



Faculty of Health and Medical Sciences



NEW TECHNOLOGIES - AN INTRODUCTION

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University of Copenhagen
Denmark**



FORENSIC MEDICINE – COPENHAGEN - DENMARK



DEPARTMENT

Staff	
Forensic genetics	100
Forensic chemistry	40
Forensic pathology	40
• Forensic anthropology	
• Forensic odontology	
Other	50
Total	230

FORENSIC GENETICS

Crime cases	25,000
Reference samples	12,000
Paternity cases	1,000
Immigration cases	50



FORENSIC GENETIC PERSPECTIVES OF MASSIVELY PARALLEL SEQUENCING



ALSO CALLED

- SECOND GENERATION SEQUENCING
- NEXT GENERATION SEQUENCING

MAY BE USED FOR

- • STRs FOR IDENTIFICATION / RELATIONSHIP TESTING
- • SNPs FOR IDENTIFICATION / RELATIONSHIP TESTING
 - WHOLE mt-GENOME
- • SNPs FOR PHENOTYPICAL TRAITS
- • SNPs FOR ANCESTRY
 - GENETIC DISEASES, e.g.
 - HEART
 - PSYCHIATRIC
 - SKIN, etc.
 - MICROBIAL IDENTIFICATION
 - TARGETED, WHOLE EXOME, WHOLE GENOME SEQUENCING





MPS IN COPENHAGEN



GS JUNIOR (2009) - RETIRED



MISEQ (2013) + FORENSEQ (2014)

- FORENSIC SNPs-STRs
- mtDNA SEQUENCING
- GENES, e.g. HEART
- mRNA/miRNA/metDNA



ION TORRENT PGM (2013) - THREE

- HID-Ion AmpliSeq™ Identity Panel
- HID-Ion AmpliSeq™ Ancestry Panel
- LT STR SEQUENCING



NEXTSEQ 500 (2015)

- WHOLE GENOME SEQUENCING
- WHOLE EXOME SEQUENCING
- GENES, e.g. HEART, PSYCHIATRY
- mRNA/miRNA/metDNA



ION S5 NGS SYSTEM (2016)

- FORENSIC GENETICS – VALIDATION



SUPPLEMENTARY SNP MULTIPLEX FOR PATERNITY & RELATIONSHIP TESTING

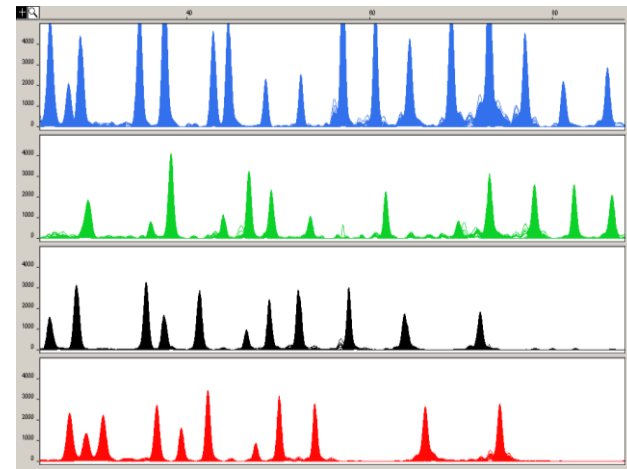
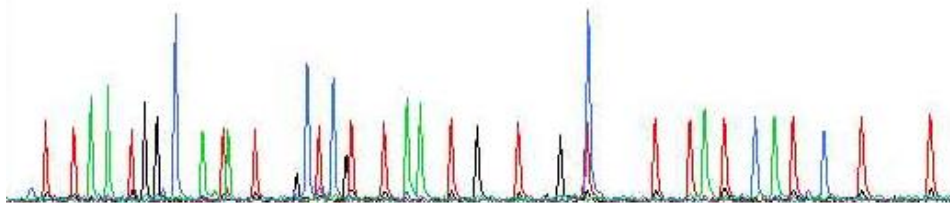


2007 -2015

STANDARD INVESTIGATION

SUPPLEMENTARY INVESTIGATION

15-17 STRs



49 SNPs

SNAPSHOT & CE



SUPPLEMENTARY MPS SNP MULTIPLEX FOR PATERNITY & RELATIONSHIP TESTING

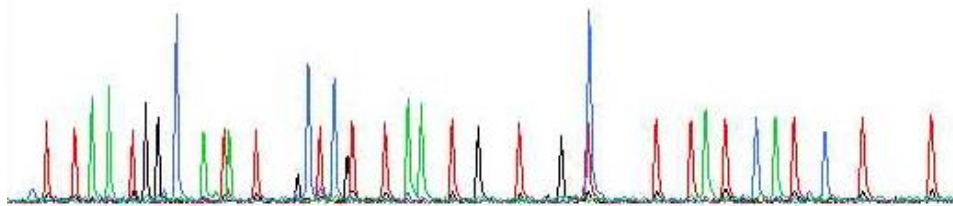


STANDARD INVESTIGATION
17 STRs

FROM 2016
SUPPLEMENTARY INVESTIGATION

HID-ION AMPLISEQ™ IDENTITY PANEL

- **124 SNPs**
- **90 AUTOSOMAL SNPs**
- **34 Y CHROMOSOME SNPs**



VALIDATION OF THE HID-ION AMPLISEQ™ IDENTITY PANEL – RELATIONSHIP TESTING



SAMPLES TYPINGS OF

- UNRELATED DANES
- RELATIONSHIP TRIOS AND DUOS
- ARTIFICIAL DNA MIXTURES (1:1, 1:3, 1:6, 1:12, 1:24, 1:48)

ALL SAMPLES WERE PREVIOUSLY TYPED WITH THE SNPFORID ASSAY (PCR-SBE-CE)

SAMPLE MATERIAL

- PURIFIED DNA (~1 NG)
- FTA CARD PUNCHES (D = 1.1 mm, BUCCAL SWABS)

CHANGES TO THE MANUFACTURER'S PROTOCOL

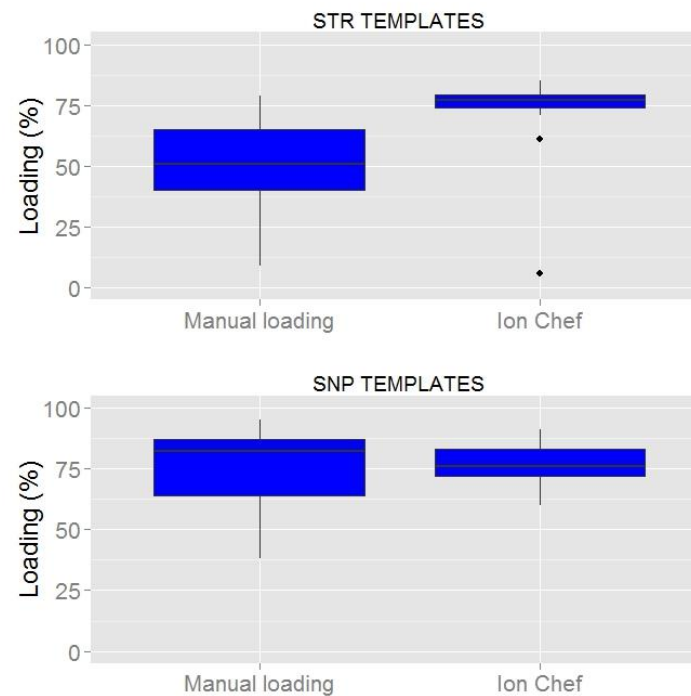
- 24 CYCLES PCR
- LIBRARIES PER CHIP:
5 FOR ION314, 18 FOR ION316, 40 FOR ION318

HID-ION AMPLISEQ™ IDENTITY PANEL



THE ION CHEF™

- LESS HANDS-ON AND FEWER PIPETTING STEPS
- MORE CONSISTENT LOADING

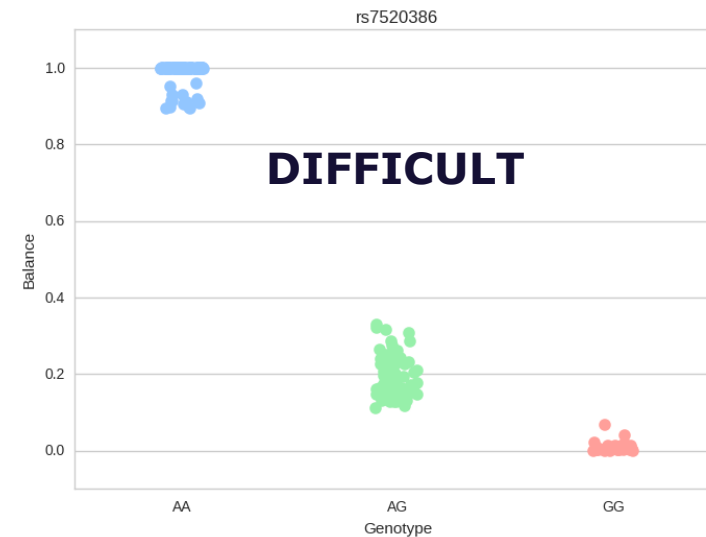
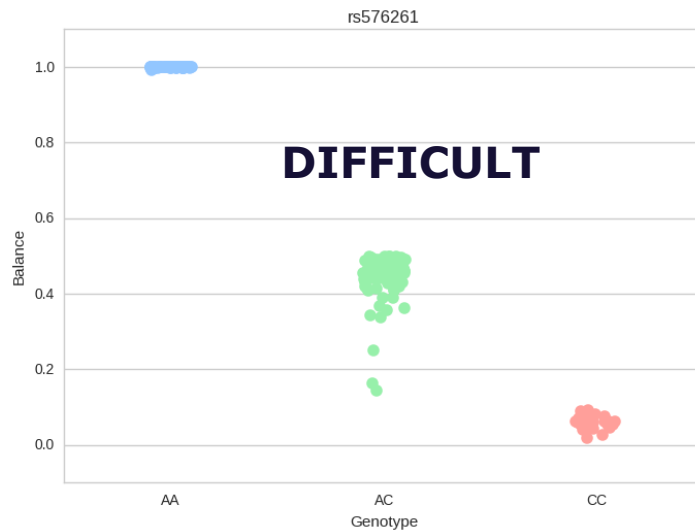


VALIDATION OF THE HID-ION AMPLISEQ™ IDENTITY PANEL

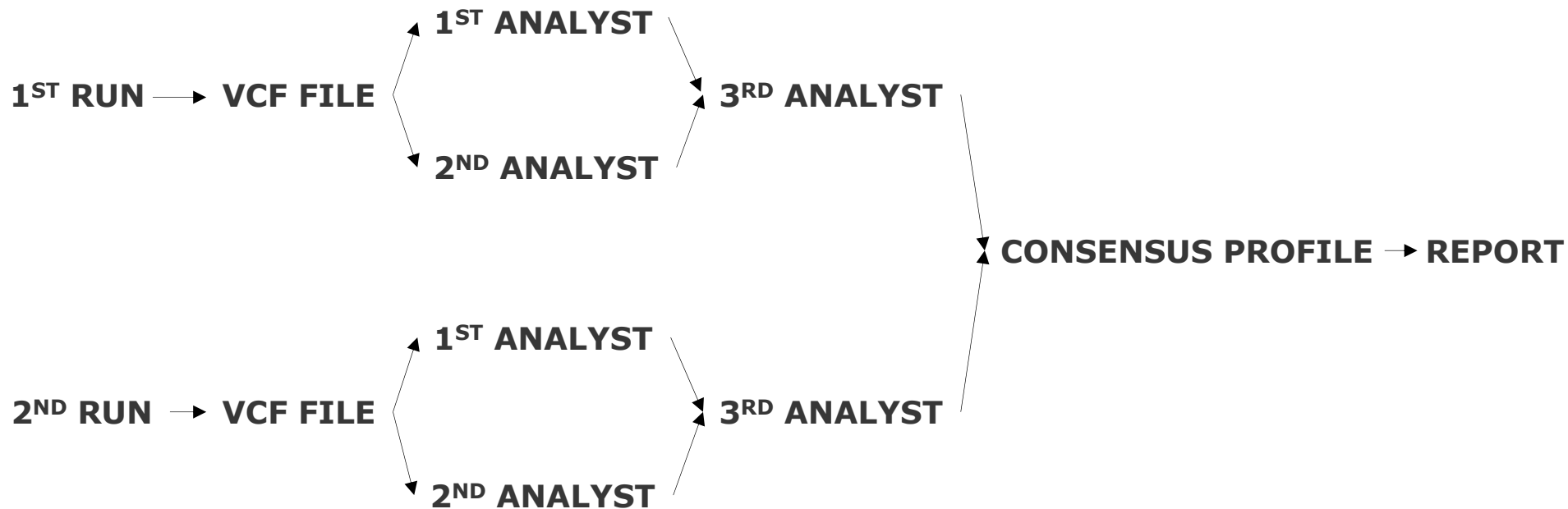


SEQUENCING PERFORMANCE FOR EACH LOCUS

- **NOISE**
- **COVERAGE**
- **HETEROZYGOTE BALANCE**



VALIDATION OF THE HID-ION AMPLISEQ™ IDENTITY PANEL



PERFORMED WITH A FEW CLICKS WITH A MOUSE



RESULTS OF VALIDATION OF THE HID-ION AMPLISEQ™ IDENTITY PANEL



- MENDELIAN INHERITANCE OF 88 AUTOSOMAL AND 34 Y-SNPS
- LR CALCULATION BASED ON 39 SNPforID SNPS (DNAVIEW)
- TYPICAL PI (GEOMETRIC MEAN) $> 10^4$
- MATCH PROBABILITY $< 10^{-6}$ (NAÏVE CALCULATION $< 10^{-13}$)

THE VALIDATION REPORT FOR RELATIONSHIP TESTING WAS APPROVED BY THE ACCREDITING BODY IN DENMARK - DANAK

PROFICIENCY TESTS FOR HID-SNPS

- NORDIC - DENMARK, SWEDEN, NORWAY
- THREE RELATIONSHIP CASES PER YEAR
- THE SNPforID CONSORTIUM/EUROFORGEN
DENMARK, SWEDEN, NORWAY, COLOGNE, SANTIAGO, LONDON
FOUR SAMPLES PER YEAR

BEGINNING OF S5 VALIDATION



ION AMPLISEQ™ PANELS

HID-ION AMPLISEQ™ ANCESTRY PANEL (165 SNPs)

123 SELDIN SNPs

55 KIDD SNPs

HID-ION AMPLISEQ™ IDENTITY PANEL (124 SNPs)

48 SNPFORID SNPs

43 IISNPs

34 UPPER-CLADE Y-SNPs

ION AMPLISEQ™ GLOBALFILER® MIXTURE ID PANEL (113 LOCI)

29 AUTOSOMAL STRs

1 Y-STR

45 SNPs

2 Y-INDELS

36 MICROHAPLOTYPES (SNPs)



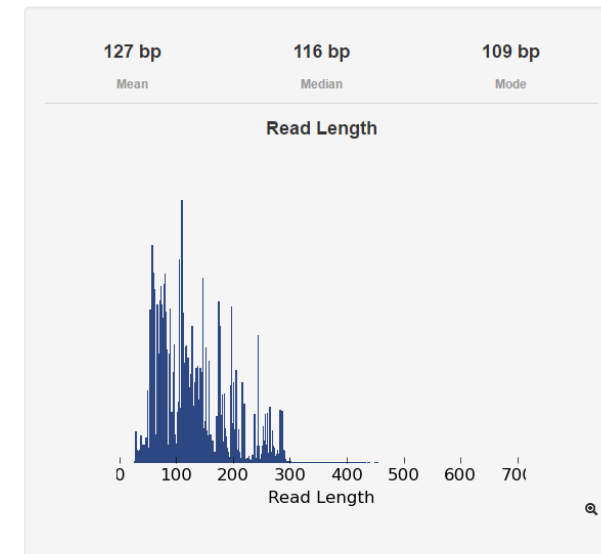
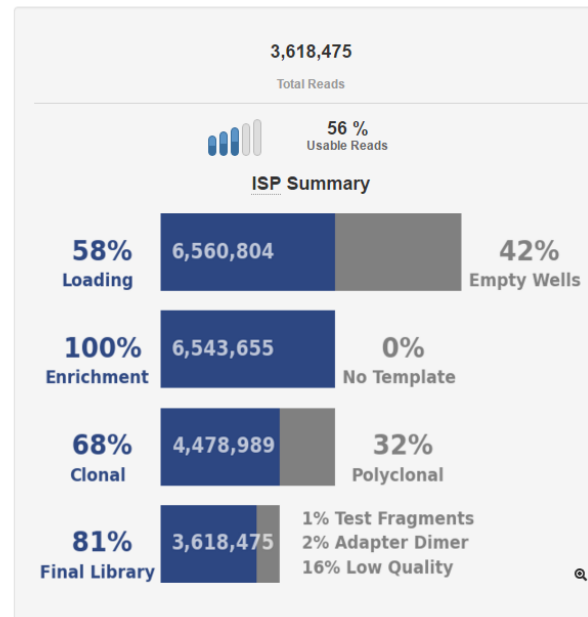
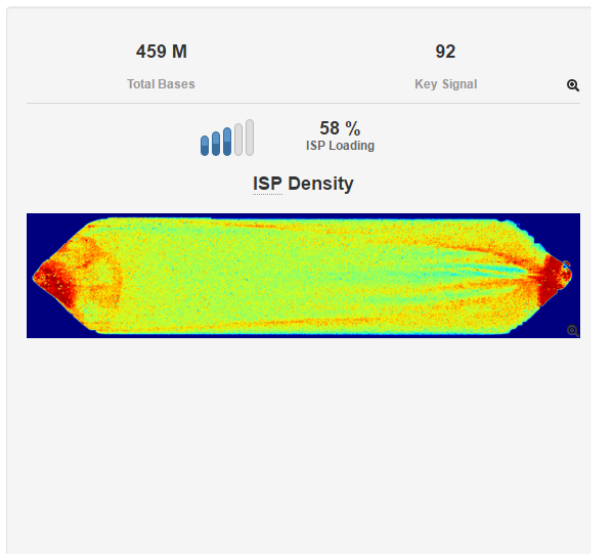
SUMMARY FROM THE CONCORDANCE STUDY



Run Summary: R_2016_05_03_09_04_37_user_S5-00565-4-20160502_Concordance_MAJ

Reports :

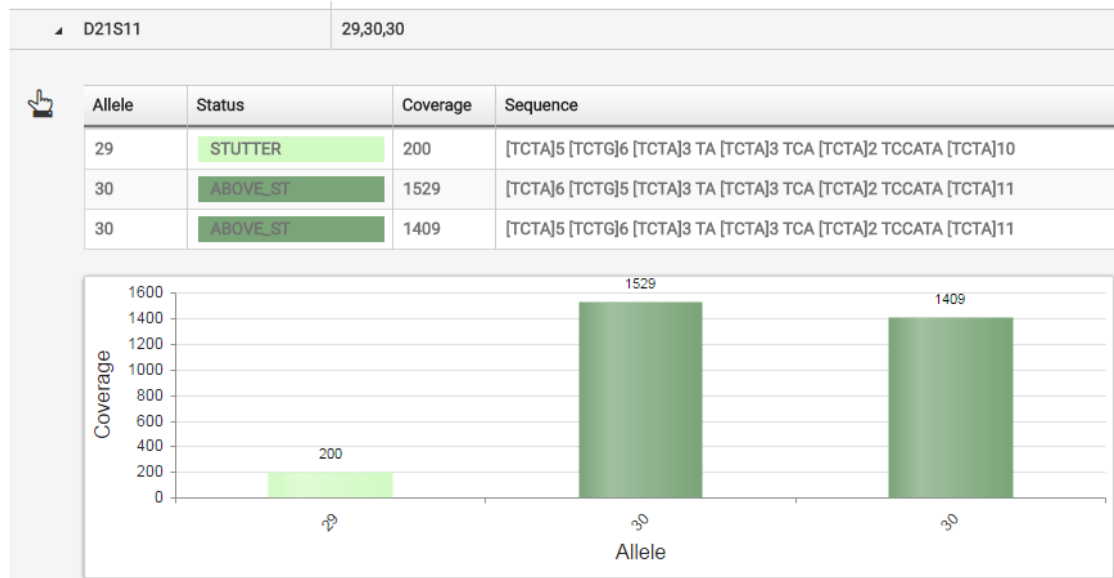
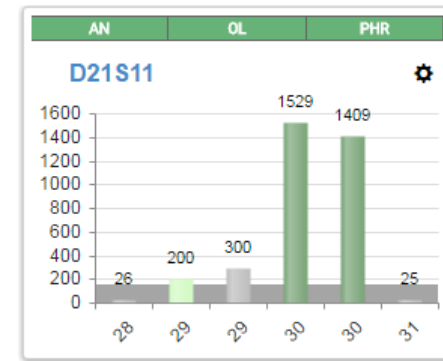
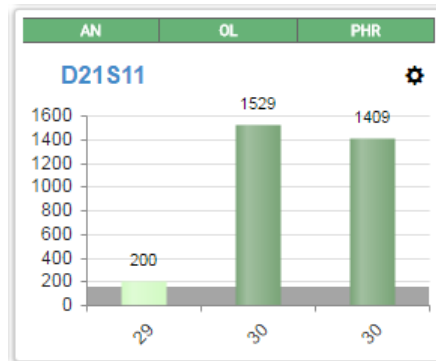
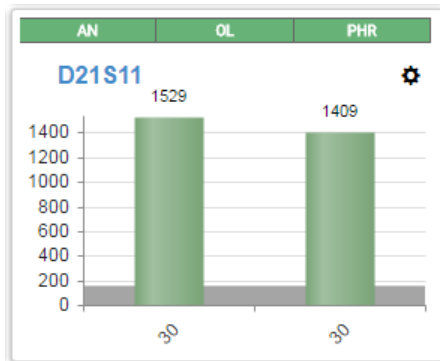
Read Summary: Unaligned





THE CONVERGE™ SOFTWARE – S5

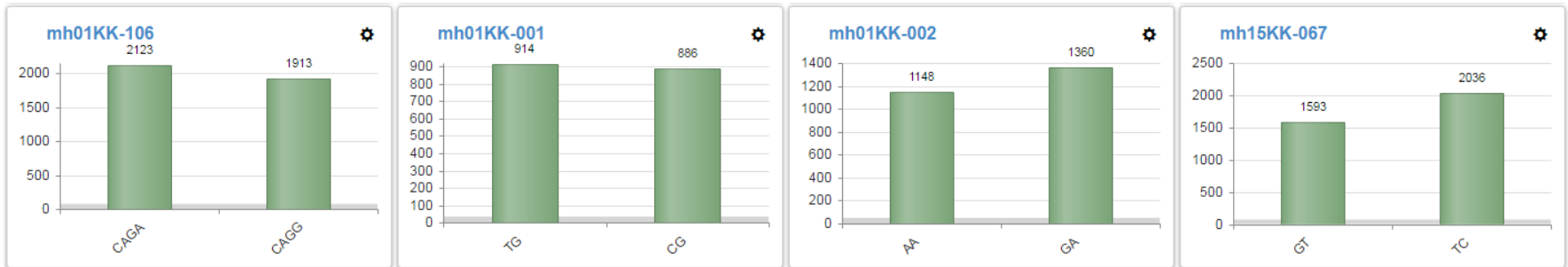
STRs: A FEW VIEWS



THE CONVERGE™ SOFTWARE – S5



MICROHAPLOTYPES



SNPs

Position	Locus	Genotype	QC	Coverage	Allele Freq A T G C	Coverage%	IGV
chr17:80765788	rs2292972	CT	●	2607			
chr10:132698419	rs964681	CT	●	3471			
chr5:174778678	rs251934	AG	●	5117			
chr17:2919393	rs9905977	GG	●	4804			
chr2:114974	rs876724	CC	●	2742			
chr21:29679687	rs2831700	AG	●	4316			
chr13:20901724	rs1335873	AA	●	5626			
chr21:43606997	rs221956	CC	●	4712			
chr1:105717631	rs4847034	GG	●	2437			
chr7:4310365	rs6955448	CC	●	3172			

MPS SOFTWARE



PROVIDERS' SOFTWARE

- **TORRENT SUITE PLUG-INNS (SNPs AND STRs)**
- **FORENSEQ UNIVERSAL ANALYSIS SOFTWARE**

STRAIT RAZOR V2.0

- **WARSHAUER ET AL., FSI GENET 2015;14: 182-86**

MYFLQ

- **VAN NESTE ET AL., FSI GENET 2015; 15: 2-7**

STRinNGS

- **FRIIS ET AL., FSI GENET 2015; 21: 68-75**

OTHERS



MPS STR SEQUENCES - SOFTWARE



Forensic Science International: Genetics 21 (2016) 68–75



Contents lists available at [ScienceDirect](#)

Forensic Science International: Genetics

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



Research paper

Introduction of the Python script STRinNGS for analysis of STR regions in FASTQ or BAM files and expansion of the Danish STR sequence database to 11 STRs



Susanne L. Friis¹, Anders Buchard¹, Eszter Rockenbauer, Claus Børsting*, Niels Morling

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

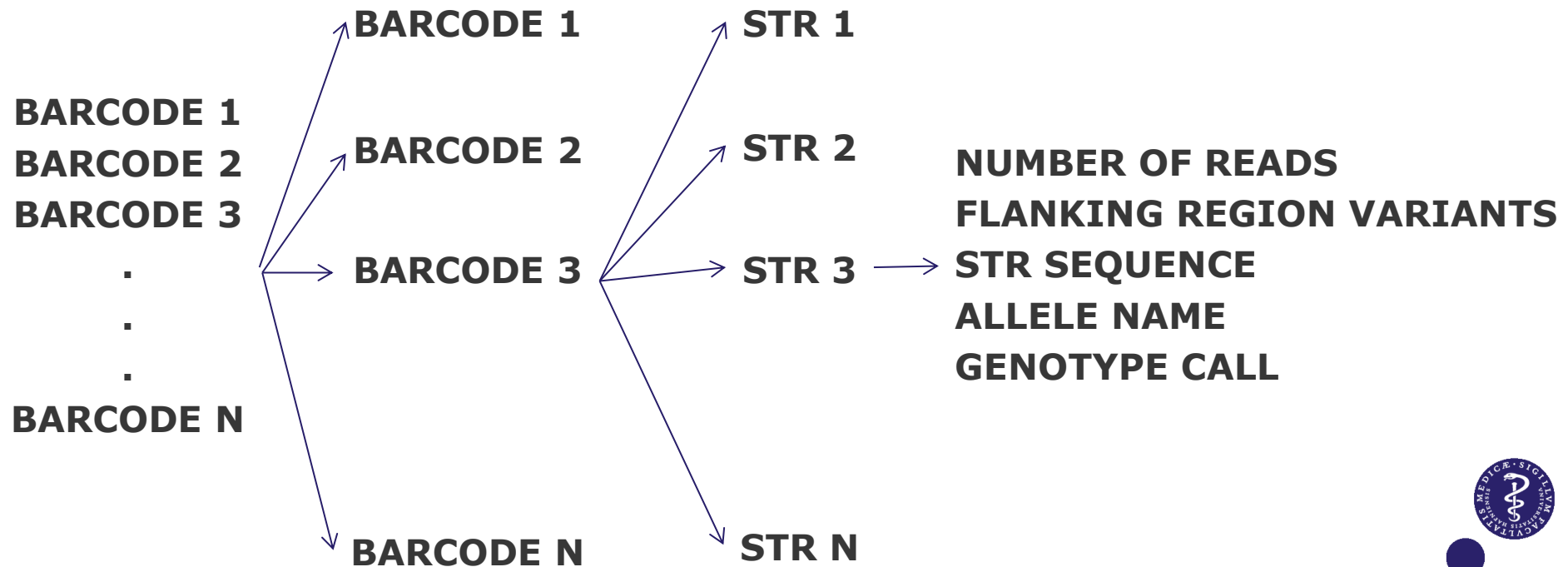


STRinNGS – STR SEQUENCING DATA



PURPOSE

- **AUTOMATED ANALYSIS AND NAMING OF STR SEQUENCES FOR DATABASING**
- **AUTOMATED ANALYSIS OF FLANKING REGIONS (SNP-STR ALLELES)**
- **WORKS WITH FASTQ, BAM FILES, ETC.**



STRinNGS – STR SEQUENCING DATA



NOMENCLATURE

- LOCUS NAME (USED IN FORENSIC GENETICS)
- LENGTH OF REPEAT REGION/LENGTH OF REPEAT UNIT
- SEQUENCE(S) OF SUBREPEAT(S) FOLLOWED BY THE NUMBER OF SUBREPEATS
- VARIATION IN THE FLANKING REGIONS

EXAMPLES

TH01[9]AATG[9]

D5S818[12]AGAT[9]ACAT[1]AGAT[2]RS25768[T]RS73801920[G]

(GELARDI ET AL., FSI GENET 2014; 12: 38-41)

**WILL BE ADJUSTED TO THE NOMENCLATURE OF THE ISFG
RECOMMENDATIONS 2016**



STR NOMENCLATURE

ISFG RECOMMENDATIONS 2016



Forensic Science International: Genetics 22 (2016) 54–63



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Contents lists available at ScienceDirect

Forensic Science International: Genetics

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



Massively parallel sequencing of forensic STRs: Considerations of the DNA commission of the International Society for Forensic Genetics (ISFG) on minimal nomenclature requirements



Walther Parson^{a,b,*}, David Ballard^c, Bruce Budowle^{d,e}, John M. Butler^f,
Katherine B. Gettings^f, Peter Gill^{g,h}, Leonor Gusmão^{i,j,k}, Douglas R. Hares^l, Jodi A. Irwin^l,
Jonathan L. King^d, Peter de Knijff^m, Niels Morlingⁿ, Mechthild Prinz^o,
Peter M. Schneider^p, Christophe Van Neste^q, Sascha Willuweit^r, Christopher Phillips^s



ANCESTRY INFORMATIVE MARKERS (AIMs)



- **LINEAGE MARKERS (HAPLOGROUPS OF MTDNA AND Y-STR)**
- **SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)**
- **INDELS (INSERTION / DELETIONS)**
- **SEVERAL HUNDREDS MARKERS ANALYSED**
- **CONTINENTAL RESOLUTION ESTABLISHED
(EAST ASIA – EUROPE – AFRICA – OCEANIA)**
- **FINER RESOLUTION EMERGING**

Ancestry Analysis in the 11-M Madrid Bomb Attack Investigation

Christopher Phillips^{1,2,*}, Lourdes Prieto³, Manuel Fondevila¹, Antonio Salas¹, Antonio Gómez-Tato⁴, José Álvarez-Dios⁴, Antonio Alonso⁵, Alejandro Blanco-Verea¹, María Brión², Marta Montesino³, Ángel Carracedo^{1,2}, María Victoria Lareu¹

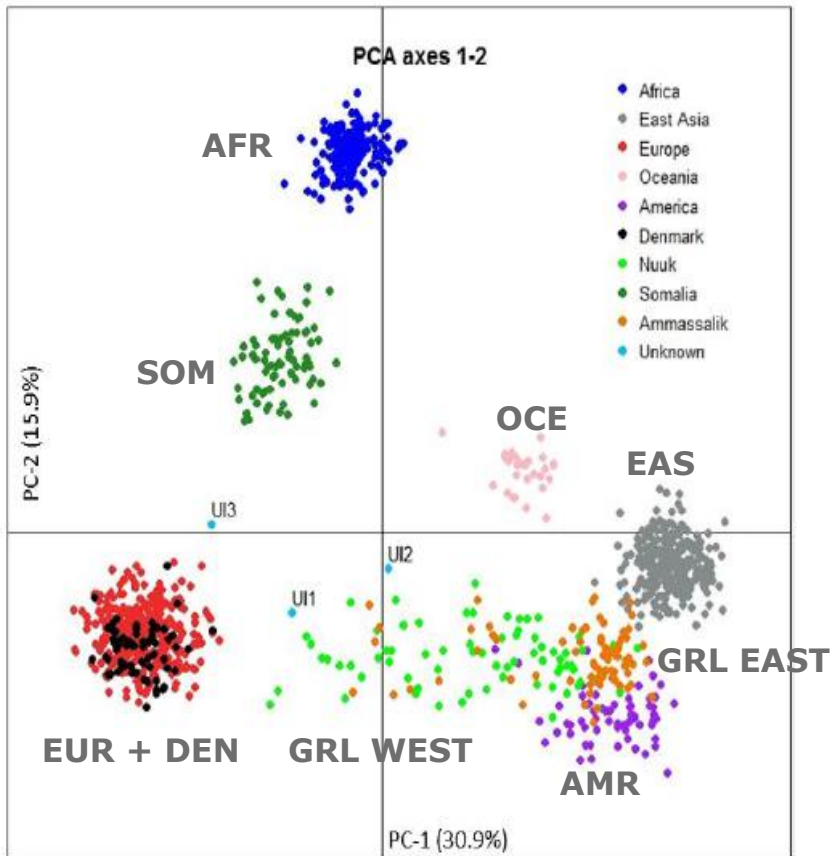
1 Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain, 2 Genomic Medicine Group, CIBERER, University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain, 3 University Institute of Research Police Sciences (IUICP),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



ANCESTRY BY SNP TYPING OF AIMs



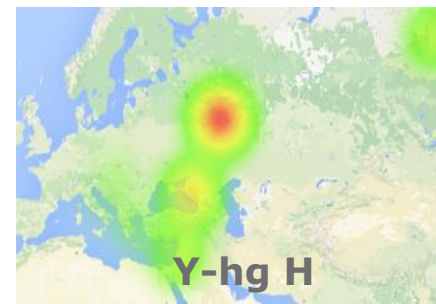
EUROFORGEN SNPs



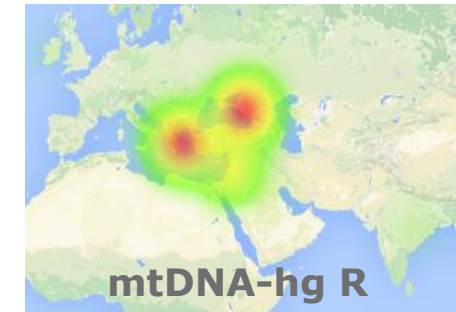
LIFE TECHNOLOGY – MPS SNPs

KIDD SNPs

UI1



SELDIN SNPs



UI3

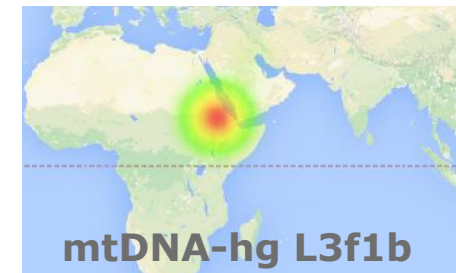
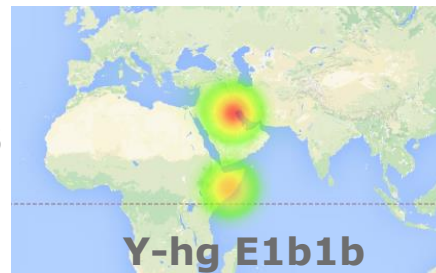
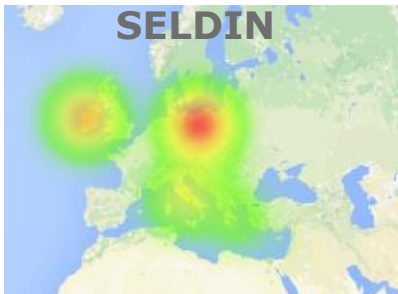
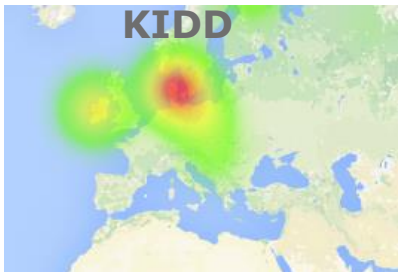


Figure 14: A 2-Dimensional PCA plot of Danes, Somali, Nuuk and Ammassalik Greenlanders and the five continental group training set (AFR, EUR, EASN, AME and OCE). Three unidentified individuals from casework are added to the PCA plots. (Population ID: Unknown, sample ID: UI1, UI2 and UI3). Principal Component 1 and 2 (PC-1 and PC-2) constitutes 30.9 % and 15.9 % of the variation, respectively.



ANCESTRY, TRAITS, AND SNPs

FREDERIK



Phenotype Prediction ILLUMINA

(Sample)

1_FTP

5.0 - Copenhagen A51KG

HAIR COLOR RESULTS



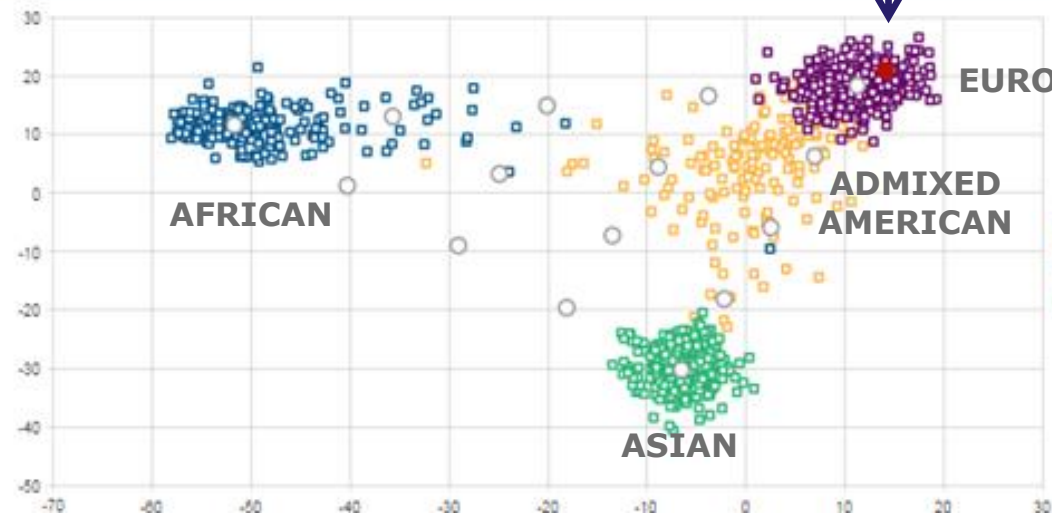
EYE COLOR RESULTS

0.04 Intermediate

0.02 Brown

0.94 Blue

ANCESTRY RESULTS



GREENLANDERS



Rasmussen et al. Nature 2010; 463: 757-62.





RESEARCH ARTICLE

Peopling of the North Circumpolar Region – Insights from Y Chromosome STR and SNP Typing of Greenlanders

Jill Katharina Olofsson*, Vania Pereira, Claus Børsting, Niels Morling

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences,
University of Copenhagen, Copenhagen, Denmark

DOI:10.1371/journal.pone.0116573
January 30, 2015



Y CHROMOSOMES IN GREENLANDERS



Approximately 40% of the analyzed Greenlandic Y chromosomes were of European origin (I-M170, R1a-M513 and R1b-M343)

Y chromosomes of European origin were mainly found in individuals from the west and south coasts of Greenland, which is in agreement with the historic records of the geographic placements of European settlements in Greenland.

Two Inuit Y-chromosomal lineages, Q-M3 (xM19, M194, L663, SA01 and L766) and Q-NWT01 (xM265) were found in 23% and 31% of the male Greenlanders, respectively.



AIMs IN GREENLANDERS



Forensic Science International: Genetics 24 (2016) 60–64



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Research paper

Frequencies of HID-ion ampliseq ancestry panel markers among greenlanders

Gonçalo Espregueira Themudo*, Helle Smidt Mogensen, Claus Børsting, Niels Morling

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark



GREENLANDERS - EUROFORGEN SNPs

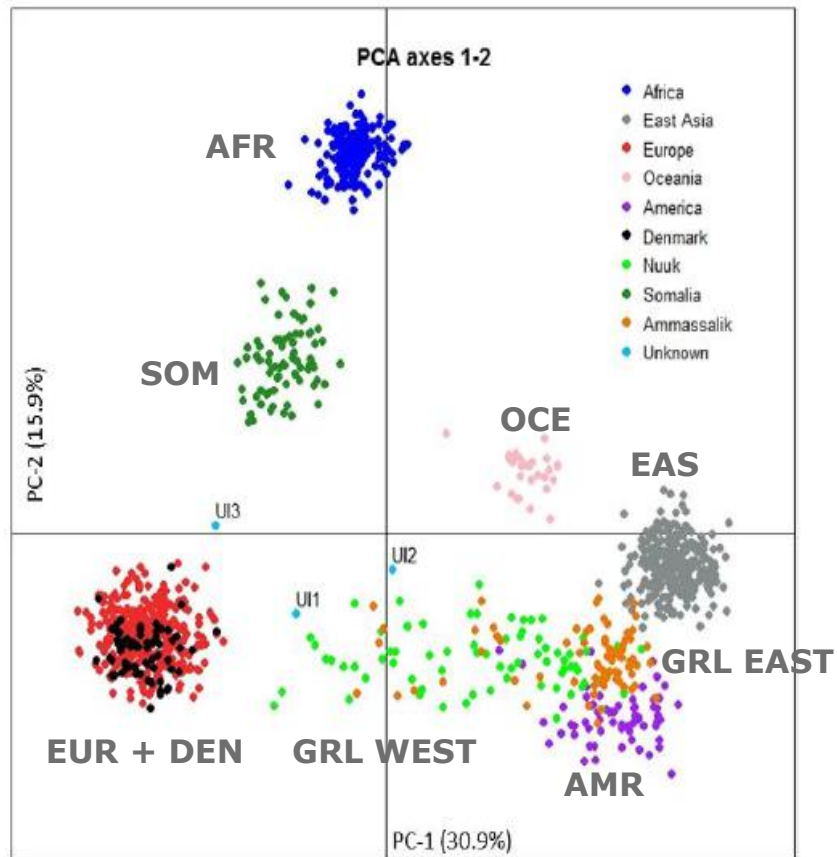


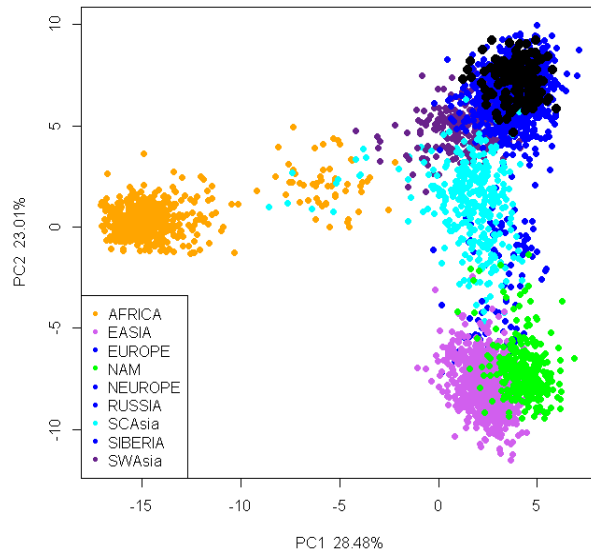
Figure 14: A 2-Dimensional PCA plot of Danes, Somali, Nuuk and Ammassalik Greenlanders and the five continental group training set (AFR, EUR, EASN, AME and OCE). Three unidentified individuals from casework are added to the PCA plots. (Population ID: Unknown, sample ID: UI1, UI2 and UI3). Principal Component 1 and 2 (PC-1 and PC-2) constitutes 30.9 % and 15.9 % of the variation, respectively.



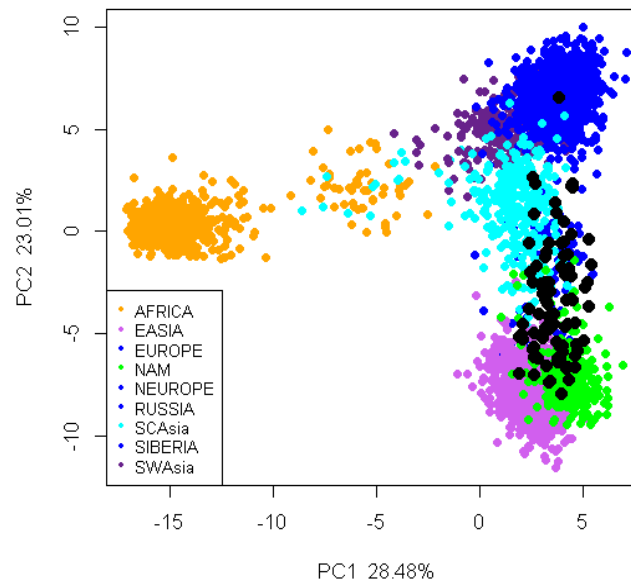
KIDD AIM SNPs (55 - LT ANCESTRY PANEL)



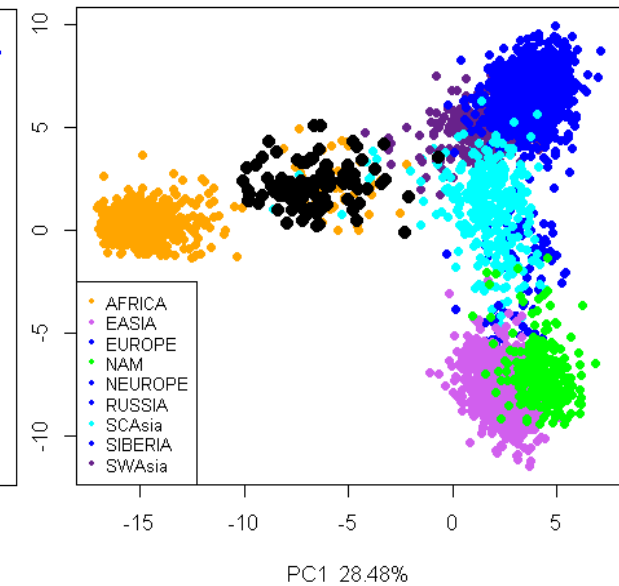
55 AIMs DK



55 AIMs GRL



55 AIMs SOM



GREENLANDERS - KIDD AIM SNPs



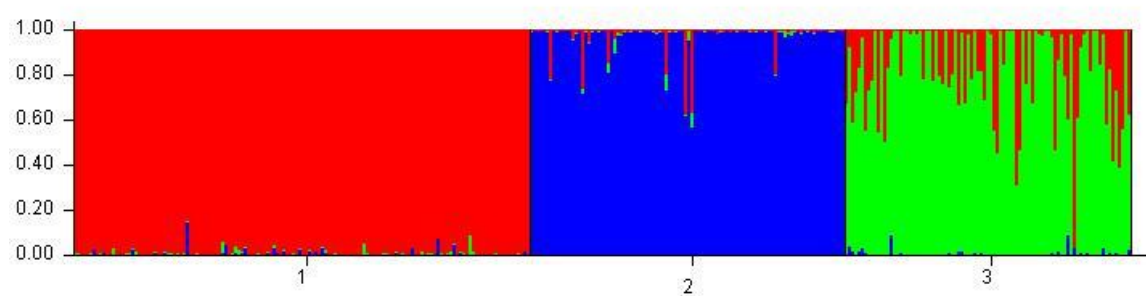
SELDIN SNPs (123 – LT ANCESTRY PANEL) STRUCTURE



DANES
142

SOMALIS
98

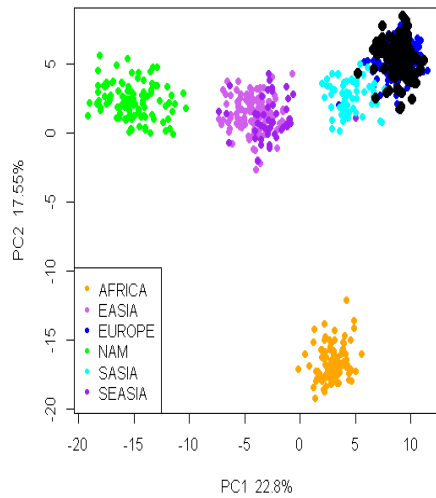
GREENLANDERS
89



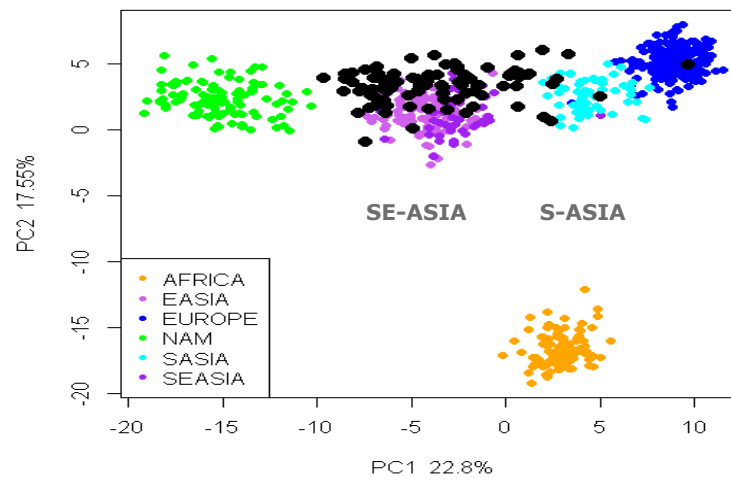
SELDIN SNPs (123 – LT ANCESTRY PANEL)



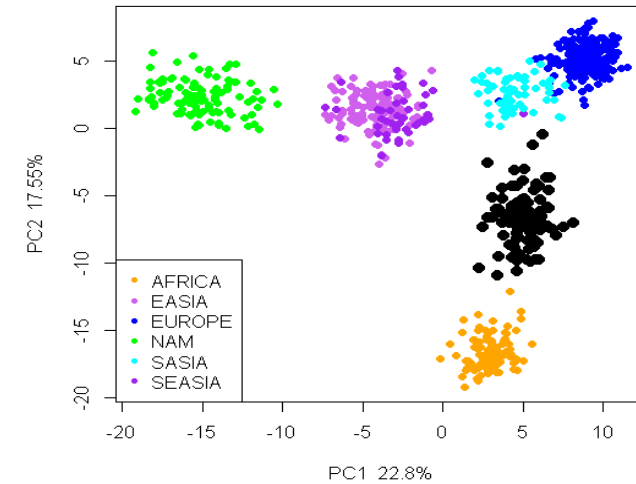
123 AIMs DK



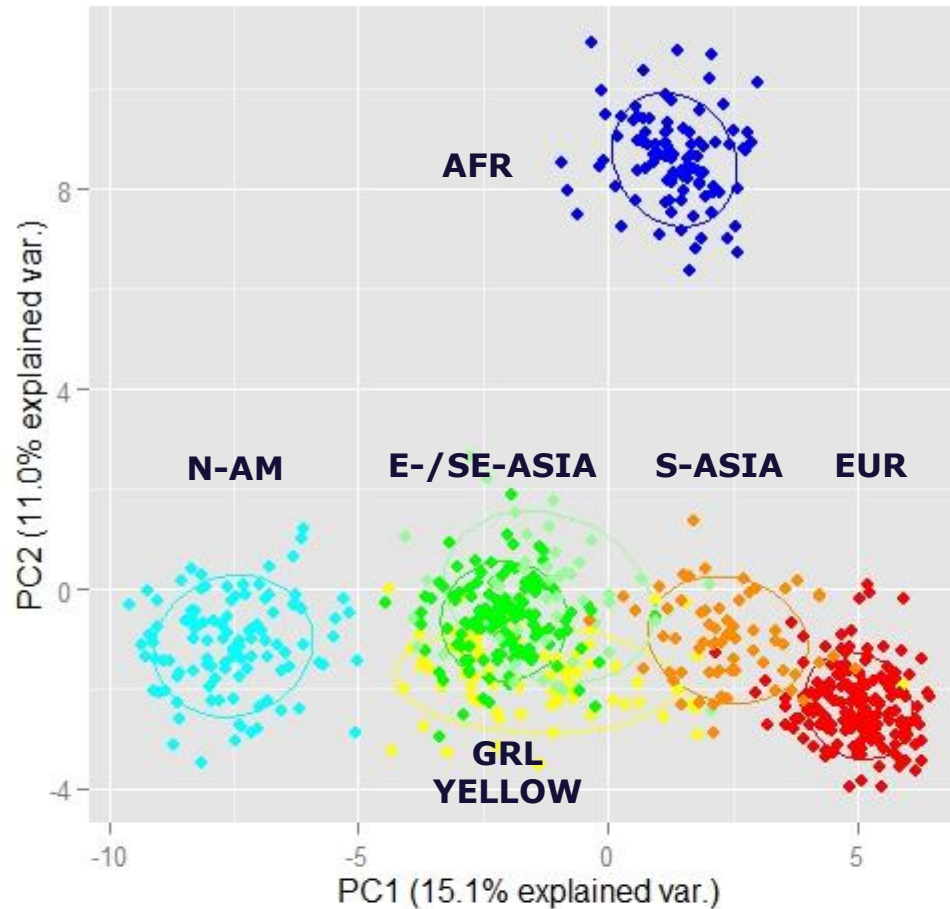
123 AIMs GRL



123 AIMs SOM

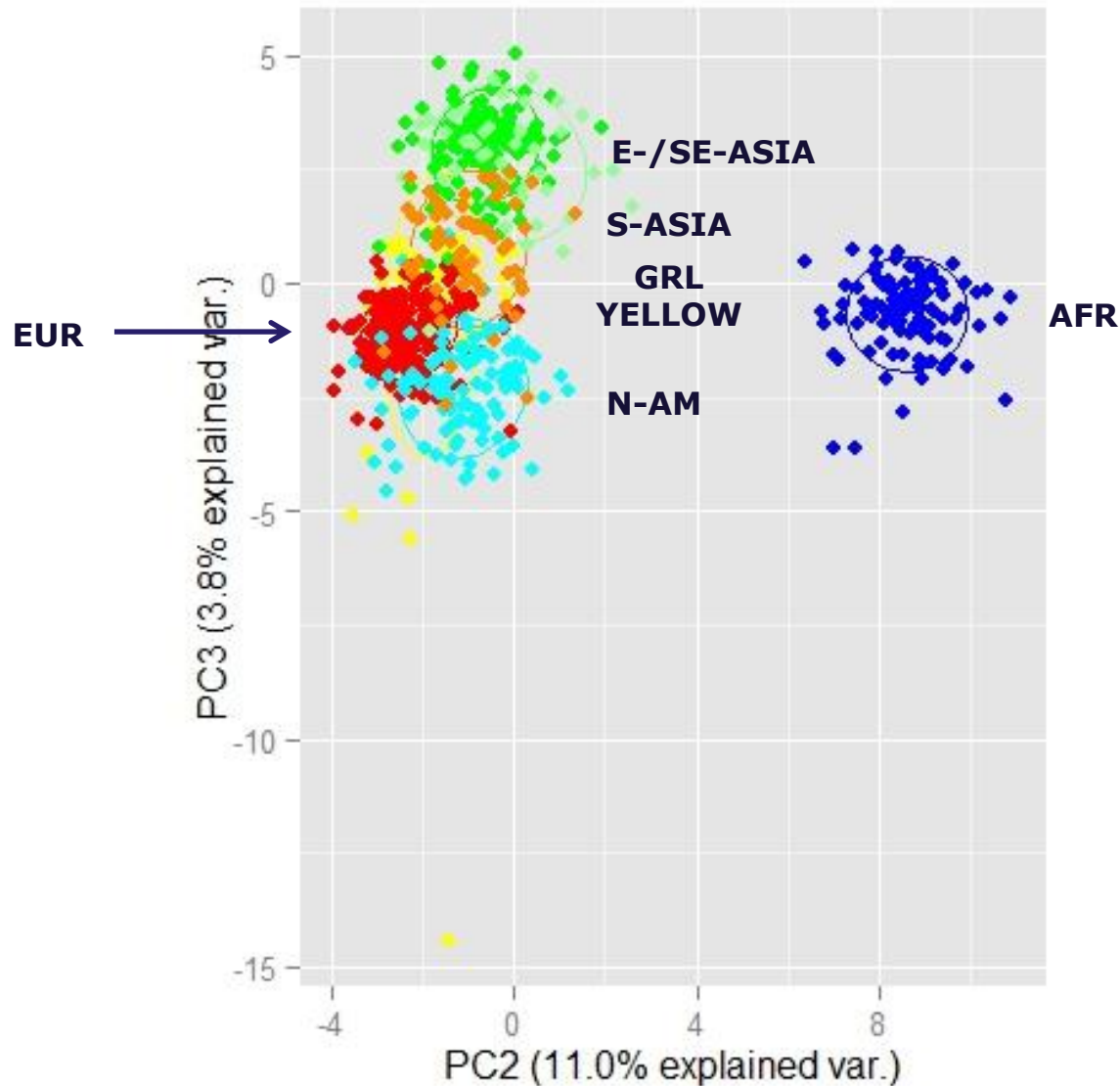


GREENLANDERS – NEW ANALYSIS AFTER HAVING LEARNED THE INUIT FREQUENCIES



groups ◆ AFRICA ◆ EASIA ◆ EUROPE ◆ Greenland ◆ NAM ◆ SASIA ◆ SEASIA

GREENLANDERS – NEW ANALYSIS WITH PC3



GREENLANDERS - NEXT MOVE



SCRUTINIZE DATA FROM CLINICAL INVESTIGATIONS, ETC.

WHOLE GENOME SEQUENCING OF INUIT

NEW SEQUENCING METHODS WITHOUT PCR



THIRD GENERATION DNA SEQUENCING – SINGLE MOLECULES

PACBIO *RS* – PACIFIC BIOSYSTEMS

SINGLE MOLECULE REAL TIME SEQUENCING

<http://www.pacificbiosciences.com/>



OXFORD NANOPORE TECHNOLOGIES

GridION – MinION SYSTEM

<http://www.nanoporetech.com/technology>



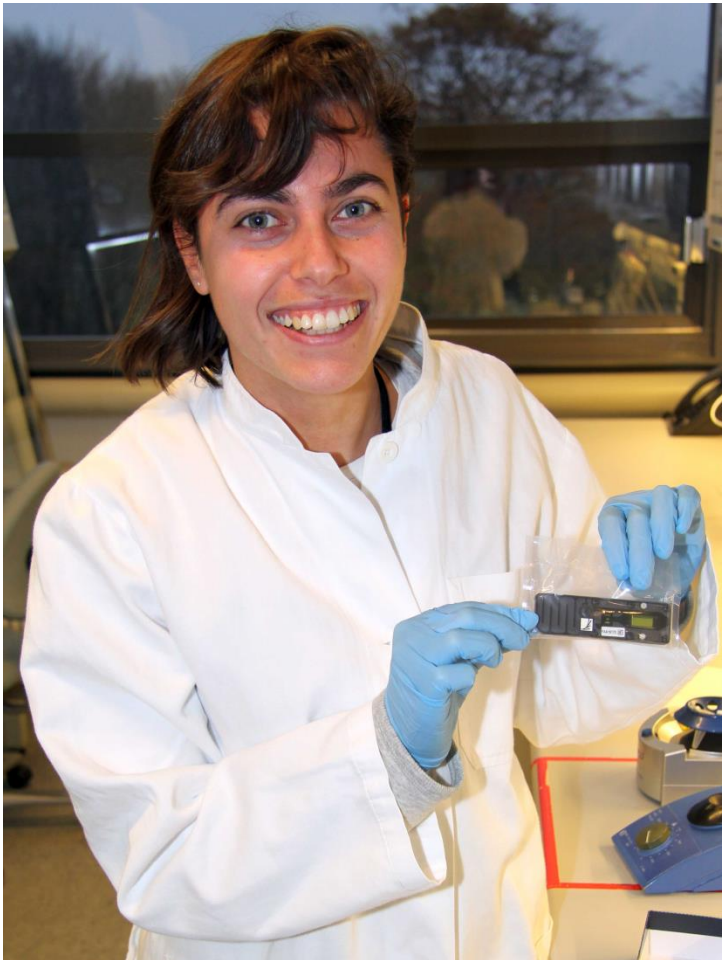
HELICOS GENETIC ANALYSIS SYSTEM

HELISCOPE SINGLE MOLECULE SEQUENCER

<http://www.helicosbio.com>



MinION – Oxford NANOPORE Technologies



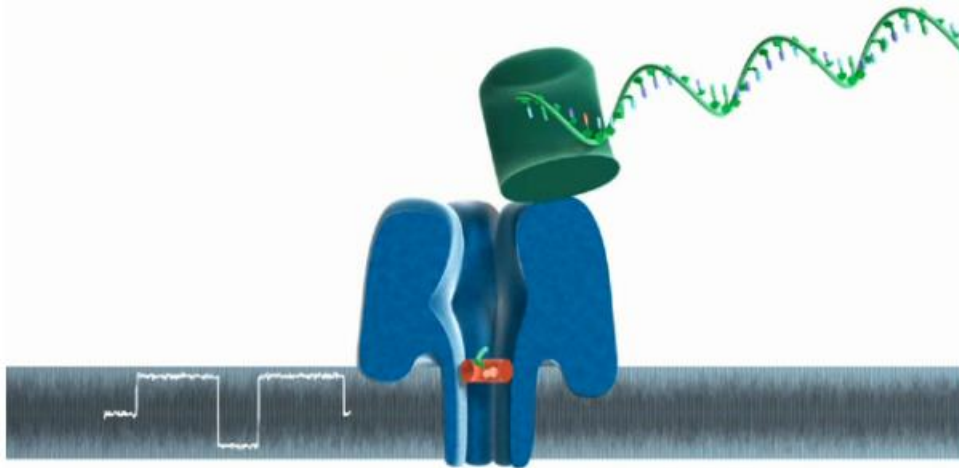
<https://nanoporetech.com/technology/the-minion-device-a-miniaturised-sensing-system/the-minion-device-a-miniaturised-sensing-system>



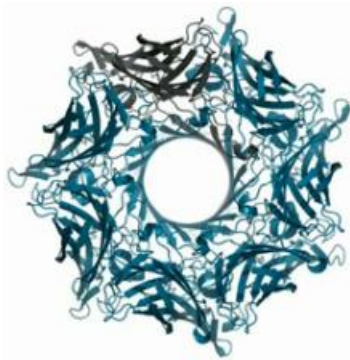
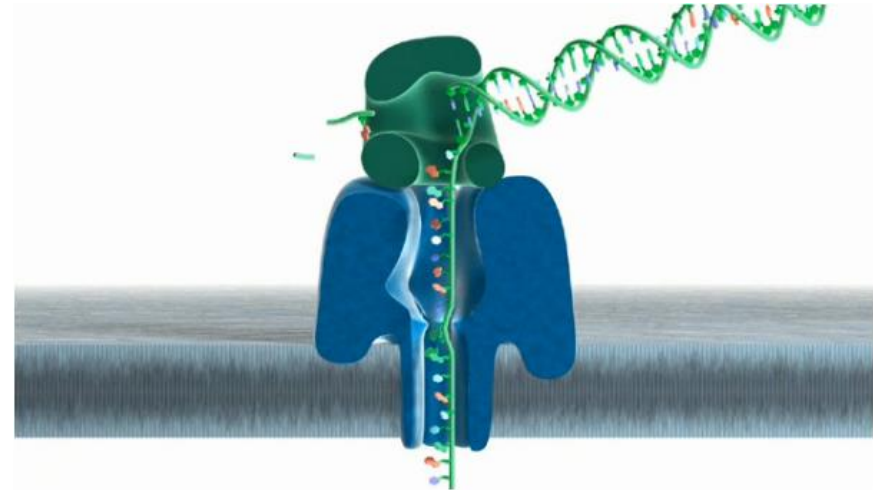
OXFORD NANOPORE TECHNOLOGIES



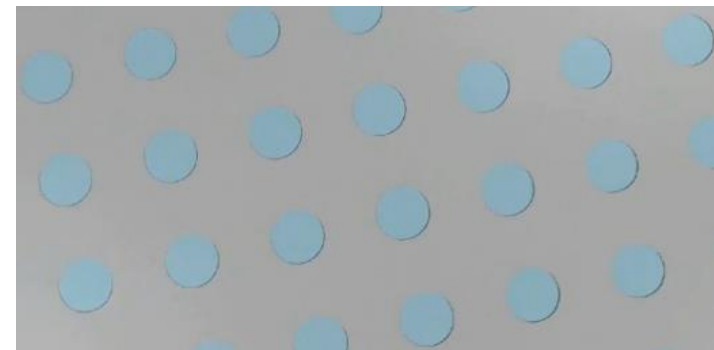
EXONUCLEASE SEQUENCING



STRAND SEQUENCING



α -HEMOLYSIN



ARRAY WITH MULTIPLE UNITS

FORENSIC GENETIC MPS CHALLENGES



- **AUTOMATION OF**
 - **LABORATORY PROCEDURES**
 - **DATA ANALYSIS – INCLUDE FLANKING REGIONS (OPTION)**
- **USE INTERNATIONAL NOMENCLATURE**
- **ESTABLISH MPS POPULATION DATABASES FOR STR, SNPs, etc.**
- **DEVELOP RELEVANT MPS KITS**
 - **SMALL NUMBERS OF MARKERS FOR SCREENING AND ADDITIONAL TESTING**
 - **LARGE NUMBERS OF MARKERS ± PHENOTYPIC AND/OR ANCESTRY MARKERS**
 - **PHENOTYPE KITS**
 - **AIMs KITS**
- **IMPLEMENT MPS IN CASEWORK**



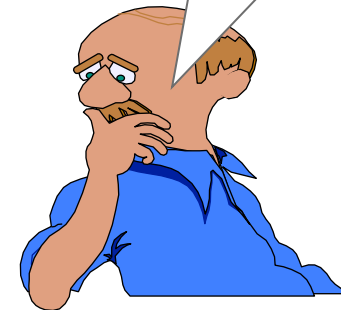
NEW DNA SEQUENCING METHODS IN FORENSIC GENETICS



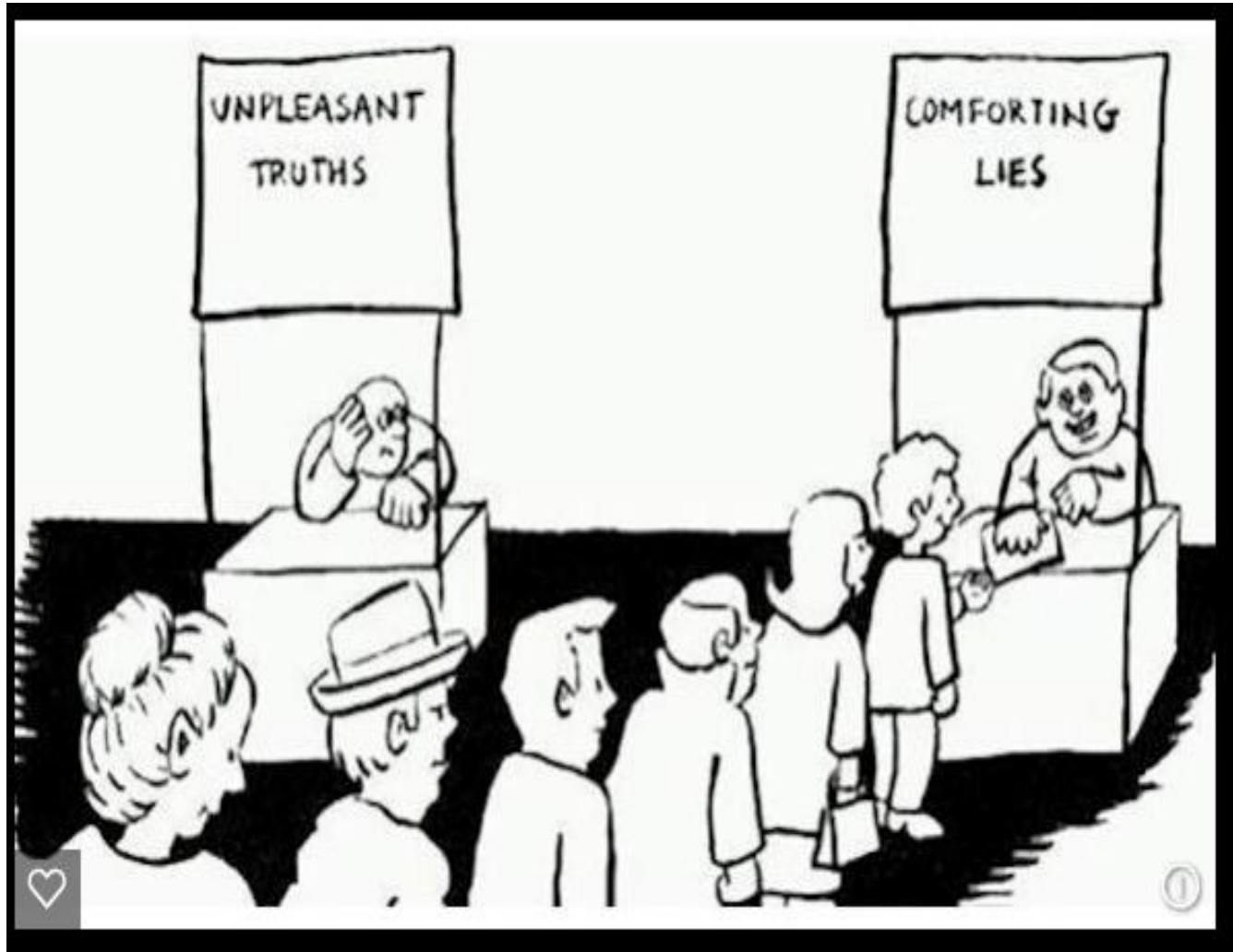
CRITICAL TESTING OF

- RELIABILITY
- SENSITIVITY
- SPECIFICITY
- RISK OF CONTAMINATION
- RESOLUTION OF DNA MIXTURES
- WEIGHT OF THE EVIDENCE
- etc.

*Be careful with
new technologies
in forensic genetics*



WHAT DO WE WANT?



KINDLY PROVIDED BY CECILIA CROUSE, PALM BEACH SHERIFF'S CRIME LAB



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and
Enthusiastic students and guests from all over the world



THANK YOU FOR YOUR ATTENTION TO A VIKING

