

SE33 Variant Alleles: Sequences and Implications

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Among the 23 short tandem repeat (STR) loci commonly used in commercial STR kits, SE33 is by far the most polymorphic locus possessing 53 detectable alleles and 292 observed genotypes in 938 unrelated U.S. population samples (a subset of data from Hill et al. 2011). A review of the SE33 literature has found more than 170 alleles when internal sequence rearrangements are included (Butler 2011). The high degree of variation with SE33 can potentially impact PCR primers and amplicon mobility. U.S. population sample sets have been tested with PowerPlex ESX 17 and ESI 17 as well as NGM SElect and the widely used primers (Polymeropoulos et al. 1992) to explore any concordance issues between kits possessing primers in different positions. A G→A mutation 68 bp downstream of the repeat region has been detected in several samples that can cause a mobility shift in PowerPlex ESI 17 and ESSplex SE relative to PowerPlex ESX 17 and NGM SElect SE33 alleles. The observed frequencies and potential implications of flanking region differences are presented here.

23 STR loci present in STR kits rank ordered by their variability across 938 unrelated U.S. population samples

STR Locus	Alleles Observed	Genotypes Observed	Het. (obs)	P _i value N = 938
SE33	53	292	0.9360	0.0069
Penta E*	20	114	0.8799	0.0177
D2S1338	13	68	0.8785	0.0219
D1S1656	15	92	0.8934	0.0220
D18S51	21	91	0.8689	0.0256
D12S391	23	110	0.8795	0.0257
FGA	26	93	0.8742	0.0299
Penta D*	16	71	0.8754	0.0356
D21S11	25	81	0.8358	0.0410
D19S433	16	76	0.8124	0.0561
D8S1179	11	45	0.7878	0.0582
vWA	11	38	0.8060	0.0622
D7S820	11	32	0.8070	0.0734
TH01	8	24	0.7580	0.0784
D16S539	9	28	0.7825	0.0784
D13S317	8	29	0.7655	0.0812
D10S1248	12	39	0.7825	0.0837
D2S441	14	41	0.7772	0.0855
D3S1358	11	30	0.7569	0.0873
D2Z1045	11	42	0.7697	0.0933
CSF1PO	9	30	0.7537	0.1071
D5S818	9	34	0.7164	0.1192
TPOX	9	28	0.6983	0.1283

*Penta D and Penta E were only examined at 656 samples in this data set; 10 additional loci beyond the current CODIS 13 core loci shown in blue font; data are a subset of Hill et al. (2011)

Summary of Observations

A total of 22 discordant results have been observed with SE33 testing involving five different kits examined at NIST (ESX 17, ESI 17, NGM SElect, ESSplex SE, and the SE33 monoplex which contains the same primers as SEfiler and PowerPlex ES). In some cases, more than 1500 U.S. population samples have been examined although most of the focus to-date has been on a subset of 663 U.S. Caucasian, African American, and U.S. Hispanic samples.

Four samples (involving alleles 24.2, 25.2, 26.2, and 27.2) possess a C→T SNP 110 bp upstream of the repeat region that results in allele dropout when using the SE33 monoplex (SEfiler and PowerPlex ES) forward primer but correct genotypes with all other kits.

In a single sample, a 3 bp deletion of TTTG that is 28-30 bp downstream of the repeat region creates a "28.3" allele with ESX 17, ESI 17, and ESSplex SE kits or a "29.2" allele with the NGM SElect and SE33 monoplex (and thus SEfiler and PowerPlex ES kits).

A 4 bp deletion of AAAA that is 85-88 bp upstream of the repeat region (also reported by Rolf et al. 2011) impacts ESSplex SE forward primer annealing. Four occurrences of this flanking region mutation have been observed with alleles 13, 15, and 17 (twice).

A C→T SNP 60 bp downstream of the repeat region was observed once in an allele 25.2. This flanking mutation resulted in allele dropout with ESX 17 and a +1 base size migration shift in ESI 17 and ESSplex SE (see below).

A G→A SNP 68 bp downstream of the repeat region was observed in 11 samples [containing alleles 12.2, 13.2 (3x), 15.2 (3x), 16.2 (3x), and 23.2] from the NIST data set, 4 samples [containing alleles 14.2 (2x), 16.2, and 19.2] supplied by two German labs, and 5 samples (containing alleles 13.2, 21.2, 22.2, and 24.2) supplied by Applied Biosystems. This mutation led to a migration shift of approximately +1 base for ESI 17 and ESSplex SE amplicons relative to NGM SElect and ESX 17 amplicons (see below). The newly developed ESI 17 Pro primers corrected this migration shift and re-established full concordance with ESX 17 and NGM SElect on these samples.

No primer pair is immune to potential problems with the highly polymorphic SE33 locus.

Reported SE33 Alleles in the Literature or Identified through NIST Allele Sequencing

Shaded alleles have the same size but different internal sequence structures

Allele (Repeat #)	ABI Sefiler	Promega ESX 17	Promega ESI 17	Repeat Motif Patterns										Reference					
				5' flanking	central repeat					3' flanking									
				AAAG	AG	AAAG	AG	AAAG	AG	AAAG	AG	AAAG	AG	AAAG	AG	AAAG	AG		
3	197 bp	258 bp	300 bp														STRBase		
4.2	203 bp	264 bp	306 bp														RF-ESI ladder		
6.3	212 bp	273 bp	315 bp	2	1	3	1	7	0	0	0	0	0	0	0	0	Lászik et al. (1997)		
7	213 bp	274 bp	316 bp														Rolf et al. (2001)		
7.3	216 bp	277 bp	319 bp	2	1	3	1	8	0	0	0	0	0	0	0	0	Dauber et al. (2004)		
8	217 bp	278 bp	320 bp														RF-ESI ladder		
8.1	218 bp	279 bp	321 bp														Lászik et al. (2001)		
9(a)	221 bp	282 bp	324 bp	2	1	3	1	9	0	0	0	0	0	0	1	0	3	Dauber et al. (2009)	
9(b)	221 bp	282 bp	324 bp	2	1	3	1	9	0	0	0	0	0	1	1	2	1	Kline et al. (2010)	
9.2	223 bp	284 bp	326 bp														Lászik et al. (2001)		
10	225 bp	286 bp	328 bp														RF-ESI ladder		
10.2	227 bp	288 bp	330 bp	2	1	0	0	18	0	0	0	0	0	0	1	0	3	1	Dauber et al. (2009)
10.3	228 bp	289 bp	331 bp														Urqhart et al. (1993)		
11	229 bp	290 bp	332 bp														RF-ESI ladder		
11.2	231 bp	292 bp	334 bp	2	1	0	0	15	0	0	0	0	0	0	1	0	3	1	Dauber et al. (2004)
12	233 bp	294 bp	336 bp	2	1	3	1	12	0	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)
12.2	235 bp	296 bp	338 bp	2	1	3	1	13	0	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)
13	237 bp	298 bp	340 bp														RF-ESI ladder		
13.2	239 bp	300 bp	342 bp	2	1	3	0	14	0	0	0	0	0	1	0	3	1	Rolf et al. (1997); Kline et al. (2010)	
13.3	240 bp	301 bp	343 bp														Poetsch et al. (2010)		
14(a)	241 bp	302 bp	344 bp	2	1	3	1	14	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
14(b)	241 bp	302 bp	344 bp	2	1	3	1	14	0	0	0	0	0	1	1	2	1	Kline et al. (2010)	
14.1	242 bp	303 bp	345 bp														Poetsch et al. (2010)		
14.2	243 bp	304 bp	346 bp	2	1	3	0	15	0	0	0	0	0	1	0	3	1	Kline et al. (2010)	
14.3	244 bp	305 bp	347 bp																
15	245 bp	306 bp	348 bp	2	1	3	1	15	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
15.2	247 bp	308 bp	350 bp														Lászik et al. (2001)		
16(a)	249 bp	310 bp	352 bp	2	1	3	1	16	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
16(b)	249 bp	310 bp	352 bp	2	1	3	1	16	0	0	0	0	0	1	1	2	1	Kline et al. (2010)	
16.1	250 bp	311 bp	353 bp														Berti et al. (2010)		
16.2	251 bp	312 bp	354 bp														Lászik et al. (2001)		
16.3	252 bp	313 bp	355 bp														Eyed et al. (2005)		
17	253 bp	314 bp	356 bp	2	1	3	1	17	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
17.2	255 bp	316 bp	358 bp														Lászik et al. (2001)		
17.3	256 bp	317 bp	359 bp														Poetsch et al. (2010)		
18	257 bp	318 bp	360 bp	2	1	3	1	18	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
18.2	259 bp	320 bp	362 bp	2	1	3	1	9	1	8	0	0	0	1	1	2	1	Dauber et al. (2009)	
18.3	260 bp	321 bp	363 bp														Eyed et al. (2005)		
19(a)	261 bp	322 bp	364 bp	2	1	3	1	19	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
19(b)	261 bp	322 bp	364 bp	2	1	3	1	19	0	0	0	0	0	1	1	2	1	Kline et al. (2010)	
19.2	262 bp	323 bp	365 bp	2	1	3	1	10	1	0	8	0	0	1	1	2	1	Rolf et al. (1997)	
19.3	264 bp	325 bp	367 bp														Berti et al. (2010)		
20(a)	265 bp	326 bp	368 bp	2	1	3	1	20	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
20(b)	265 bp	326 bp	368 bp	2	1	3	1	20	0	0	0	0	0	1	1	2	1	Kline et al. (2010)	
20.2	267 bp	328 bp	370 bp	2	1	3	1	11	0	8	0	0	0	1	1	2	1	Rolf et al. (1997)	
20.3	268 bp	329 bp	371 bp																
20.4	269 bp	330 bp	372 bp	2	1	3	1	21	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
21.1	270 bp	331 bp	373 bp																
21.2(a)	271 bp	332 bp	374 bp	2	1	3	1	9	1	0	11	0	0	1	1	2	1	Rolf et al. (1997)	
21.2(b)	271 bp	332 bp	374 bp	2	1	3	1	11	0	9	0	0	0	1	1	2	1	Rolf et al. (1997)	
21.2(c)	271 bp	332 bp	374 bp	2	1	3	1	7	0	7	0	11	0	0	1	2	1	Kline et al. (2010)	
22(a)	273 bp	334 bp	376 bp	2	1	3	1	22	0	0	0	0	0	1	0	3	1	Rolf et al. (1997)	
22(b)	273 bp	334 bp	376 bp	2	1	3	1	21	0	0	0	0	0	1	1	3	1	Rolf et al. (1997)	
22.2(a)	275 bp	336 bp	378 bp	2	1	3	1	7	1	0	14	0	0	1	1	2	1	Rolf et al. (1997)	
22.2(b)	275 bp	336 bp	378 bp	2	1	3	1	8	5	12	0	0	0	1	2	1	Rolf et al. (1997)		
22.2(c)	275 bp	336 bp	378 bp	2	1	3	1	9	1	0	12	0	0	1	2	1	Rolf et al. (1997)		
22.2(d)	275 bp	336 bp	378 bp	2	1	3	1	10	1	0	11	0	0	1	2	1	Rolf et al. (1997)		
22.2(e)	275 bp	336 bp	378 bp	2	1	3	1	11	1	0	10	0	0	1	2	1	Rolf et al. (1997)		
22.2(f)	275 bp	336 bp	378 bp	2	1	3	1	12	1	0	9	0	0	1	1	2	1	Rolf et al. (1997)	
22.3	276 bp	337 bp	379 bp														Poetsch et al. (2010)		
23	277 bp	338 bp	380 bp														Lászik et al. (2001)		
23.2(a)	279 bp	340 bp	382 bp	2	1	3	1	7	1	0	15	0	0	1	1	2	1	Rolf et al. (1997)	
23.2(b)	279 bp	340 bp	382 bp	2	1	3	1	8	1	0	14	0	0	1	1	2	1	Rolf et al. (1997)	
23.2(c)	279 bp	340 bp	382 bp	2	1	3	1	9	1	0	13	0	0	1	1	2	1	Rolf et al. (1997)	
23.2(d)	279 bp	340 bp	382 bp	2	1	3	1	10	3	0	12	0	0	1	1	2	1	Rolf et al. (1997)	
23.2(e)	279 bp	340 bp	382 bp	2	1	3	1	10	1	0	12	0	0	1	1	2	1	Rolf et al. (1997)	
23.2(f)	279 bp	340 bp	382 bp	2	1	3	1	11	0	11	0	0	0	1	1	2	1	Rolf et al. (1997)	
23.2(g)	279 bp	340 bp	382 bp	2	1	3	1	12	1	0	10	0	0	1	1	2	1	Rolf et al. (1997)	
24	281 bp	342 bp	384 bp														Lászik et al. (2001)		
24.2(a)	283 bp	344 bp	386 bp	2	1	3	1	5	1	0	18	0	0	1	1	2	1	Rolf et al. (1997)	
24																			