



Faculty of Health and Medical Sciences

Analysis of next generation sequencing data of 9 STRs and Amelogenin in 202 Somalis using the STRinNGS v1.0 software

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Aims



- To sequence samples from unrelated Somalis
- To test the performance of an NGS assay
- To use STRinNGS v1.0 software for allele calling
- To compare results with a previous study*
- To find population specific markers
- To calculate typical paternity indexes

*Friis *et al.*, (2016) FSI genet. 21, 68-75



STR sequencing using the Ion PGM



Ion Torrent™ HID STR 10-plex assay

- 10-plex STR kit with forensic core STRs designed for the Ion PGM
- Amplicon sizes 70-170 bp
 - AMEL
 - CSF1PO
 - D16S539
 - D3S1358
 - D5S818
 - D7S820
 - D8S1179
 - TH01
 - TPOX
 - vWA

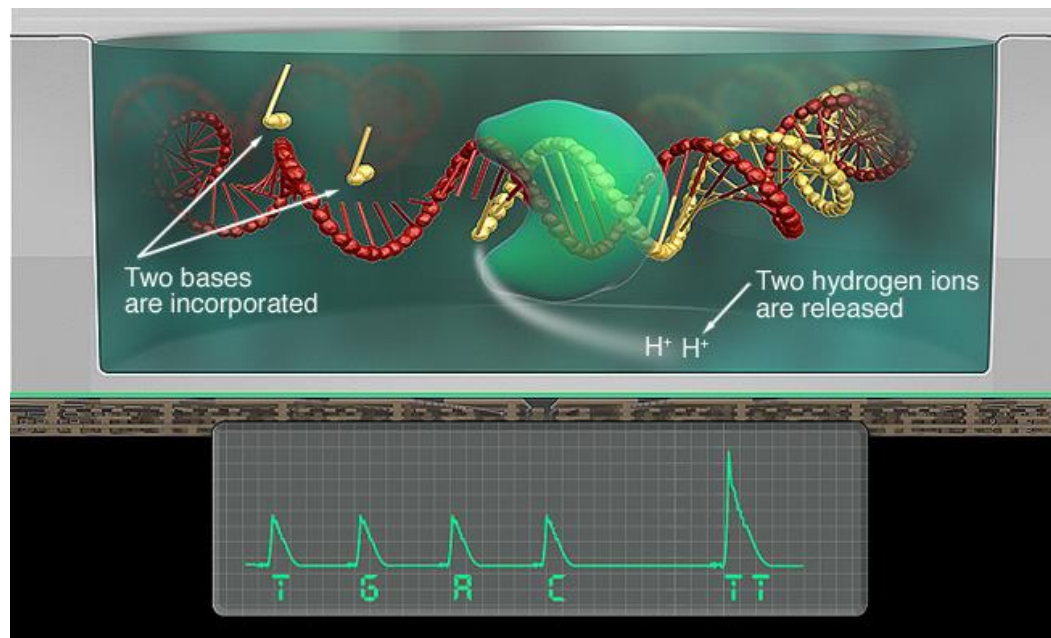




STR sequencing using the Ion PGM

The Ion Torrent™ HID STR 10-plex assay

- Amplification with multiplex PCR
- Ligation of barcoded adaptors
- Quantification, emPCR and sequencing*



*Semi-conductor technology (Rothberg et al. 2011)

Dias 4



STR sequencing using the Ion PGM



Samples

- 202 unrelated Somalis from paternity and immigration cases in Denmark

Data analysis

- STRinNGS v1.0 software

Control

- ISO17025 accredited STR typing (AmpFLSTR[®] Identifiler[®] PCR Amplification Kit)



Data analysis and control



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- Control
 - 4 samples where allele D7S820 10.1 (PCR-CE) had a sequence with just 10 repeats with NGS
 - 101 'homozygous' PCR-CE genotypes (5.5% of total) were heterozygous with PCR-NGS
- SNPs
 - 5 SNPs in the flanking regions
- Allele distribution
 - 13 alleles were observed in Somalis but not in Danes*
 - 16 alleles were observed in Danes but not in Somalis*
- New alleles
 - 9 novel alleles not reported in STRbase or in previous study*

*Friis *et al.*, (2016) FSI genet. 21, 68-75.



STRinNGS v1.0 software



In-house developed application (Python script)*

- Determines flanking regions
- Calls SNP-STR haplotypes
- Plots the predicted alleles
- Names alleles, e.g. "D7S820[10]GATA[10]rs16887642[C]"**
- Available on request
- New version v2.0 is under development (more STRs, better genotype prediction)

*Friis *et al.*, (2016) FSI genet. 21, 68-75.

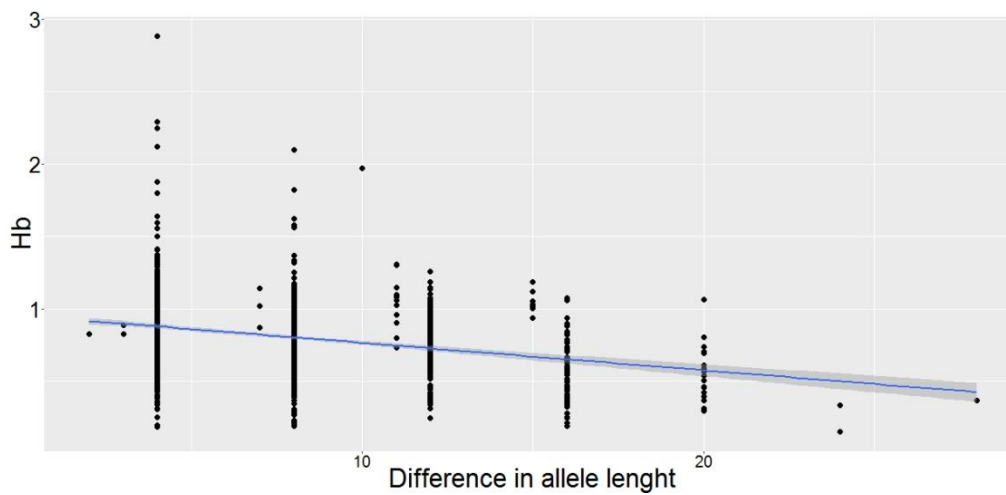
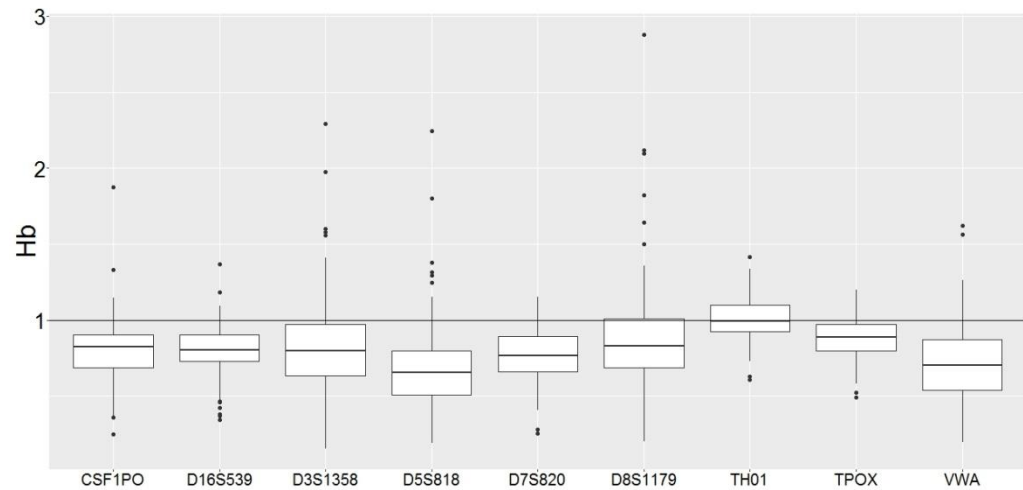
**Gelardi *et al.*, Forensic Sci. Int. Genet. (2014) 12, 38-41.



Heterozygote balance



Heterozygote balance
 $H_b = \text{Reads}(\text{HMW}/\text{LMW})$



Allele diversity in the 9 STRs

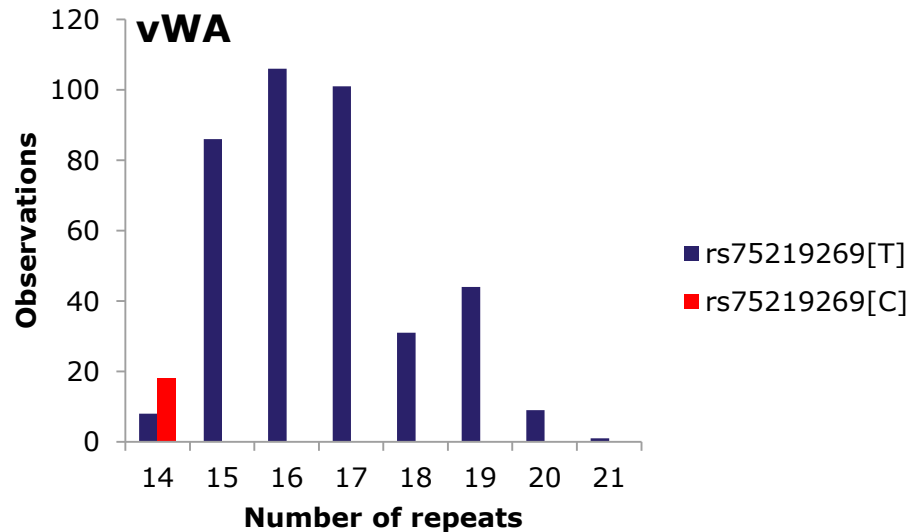
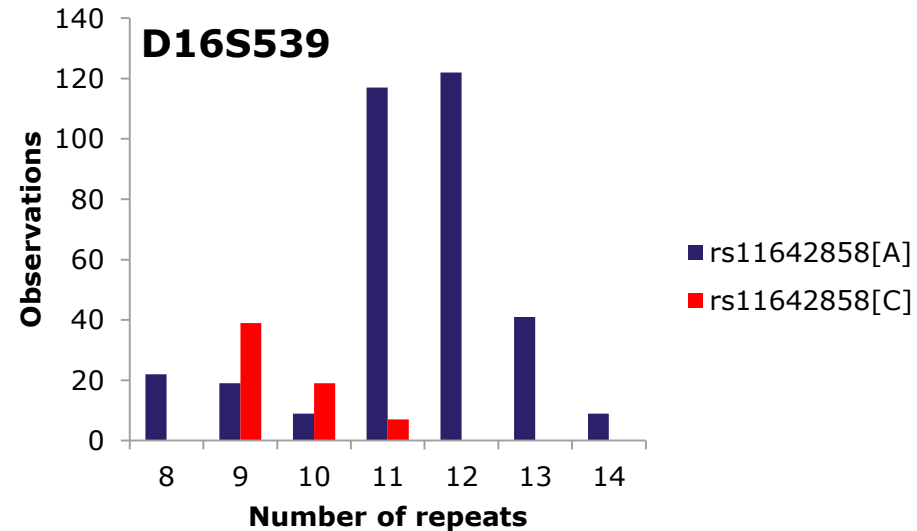
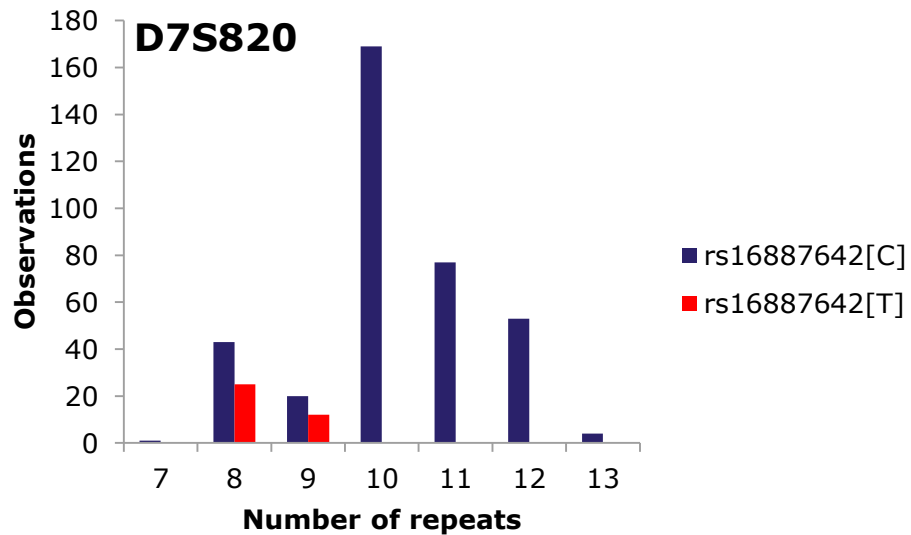


STR locus	PCR-CE	NGS	SNP
TH01	6	6	-
TPOX	6	6	-
CSF1PO	8	9	-
D7S820	8	9	rs16887642
D16S539	7	10	rs11642858
D5S818	8	17	rs25768 & rs73801920
D8S1179	10	22	-
D3S1358	8	18	-
vWA	8	19	rs75219269

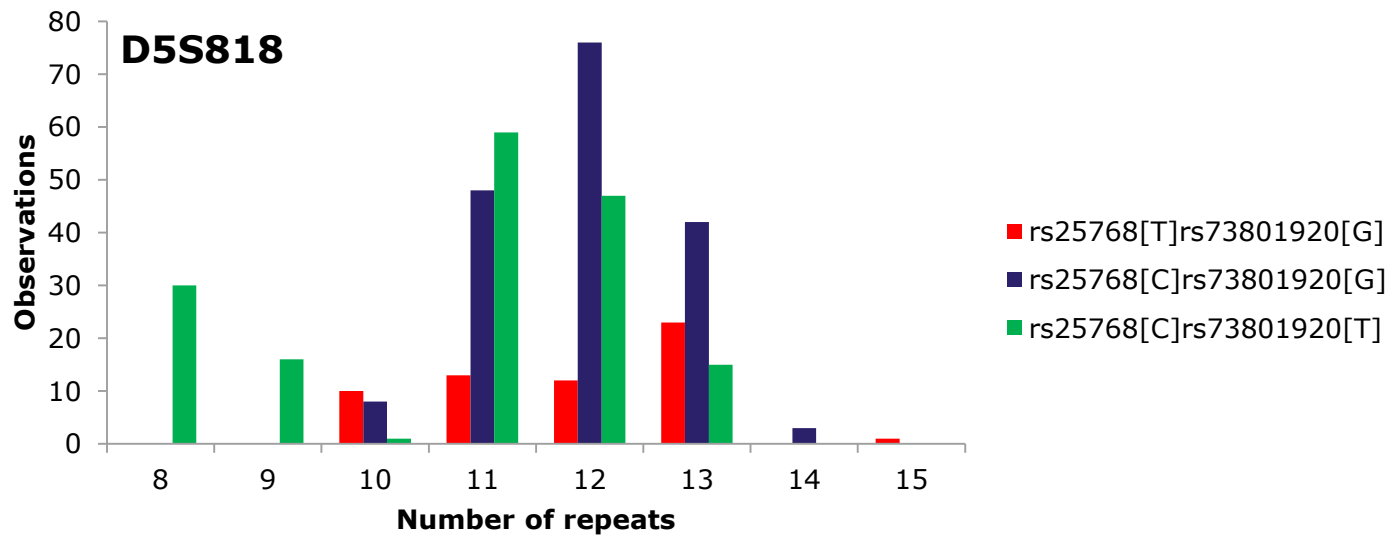




SNP-STR alleles in Somalis

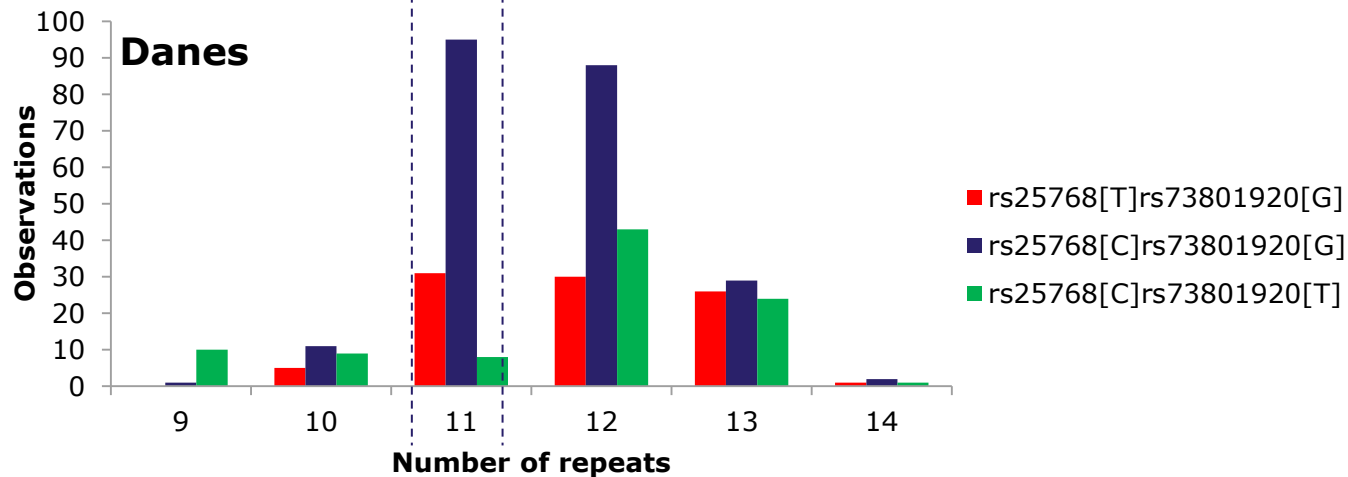
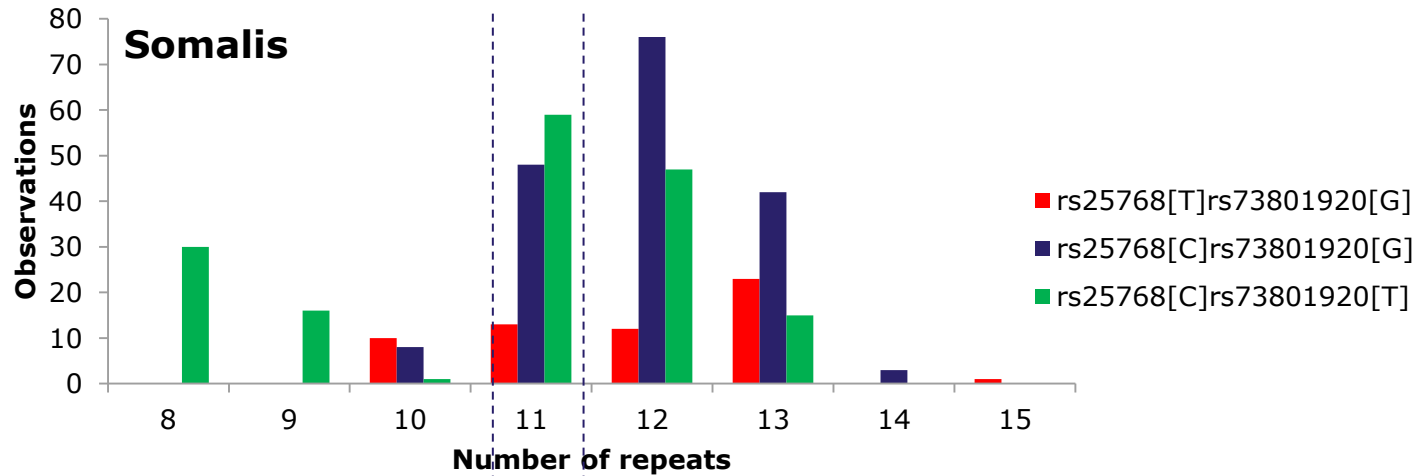


SNP-STR alleles in Somalis





D5S818 in Somalis and Danes



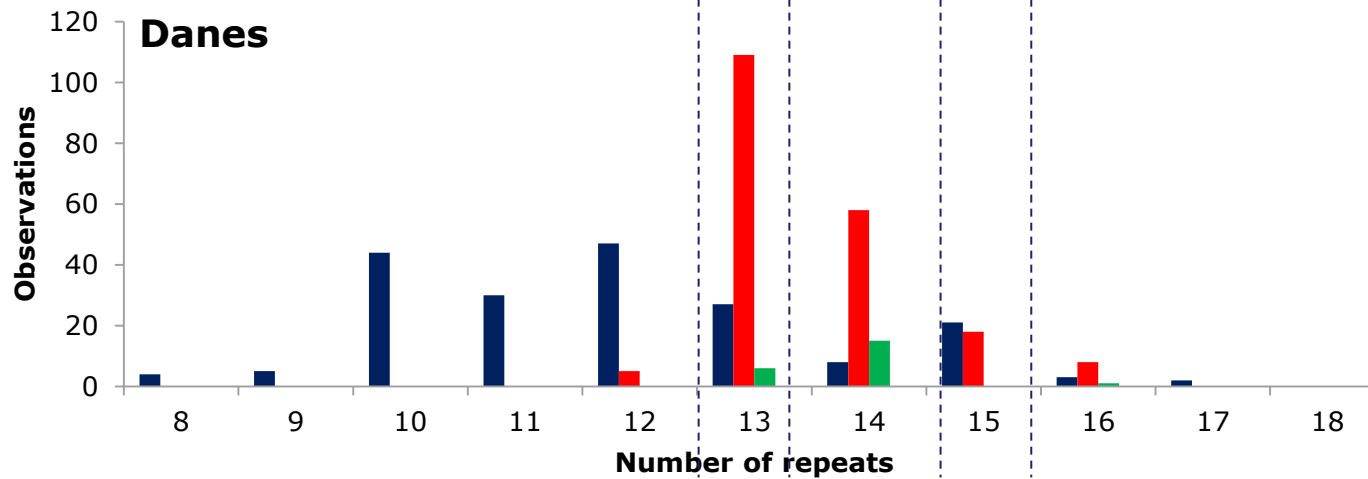
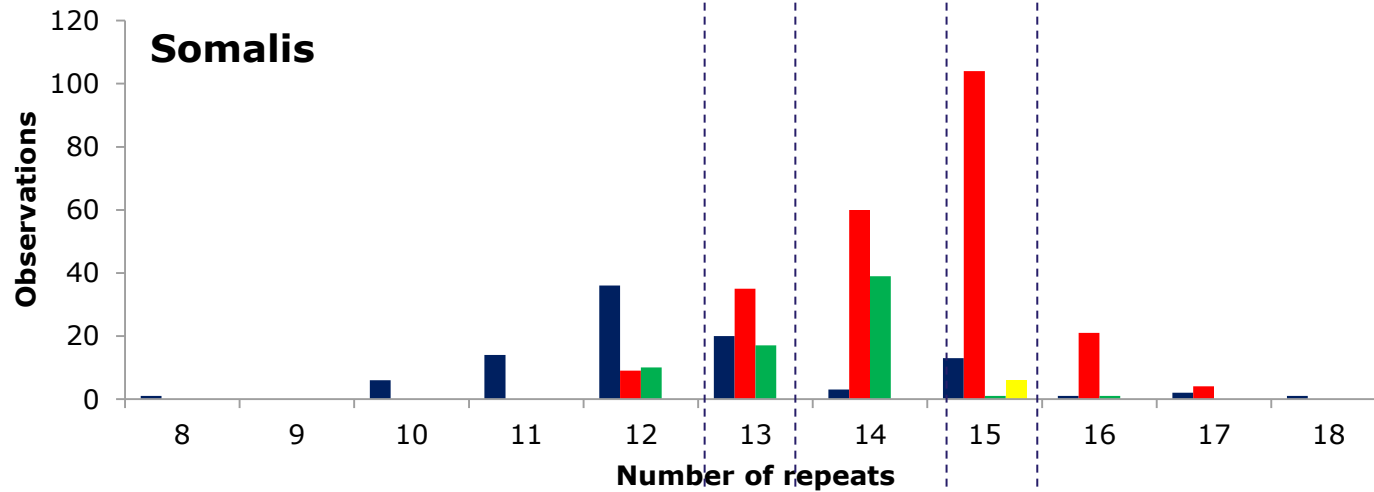
Allele diversity in the 9 STRs



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CSF1PO	8	9	-
D7S820	8	9	rs16887642
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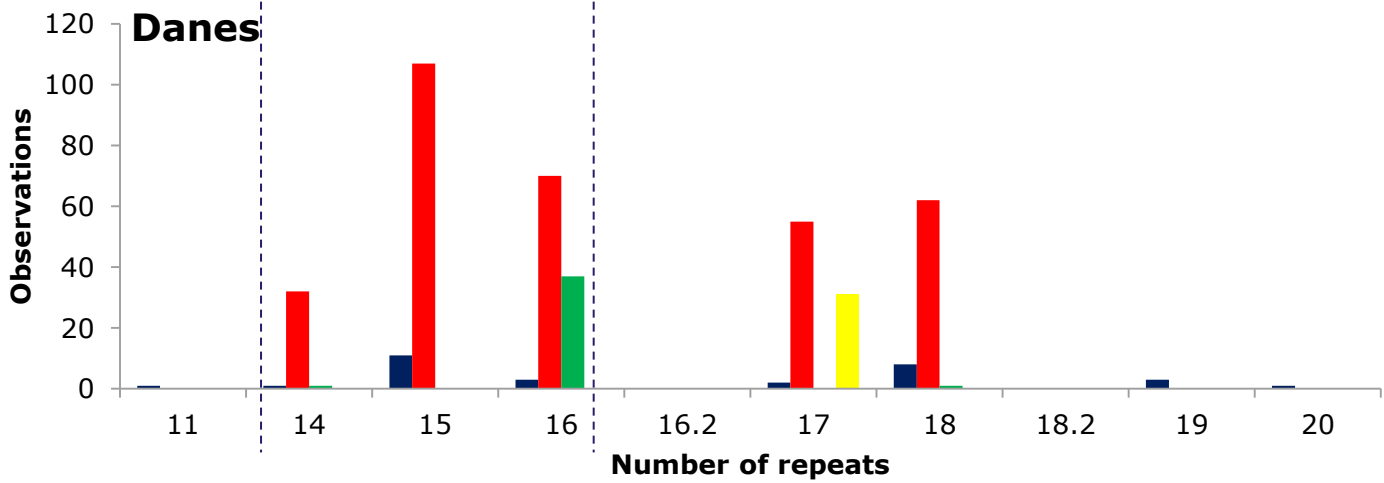
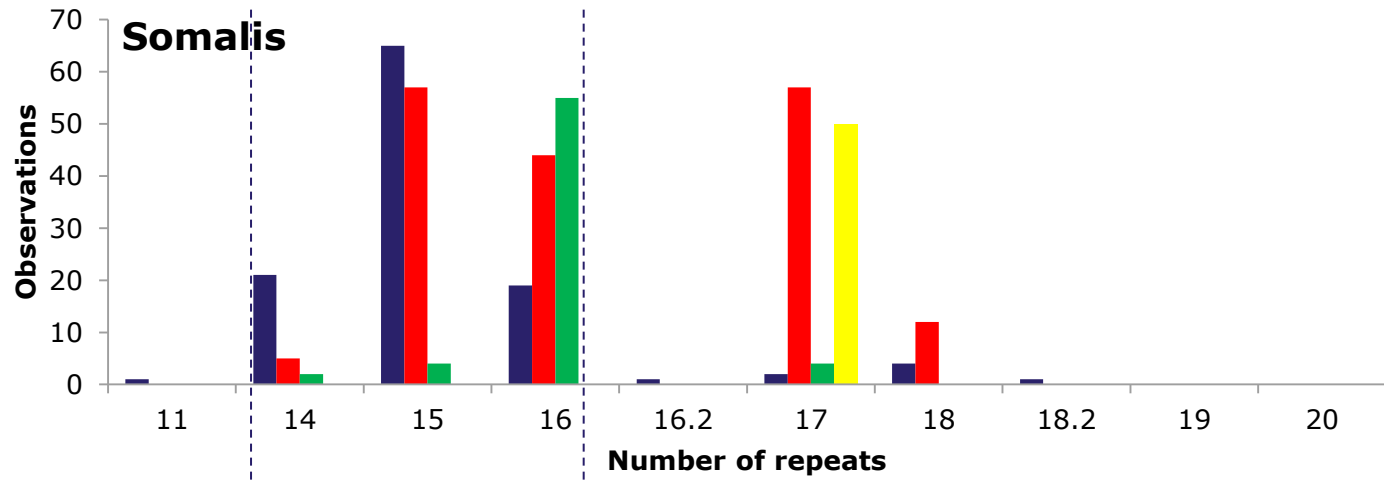


D8S1179



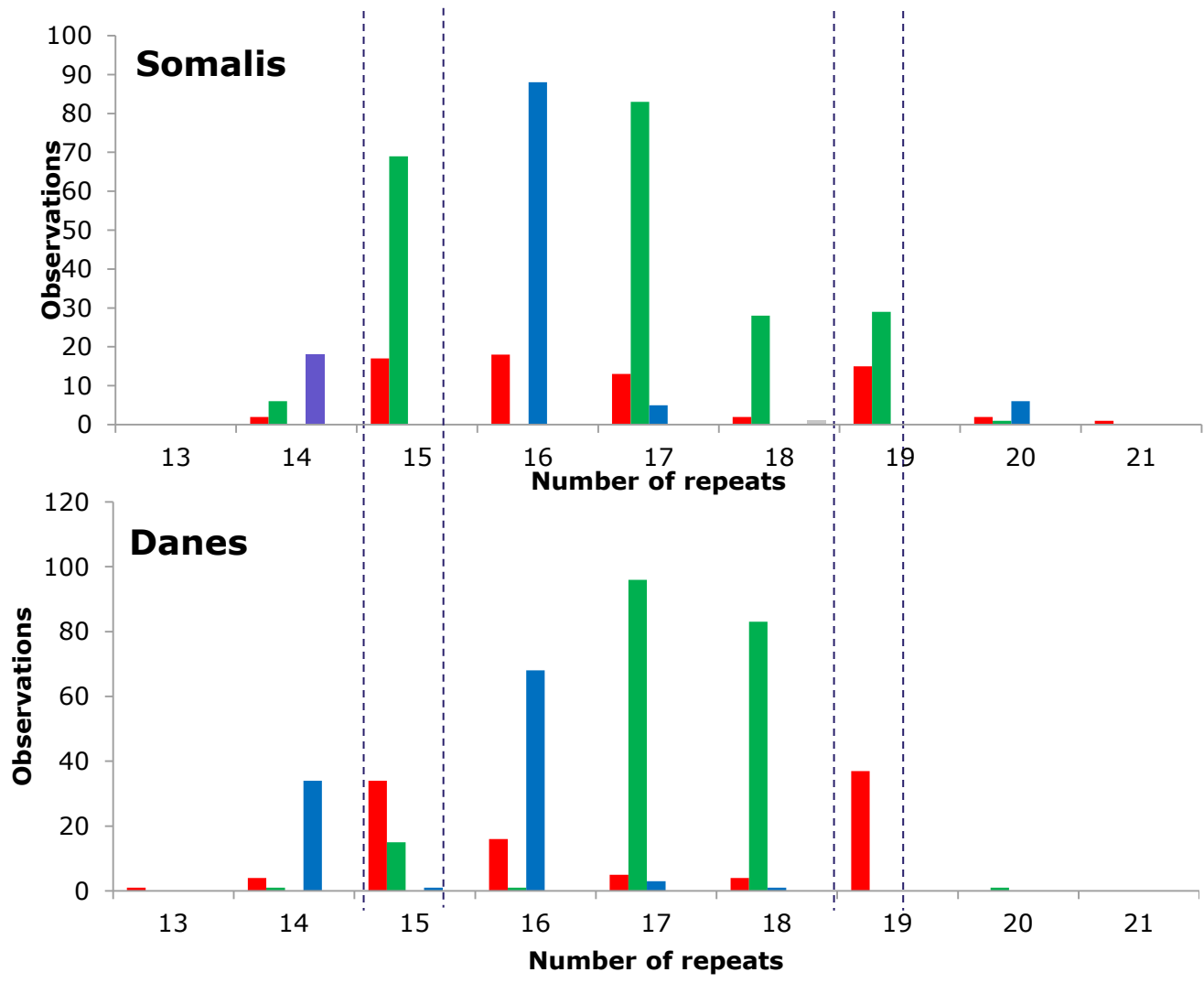


D3S1358





vWA





Forensic statistic parameters

Forensic statistics – Somalis

	PCR-CE	NGS
Mean match probability	5,88E-10	4,17E-12
Mean power of discrimination	>99,9999	>99,9999
PI geometric mean (trio)	7.150	125.000
PI geometric mean (duo)	331	3.080

Forensic statistics – Danes*

	PCR-CE	NGS
Mean match probability	5,88E-10	1,11E-11
Mean power of discrimination	>99,9999	>99,9999
PI geometric mean (trio)	6.130	77.400
PI geometric mean (duo)	293	2.130

*Friis *et al.*, (2016) FSI genet. 21, 68-75.



Conclusions



- Ion Torrent™ STR 10plex assay performed well
- STRinNGS v1.0 software gave correct allele calls
- Alleles with not yet reported sequence compositions were identified
- Population specific markers were identified
- Improved the power of genetic evidence in paternity cases
- Applied Biosystems™ Precision ID GlobalFiler™ NGS STR Panel (21 STRs, AMEL, Y-indel)



Acknowledgement



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Thank you for your attention

