Faculty of Health and Medical Sciences

Sequence variation in the short tandem repeat system SE33 discovered by next generation sequencing

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Aims

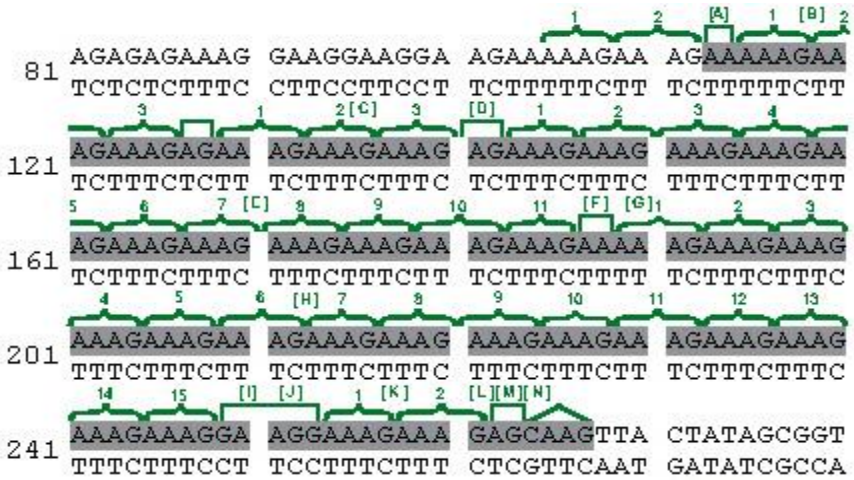
- Sequence SE33 with an NGS assay
- Use Roche Junior Platform
- Search for sequence variation
- Identify new allele variants
- Find population specific alleles
- Test usefulness of higher allele diversity in paternity cases

SE33

- Core locus in the European Standard Set of STRs
- Included in several commercial STR typing kits
  - E.g. NGM Select, ESI17
- Complex and highly variable sequence
- Consisting of four nucleotide AAAG units interrupted by AA and AG units
- Wide span of allele length variation (3 to 39 four repeat units)

Challenges with SE33

- No commercially available NGS kits to sequence SE33
- Several polyA stretches difficult to sequence with presently used NGS techniques
- Alleles can be up to 343 bp ↔ most NGS machines produce ≤ 300 bp reads



GS Junior 454

- Pyrosequencing technology
- 200 cycles of nucleotide addition (A, T, C and G in fixed order)
- Read length ≤ 800 bases
- Accuracy of 99% for reads of ≤ 400bp
- Several successful STR sequencing studies*

*Fordyce et al. 2011, Van Neste et al. 2012, Dalsgaard et al. 2014; Rockenbauer et al. 2014; Gelardi et al. 2014; Scheible et al. 2014

Samples

Samples

203 samples from Danes(144), Somalis(81) and Greenlanders(58)

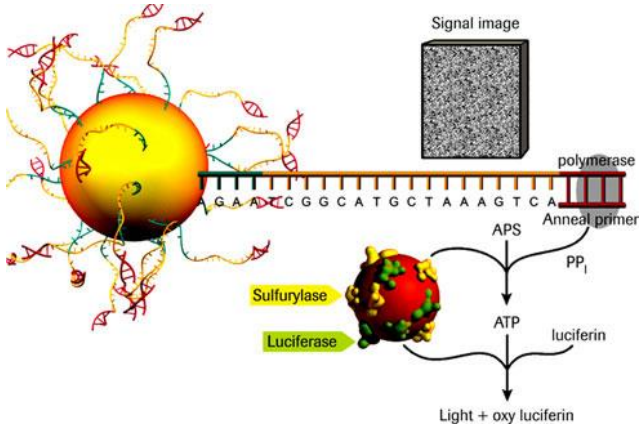
- 188 from unrelated individuals
- 5 trios with an unsolved genetic inconsistency in SE33 between parent and child

Control

- ISO17025 accredited STR typing (AmpFLSTR® NGMSelect® PCR Amplification Kit)

STR sequencing

- Amplicon sequencing with Multiplex IDentifiers (MIDs) in the PCR primer sequence
- Quantification and pooling amplicons into a library
- emPCR and sequencing



Data analysis

- Generally low sequencing quality
- Standard filters were switched off to get more reads in output file
- "Filters_off_amplicons"-pipeline*:
  - Sorting by MIDs
  - Filtering by flanking sequences
- Both flanking sequence must be present (optional)
- Alignment in BioEdit (Ibis Biosciences)

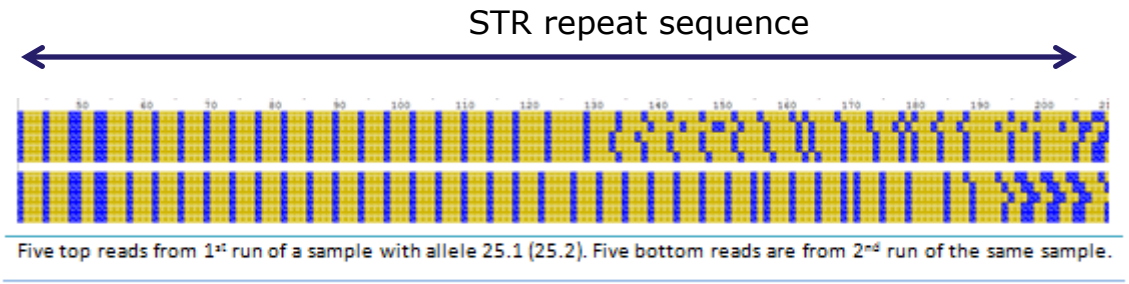
*in-house Python-based algorithm

Results of allele calling

- Total sample coverage 4,643 (before) and 318 (after filtering)
- 10-20% forward 80-90% reverse strand reads
- 57 samples were sequenced twice
- Low-quality samples were reanalysed with only one flanking sequence
- 295 correct allele calls (from 394 expected alleles)
- ~20% called with an insecurity of +/-1bp
- Most correctly called alleles had ≤ 30 repeats (~300bp incl. flanks)

Results of allele calling

- Uncertain calls:
  - Only few reads for long alleles (>30 repeats)
  - Uncertain number of ”A’s” (”T” in reverse strand read)
- Undetermined alleles:
  - lack of sequencing reads
  - inconsistent reads
  - reads deviating from PCR-CE



Five top reads from 1st run of a sample with allele 25.1 (25.2). Five bottom reads are from 2nd run of the same sample.

Results of allele calling

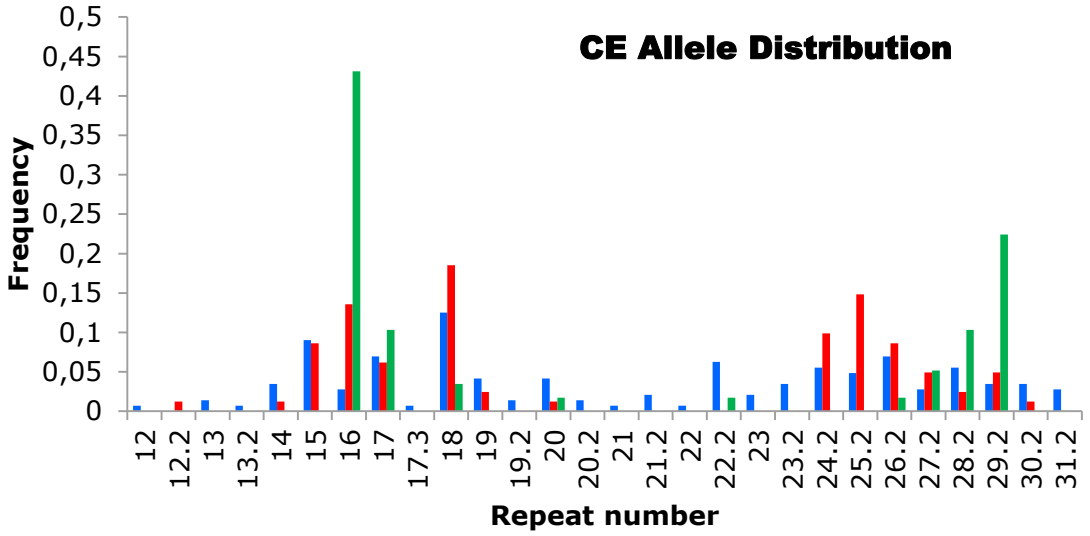
- High increase in allele variation
- 27 novel alleles not reported in STRbase
- Raised the power of discrimination by 282% compared to CE
- Identified the mutated allele in 3 out of 5 family trios

Novel alleles

Table 19 – New Alleles		
1	SE33[13]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{13}$G[AAAG]$_3$AG
2	SE33[16]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{16}$G[AAAG]$_3$AG (4bp del in flanking region)
3	SE33[17]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{17}$G[AAAG]$_3$AG (4bp del in flanking region)
4	SE33[17.3]	[AAAG]$_2$AG[AAAG]$_3$---[AAAG]$_{15}$G[AAAG]$_3$G[AAAG]$_3$AG
5	SE33[18]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{17}$GAAGG[AAAG]$_3$AG
6	SE33[21.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_8$AA[AAAG]$_{13}$GAAGG[AAAG]$_2$AG
7	SE33[21.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{12}$AA[AAAG]$_9$GAAGG[AAAG]$_2$AG
8	SE33[23]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{23}$G[AAAG]$_3$AG
9	SE33[24.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_9$A**A**[AAAG]$_{15}$GAAGG[AAAG]$_2$AG
10	SE33[25.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_7$A**A**[AAAG]$_{18}$GAAGG[AAAG]$_2$AG
11	SE33[25.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_8$AA[AAAG]$_{17}$GAAGG[AAAG]$_2$AG
12	SE33[25.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{13}$A**A**[AAAG]$_{12}$GAAGG[AAAG]$_2$AG
13	SE33[26.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_7$A**A**[AAAG]$_{19}$GAAGG[AAAG]$_2$AG
14	SE33[26.2]	[AAAG]$_2$AG[AAAG]$_3$AAG[AAAG]$_{10}$A[AAAG]$_{17}$GG----[AAAG]$_2$AG
15	SE33[26.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{12}$A**A**[AAAG]$_{14}$GAAGG[AAAG]$_2$AG
16	SE33[27.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_5$A[AAAG]$_{20}$[GAAG]$_3$G[AAAG]$_2$AG
17	SE33[27.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_5$A[AAAG]$_{22}$GAAGG[AAAG]$_2$AG
18	SE33[27.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_7$A**A**[AAAG]$_{20}$GAAGG[AAAG]$_2$AG
19	SE33[27.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_9$A**A**[AAAG]$_{18}$GAAGG[AAAG]$_2$AG
20	SE33[28.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_9$AA[AAAG]$_{17}$G[AAGG]$_3$[AAAG]$_2$AG
21	SE33[29.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_9$A[AAAG]$_{19}$G[AAGG]$_2$[AAAG]$_2$AG
22	SE33[29.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{10}$A**A**[AAAG]$_{19}$GAAGG[AAAG]$_2$AG
23	SE33[30.2]	**A**AAG]$_2$AG[AAAG]$_3$AG[AAAG]$_8$AA[AAAG]$_{22}$GAAGG[AAAG]$_2$AG
24	SE33[31.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{11}$AA[AAAG]$_{20}$GAAGG[AAAG]$_2$ (4bp del in flanking region)
25	SE33[30.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{13}$A[AAAG]$_{18}$G----[AAAG]$_2$AG
26	SE33[31.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_4$A[AAAG]$_{10}$A[AAAG]$_{17}$GAAGG[AAAG]$_2$AG
27	SE33[31.2]	[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_8$AG[AAAG]$_{23}$GAAGG[AAAG]$_2$AG

Bold black bases are missing in some reads.
Bold red bases are appear duplicated in some reads.

Allele distribution



Number of called alleles:

DK	144
SOM	81
GRL	58

PCR-CE / NGS

No. of unique alleles

	Danes	Somali	Greenlanders
Danes	12 / **43**	27 / **61**	
Somali	1 / **10**	14 / **14**	15 / **28**
Greenlanders	0 / **4**	9 / **9**	8 / **7**	9 / **15**

Mutation studies

	Rolle	NGS Allele sequence	CE length
1	M	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{11}$AA[AAAG]$_{10}$GAAGG[AAAG]$_2$	21.2
1	M	No usable sequence information obtained	(31.2)
1	C	[AAAG]$_2$A[AAAG]$_3$AAG[AAAG]$_3$AAG[AAAG]$_{19}$G[AAAG]$_3$	19
1	C	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AAG[AAAG]$_{11}$A$_x$[AAAG]$_{10}$GAAGG[AAAG]$_2$	21.2
1	F	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{18}$G[AAAG]$_3$	18
1	F	[AAAG]$_2$A[AAAG]$_3$AAG[AAAG]$_3$AAG[AAAG]$_{20}$G[AAAG]$_3$	20
2	M	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{17}$G[AAAG]$_3$	17
2	M	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{18}$G[AAAG]$_3$	18
2	C	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{17}$G[AAAG]$_3$	17
2	C	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{16}$GAAGG[AAAG]$_3$	17
2	F	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{17}$GAAGG[AAAG]$_3$	18
2	F	No usable sequence information obtained	(20)
5	M	[AAAG]$_2$A$_x$[AAAG]$_3$A$_x$G[AAAG]$_3$A$_x$G[AAAG]$_{11}$A$_x$[AAAG]$_{18}$GAAGG[AAAG]$_2$	29.2
5	M	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{13}$A[AAAG]$_{18}$G[AAAG]$_2$	30.2
5	C	[AAAG]$_2$A$_x$[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{13}$A$_x$[AAAG]$_{15}$GAAGG[AAAG]$_2$	28.2
5	C	[AAAG]$_2$A[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{13}$A[AAAG]$_{18}$G[AAAG]$_2$	30.2
5	F	[AAAG]$_2$A[AAAG]$_2$AG[AAAG]$_3$AG[AAAG]$_{11}$A[AAAG]$_{20}$G[AAAG]$_2$	30.2
5	F	[AAAG]$_2$A$_x$[AAAG]$_3$AG[AAAG]$_3$AG[AAAG]$_{14}$AA[AAAG]$_{15}$GAAGG[AAAG]$_2$	29.2

M: mother, F: father, C: child. ( ) Alleles with no sequence information were not sequenced correctly in comparison to CE results and were left out of analysis.

Conclusions

- SE33 is a promising candidate locus for NGS based genotyping
- Large sequence variation is observed
- Difficulties owing to poly-A stretches and long alleles
- The Roche Junior platform does not perform optimally
- Similar difficulties expected on other available NGS platforms
- Alternative sequencing technique/s and platform/s are needed

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