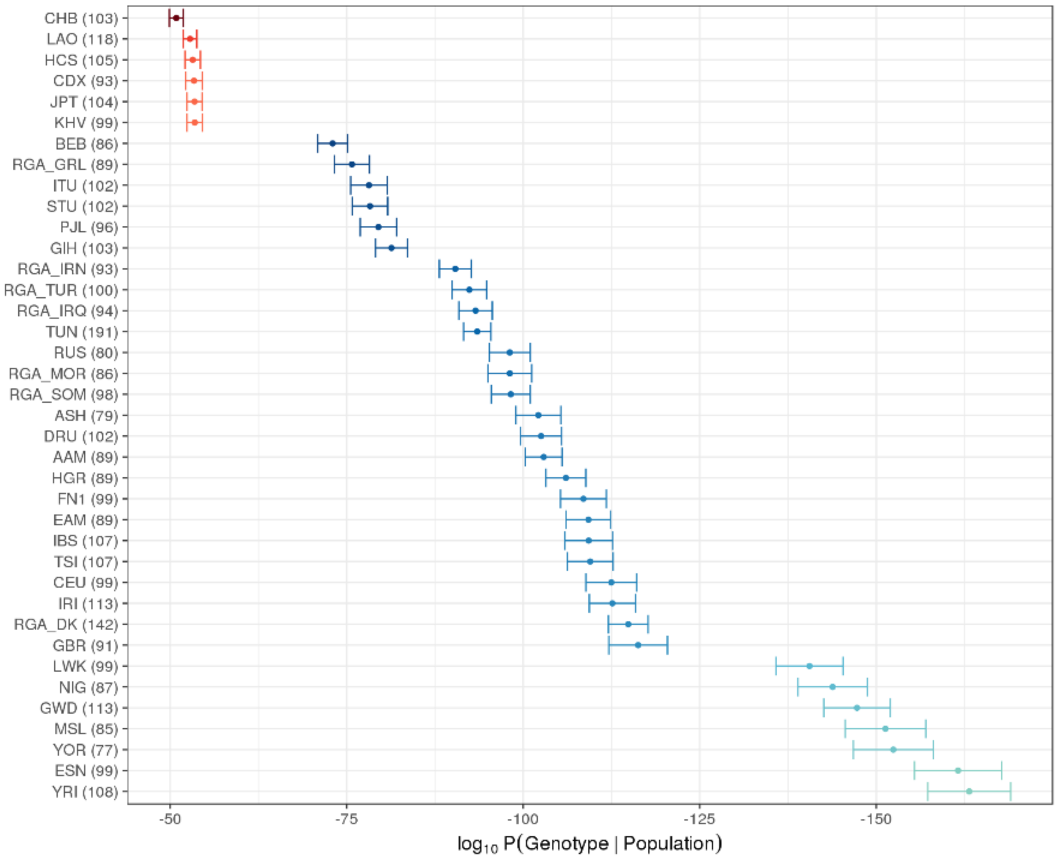
- Imported into the GenoGeographer* software
- 38 reference populations with >75 AIM profiles each
- 6 population groups with >275 AIM profiles each

z-score <1.64 for six populations and one population group

*Tvedebrink *et al*., FSI genet. suppl. (2017) 6, e463-465.

Ancestry inference in crime case work

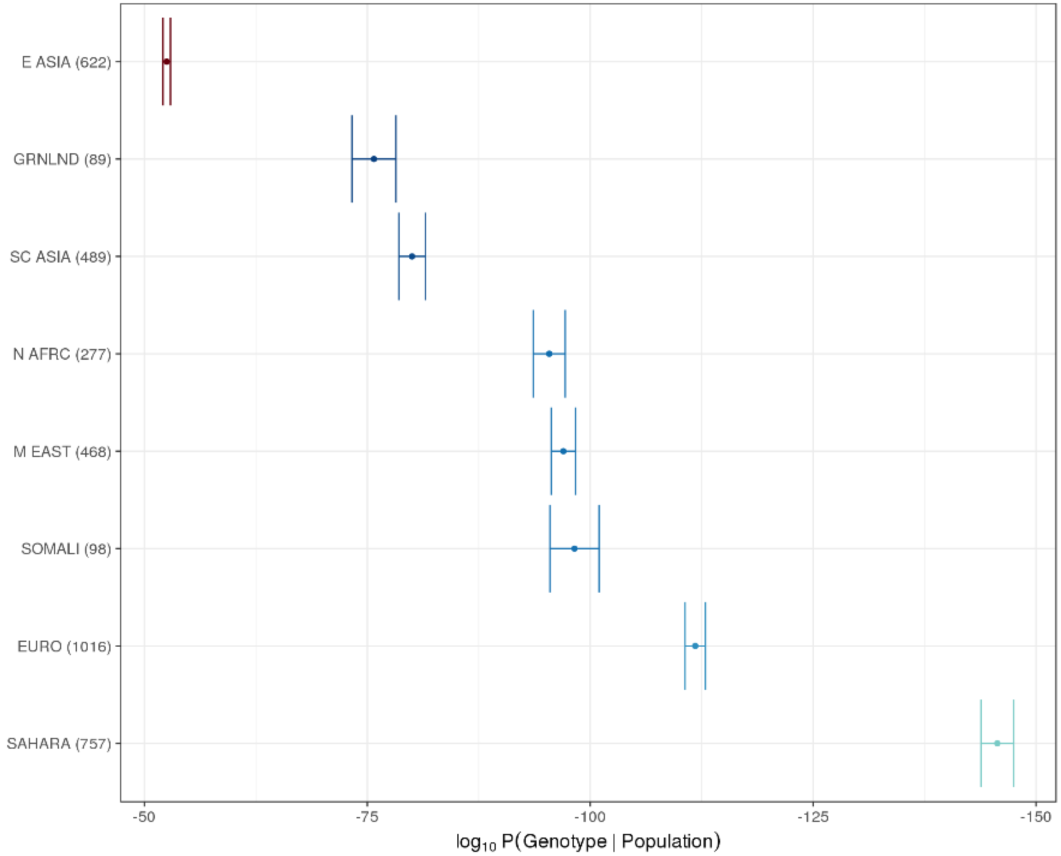
Case example: GenoGeographer output



Error bar plots for populations. The bars reflects the approximate confidence interval

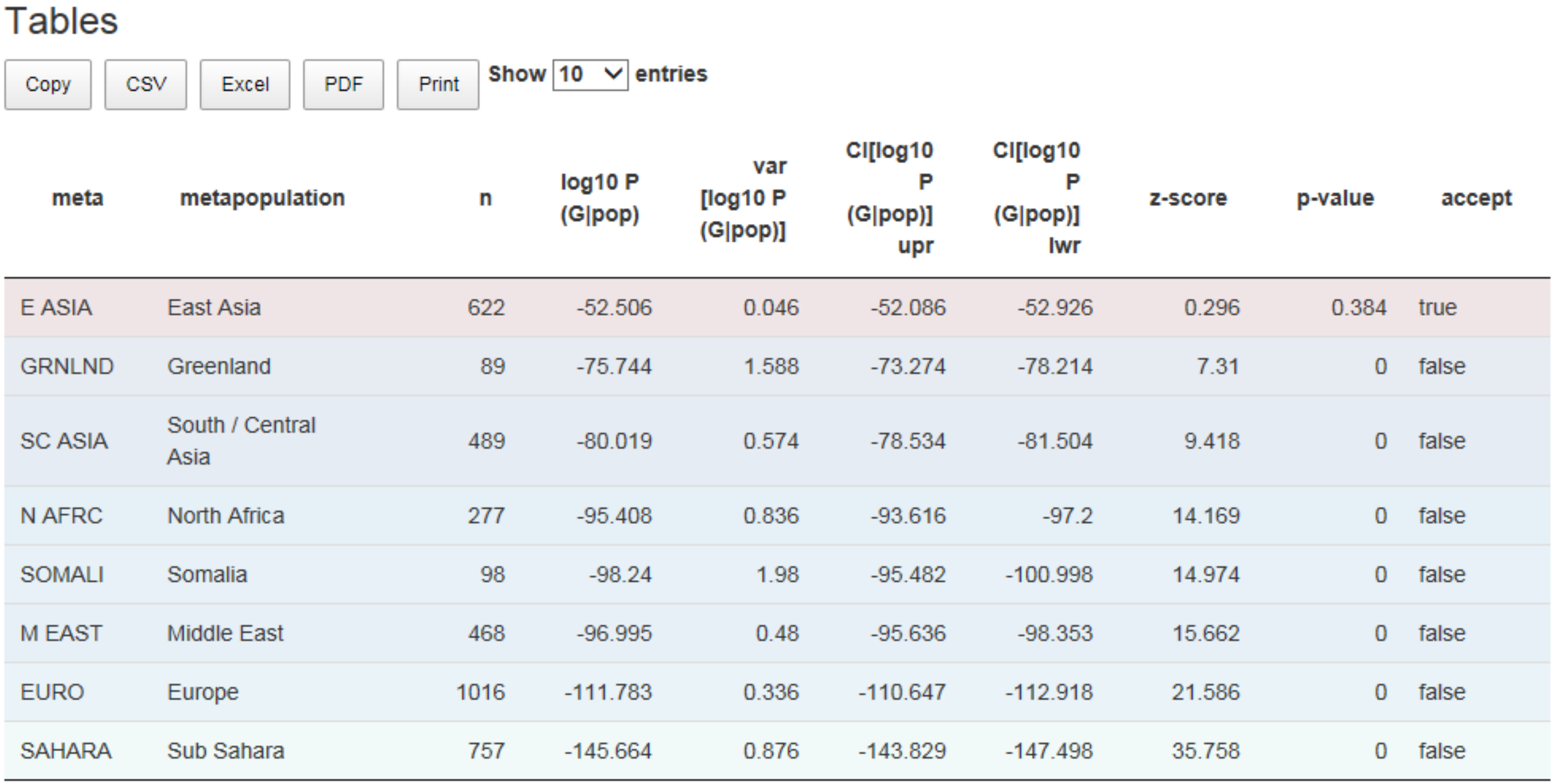
Ancestry inference in crime case work

Case example: GenoGeographer output



Ancestry inference in crime case work

Case example: GenoGeographer output



Tables

Show 10 entries

meta	metapopulation	n	log10 P (G|pop)	var [log10 P (G|pop)]	CI[log10 P (G|pop)] upr	CI[log10 P (G|pop)] lwr	z-score	p-value	accept
E ASIA	East Asia	622	-52.506	0.046	-52.086	-52.926	0.296	0.384	true
GRNLND	Greenland	89	-75.744	1.588	-73.274	-78.214	7.31	0	false
SC ASIA	South / Central Asia	489	-80.019	0.574	-78.534	-81.504	9.418	0	false
N AFRC	North Africa	277	-95.408	0.836	-93.616	-97.2	14.169	0	false
SOMALI	Somalia	98	-98.24	1.98	-95.482	-100.998	14.974	0	false
M EAST	Middle East	468	-96.995	0.48	-95.636	-98.353	15.662	0	false
EURO	Europe	1016	-111.783	0.336	-110.647	-112.918	21.586	0	false
SAHARA	Sub Sahara	757	-145.664	0.876	-143.829	-147.498	35.758	0	false

Ancestry inference in crime case work

Case example:

- LR = P(Genotype|East asian)/P(Genotype|European)
  > 10,000
- LR = P(Genotype|East asian)/P(Genotype|Greenland)
  > 10,000

Consequences:

- Police re-opened the case
- Additional information from the public

mtDNA typing in case work

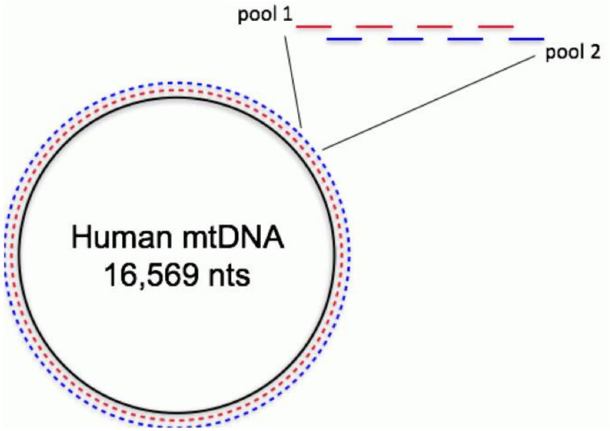
- 2002: Evaluation of Sanger sequencing assay[a] for crime case work
- 2006: Validation according to ISO17025 standard
- 2008: mtDNA investigation discontinued
- 2017: Evaluation of the Precision ID Whole mtDNA Genome Panel[b]
- 2018: On-going validation of the assay for immigration case work
  Pilot studies on selected crime case sample materials
- 2019: Validation according to ISO17025 standard?
  Report mtDNA in immigration case work?

[a] Rasmussen *et al.*, FSI (2002) 129, 209-213.
[b] Pereira *et al.*, Electrophoresis (2018) submitted.

mtDNA typing in case work

Precision ID Whole mtDNA Genome Panel
- Amplifies and sequence the entire mtDNA genome (16,569 bp)
- Two multiplex PCRs with 81 primer sets each

Used as supplementary investigation in immigration case work
- Cases with 2nd or 3rd degree relatives



mtDNA typing in case work

Preliminary analysis criteria:

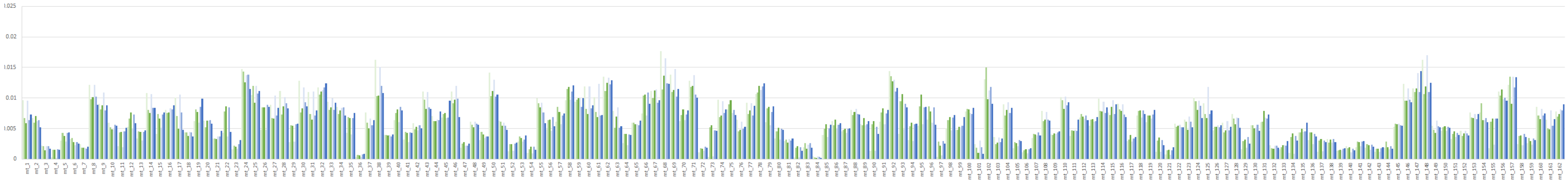
- Read depth: ≥100 reads
- Noise reads: <7%

Will not report variants in two homopolymer streches

- 302-315: acccccctcccccg
- 16,180-16,192: aaacccccctcccca

Consistent fragment balance (10-1,000 pg gDNA input)

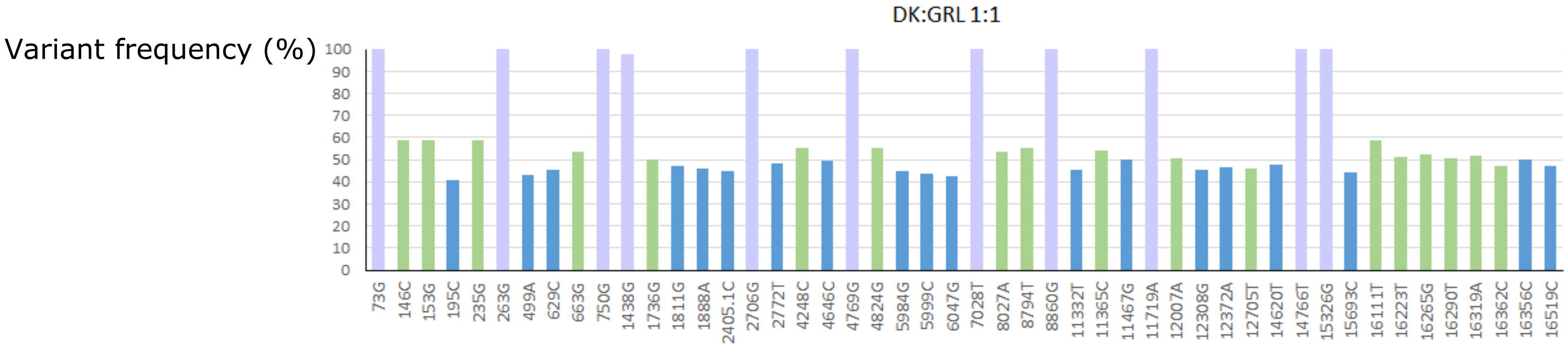
- Read depths varies by a factor of 40-100



mtDNA typing in case work

Controlled two-person mixtures

- One Dane (U4d1a) and one Greenlander (A2b1)
- Differed in 48 positions compared to the rCRS



Acknowledgements

Section of Forensic Genetics
University of Copenhagen

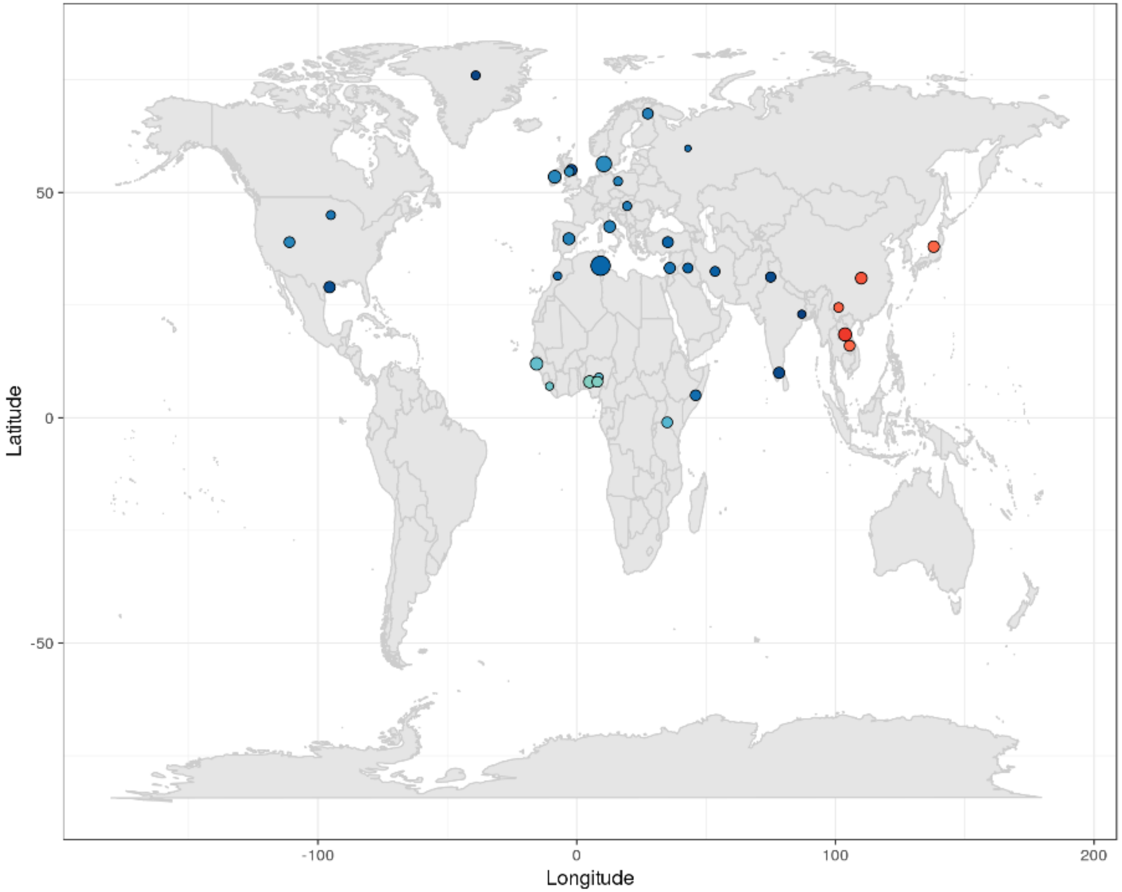
Anders Buchard
Helle S. Mogensen
Vania Pereira
Eszter Rockenbauer
Marie-Louise Kampmann
Niels Morling
Carina G. Jønck
Brian Stidsen

Department of Mathematical Sciences
University of Aalborg

Torben Tvedebrink
Poul S. Eriksen
Mikkel Andersen

Ancestry inference in crime case work

Case example: GenoGeographer output



Map of the populations geographical locations and their accept/reject status