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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 \rightarrow 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

	Alleles	Genotypes Observed	Het.	P _I value N – 938
STR LOCUS	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
D22S1045	11	42	0.7697	0.0933
CSF1PO	9	30	0.7537	0.1071
D5S818	9	34	0.7164	0.1192
ΤΡΟΧ	9	28	0.6983	0.1283

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 \rightarrow 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 TTG 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).

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

Shaded alleles have the same size but different internal sequence structures **Repeat Motif Patterns** Motif patterns based on Rolf et al. (1997) 0 0 0 AAAG AG AAAG AAAG AAAAG AAAAG AAAAG AAAAG AAAAG AAAAG G 4AGG 4G/AN AG ABI Promega Promega (Repeat #) SEfiler ESX 17 ESI 17 Reference 5' flanking 3' flanking central repeat

*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)

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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 \rightarrow 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 \rightarrow 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, 23.2, 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.

Annotated SE33 Allele 25.2 with Known Primer Positions

[AAAG]₂ AG [AAAG]₃ AG [AAAG]₉ AAAAAG [AAAG]₁₅ G AAGG [AAAG]₂ AG Repeat Motif Pattern (Rolf et al. 1997) = 2,1,3,1,9,1,0,0,15,0,0,1,1,2,1

10	20	30	40	50	60	70	80	90
CGTCTGTAATTCCA	GCTCCTAGGG?	AGGCTGAGGC	AGGATAATCG	CTTGAACCTG	GGAGGTGGAG	GCTACAGTGA	GCCGAGGTCA:	TGCCAT
+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+ • • • • + • • • •	+ • • • • + • • • • • • •	····	+++++
Sequencing	Foward	>						
	Hering et al. (2002),	, Heinrich et al. (200)4)					
TGCACTCCAATCTG	•GG <mark>CG</mark> ACAAGA0	GTGAAACTCC(GTCAAAAGAA	AGAAAGAAAG	AGACAAAGAG	AGTTAGAAAG <i>i</i>	AAAGAAAGAG <i>i</i>	AGAGAG
+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++		+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+ • • • • + • • • • • • •	····	+++++
	Forward Primer	\rightarrow	del					
Polymero	opoulos et al. 1992		Rolf et al. (2011)					
AGAGAAAGGAAGGA	AGGAAGAAAA	AGAAAGAAAA	AGAAAGAAAG	AGAAAGAAAG	AAAGAGAAAG	AAAGAAAGAA	AGAAAGAAAGi	AAAGAA
+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++	+ • • • • • • • • 	+++++++++++++++++++++++++++++++++++++++	+ • • • • • • • •	+ • • • • + • • • • • • •	+++++	→ • • • • +
					SE33 Repeat Re	egion (25.2)		
AGAAAGAAAAAGAA	AGAAAGAAAG	AAAGAAAGAA	AGAAAGAAAG	AAAGAAAGAA	AGAAAGAAAG	AAAGAAAGGA <i>i</i>	AGGAAAGAAA	GAGCAA
+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++	+ • • • • • • • • 	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+ • • • • + • • • • • • • • • • • • • •	+++++++++++++++++++++++++++++++++++++++	→ • • • • +

SE33 Repeat Region (25.2)

Hering et al. (2002), Heinrich et al. (2004)

GTTACTATAGC<mark>GGTAGGGGAGATG<u>TTG</u>TAGAAATATATATAAACCTCCTTACACCG</mark>CGAGACC<mark>G</mark>CGTCAGCCCAGCGAGCACAGAACCT

3 4.2	197 bp 203 bp	258 bp 264 bp	300 bp 306 bp			_								_				STRBase PP-ESI ladder
6.3 7 7 3	212 bp 213 bp 216 bp	273 bp 274 bp 277 bp	315 bp 316 bp 319 bp	2	1	3	1	7	0	0	0	0	0	0	0	0	3	1 Rolf <i>et al.</i> (1997) Lászik <i>et al.</i> (2001) 1 Dauber <i>et al.</i> (2004)
8 8.1	217 bp 218 bp	278 bp 279 bp	320 bp 321 bp	L		0	·	0	0	0	0	0	0	0	Ū	Ū	0	PP-ESI ladder Lászik <i>et al.</i> (2001)
9 (a) 9 (b)	221 bp 221 bp	282 bp 282 bp	324 bp 324 bp	2 2	1 1	3 3	1 1	9 9	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 1	3 2	1 Dauber et al. (2009) 1 Kline et al. (2010)
9.2 10 10 2	223 bp 225 bp 227 bp	284 bp 286 bp 288 bp	326 bp 328 bp 330 bp	2	1	0	0	18	0	0	0	0	0	0	1	0	3	Laszik <i>et al.</i> (2001) PP-ESI ladder 1 Dauber <i>et al.</i> (2009)
10.2 10.3 11	228 bp 229 bp	289 bp 289 bp 290 bp	331 bp 332 bp	Z	•	U	U	10	U	U	U	U	U	U	I	U	0	Urquhart <i>et al.</i> (1993) PP-ESI ladder
11.2 12	231 bp 233 bp	292 bp 294 bp	334 bp 336 bp	2 2	1 1	0 3	0 1	15 12	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	3 3	1 Dauber et al. (2004) 1 Rolf et al. (1997)
12.2 13 13 2	235 bp 237 bp 230 bp	296 bp 298 bp 300 bp	338 bp 340 bp 342 bp	2	1	3	0	13	0	0	0	0	0	0	1	0	3	1 Rolf <i>et al.</i> (1997) PP-ESI ladder 1 Rolf <i>et al.</i> (1997) Klipp <i>et al.</i> (2010)
13.2 13.3 14 (a)	239 bp 240 bp 241 bp	300 bp 301 bp 302 bp	343 bp 344 bp	2	1	3	1	14	0	0	0	0	0	0	1	0	3	Poetsch <i>et al.</i> (2010) 1 Rolf <i>et al.</i> (2010)
14 (b) 14.1	241 bp 242 bp	302 bp 303 bp	344 bp 345 bp	2	1	3	1	14	0	0	0	0	0	0	1	1	2	1 Kline et al. (2010) Poetsch <i>et al.</i> (2010)
14.2 14.3	243 bp 244 bp	304 bp 305 bp	346 bp 347 bp	2	1	3	0	15	0	0	0	0	0	0	1	0	3	1 Kline <i>et al.</i> (2010)
15 15.2 16 (a)	245 bp 247 bp 249 bp	306 bp 308 bp 310 bp	348 bp 350 bp 352 bp	2	1	3	1	15	0	0	0	0	0	0	1	0	3	Lászik <i>et al.</i> (1997) Lászik <i>et al.</i> (2001)
16 (b) 16.1	249 bp 250 bp	310 bp 311 bp	352 bp 353 bp	2	1	3	1	16	0	0	0	0	0	0	1	1	2	1 Kline <i>et al.</i> (2010) Berti <i>et al.</i> (2010)
16.2 16.3	251 bp 252 bp	312 bp 313 bp 214 bp	354 bp 355 bp 356 bp	2	1	2	1	17	0	0	0	0	0	0	1	0	2	Lászik <i>et al.</i> (2001) Egyed <i>et al.</i> (2005)
17 17.2 17.3	253 bp 255 bp 256 bp	314 bp 316 bp 317 bp	356 bp 358 bp 359 bp	Ζ	1	3	1	17	0	0	0	0	0	0	1	0	3	Lászik <i>et al.</i> (1997) Lászik <i>et al.</i> (2001)
18 18.2	257 bp 259 bp	318 bp 320 bp	360 bp 362 bp	2 2	1 1	3 3	1 1	18 9	0 1	0	0	0 8	0 0	0 0	1 1	0 1	3 2	1 Rolf et al. (1997) 1 Dauber et al. (2009)
18.3 19 (a)	260 bp 261 bp	321 bp 322 bp	363 bp 364 bp	2	1	3	1	19	0	0	0	0	0	0	1	0	3	Egyed <i>et al.</i> (2005) 1 Rolf <i>et al.</i> (1997) 1 Kline <i>et al.</i> (2010)
19 (b) 19.2 19.3	261 bp 263 bp 264 bp	322 bp 324 bp 325 bp	366 bp 367 bp	2	1	3	1	10	1	0	0	8	0	0	1	1	2	1 Rolf <i>et al.</i> (2010) 1 Berti <i>et al.</i> (1997)
20 (a) 20 (b)	265 bp 265 bp	326 bp 326 bp	368 bp 368 bp	2 2	1 1	3 3	1 1	20 20	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 1	3 2	1 Rolf et al. (1997) 1 Kline et al. (2010)
20.2 20.3	267 bp 268 bp	328 bp 329 bp	370 bp 371 bp	2	1	3	1	11	1	0	0	8	0	0	1	1	2	1 Rolf <i>et al.</i> (1997)
21 21.1 21.2 (a)	269 bp 270 bp 271 bp	330 bp 331 bp 332 bp	372 bp 373 bp 374 bp	2	1	3	1	21	1	0	0	11	0	0	1	1	3	1 Rolf <i>et al.</i> (1997)
21.2 (b) 21.2 (c)	271 bp 271 bp	332 bp 332 bp	374 bp 374 bp	2 2	1 1	3 3	1 1	11 7	1 0	0 7	0	9 11	0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Kline et al. (2010)
22 (a) 22 (b)	273 bp 273 bp	334 bp 334 bp	376 bp 376 bp	2 2	1	3	1	22 21	0	0 0	0 0	0	0 0	0 0	1	0	3 3	1 Rolf et al. (1997) 1 Kline et al. (2010)
22.2 (a) 22.2 (b) 22.2 (c)	275 bp 275 bp 275 bp	336 bp 336 bp 336 bp	378 bp 378 bp 378 bp	2 2 2	1 1 1	3 3 3	1 1 1	7 8 9	1 0 1	0 5 0	0 0 0	14 12 12	0 0 0	0 0 0	1 1 1	1 1 1	2 2 2	1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997)
22.2 (d) 22.2 (e)	275 bp 275 bp	336 bp 336 bp	378 bp 378 bp	2 2	1 1	3 3	1 1	10 11	1 1	0 0	0 0	11 10	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
22.2 (f) 22.3	275 bp 276 bp	336 bp 337 bp	378 bp 379 bp	2	1	3	1	12	1	0	0	9	0	0	1	1	2	1 Rolf <i>et al.</i> (1997) Poetsch <i>et al.</i> (2010)
23 23.2 (a) 23.2 (b)	277 bp 279 bp 279 bp	338 bp 340 bp 340 bp	380 bp 382 bp 382 bp	2 2	1 1	3 3	1 1	7 8	1 1	0 0	0 0	15 14	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (2001) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
23.2 (c) 23.2 (d)	279 bp 279 bp	340 bp 340 bp	382 bp 382 bp	2 2	1 1	3 3	1 1	9 10	1 0	0 3	0 0	13 12	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
23.2 (e) 23.2 (f) 23.2 (g)	279 bp 279 bp 279 bp	340 bp 340 bp 340 bp	382 bp 382 bp 382 bp	2 2 2	1 1 1	3 3 2	1 1 1	10 11 12	1 1 1	0 0	0 0	12 11 10	0 0	0 0	1 1 1	1 1 1	2 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
23.2 (g) 24 24.2 (a)	279 bp 281 bp 283 bp	340 bp 342 bp 344 bp	382 bp 384 bp 386 bp	2	1	3	1	5	1	0	0	10	0	0	1	1	2	Lászik <i>et al.</i> (1997) Lászik <i>et al.</i> (2001)
24.2 (b) 24.2 (c)	283 bp 283 bp	344 bp 344 bp	386 bp 386 bp	2 2	1 1	3 3	1 1	7 8	1 1	0 0	0 0	16 15	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
24.2 (d) 24.2 (e)	283 bp 283 bp	344 bp 344 bp 244 bp	386 bp 386 bp	2 2 2	1 1 1	3 3 2	1 1 1	10 11 12	1 1 1	0 0	0 0	13 12	0 0	0 0	1 1 1	1 1 1	2 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
24.2 (f) 24.2 (g) 25	283 bp 285 bp	344 bp 346 bp	386 bp 388 bp	2	1	3	1	6	1	0	0	16	0	0	1	1	3	1 U134C>T Kline <i>et al.</i> (2010) Lászik <i>et al.</i> (2001)
25.2 (a) 25.2 (b)	287 bp 287 bp	348 bp 348 bp	390 bp 390 bp	2 2	1	3	1	9 10	1	0	0	15 14	0	0	1	1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
25.2 (C) 25.2 (d) 25.2 (e)	287 bp 287 bp 287 bp	348 bp 348 bp 348 bp	390 bp 390 bp 390 bp	2 2 2	1 1 1	3 3 3	1 1 1	10 11 12	1 1 1	0 0 0	0 0 0	14 13 12	0 0 0	0 0 0	1 1 1	1 1 1	2 2 2	1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997)
25.2 (f) 25.2 (g)	287 bp 287 bp	348 bp 348 bp	390 bp 390 bp	2 2	1 1	3 3	1 1	14 9	1 1	0 0	0 0	10 15	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 D75C>T Kline et al. (2010)
25.2 (h) 25.3	287 bp 288 bp	348 bp 349 bp	390 bp 391 bp	2	1	3	1	6	1	0	0	17	0	0	1	2	2	1 U134C>T Kline <i>et al.</i> (2010) Berti <i>et al.</i> (2010)
26.2 (a) 26.2 (b)	289 bp 291 bp 291 bp	350 bp 352 bp 352 bp	392 bp 394 bp 394 bp	2 2	1 1	3 3	1 1	8 9	1 1	0 0	0 0	17 16	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (2001) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
26.2 (c) 26.2 (d)	291 bp 291 bp	352 bp 352 bp	394 bp 394 bp	2 2	1 1	3 3	1 1	10 11	1 0	0 0	0 1	15 14	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
26.2 (e) 26.2 (f)	291 bp 291 bp	352 bp 352 bp	394 bp 394 bp	2 2 2	1 1 1	3 3 2	1 1	11 14	1 1 1	0 0	0 0	14 11	0 0	0 0	1 1	1 1	2 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Ll124C xT
26.2 (g) 27 27.2 (a)	291 bp 293 bp 295 bp	352 bp 354 bp 356 bp	394 bp 396 bp 398 bp	2	1	3	1	6	0	0	1	18	0	0	1	1	3	Lászik <i>et al.</i> (2010) 1 Rolf <i>et al.</i> (1997)
27.2 (b) 27.2 (c)	295 bp 295 bp	356 bp 356 bp	398 bp 398 bp	2 2	1 1	3 3	1 1	8 9	1 0	0 0	0 1	18 17	0 0	0 0	1 1	1 3	2 0	1 Rolf et al. (1997) 1 Rolf et al. (1997)
27.2 (d) 27.2 (e) 27.2 (f)	295 bp 295 bp 295 bp	356 bp 356 bp 356 bp	398 bp 398 bp 398 bp	2 2 2	1 1 1	3 3 3	1 1 1	10 11 12	1 1 1	0 0	0 0	16 15 14	0 0	0 0	1 1 1	1 1 1	2 2 2	1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997)
27.2 (g) 27.2 (h)	295 bp 295 bp 295 bp	356 bp 356 bp	398 bp 398 bp	2 2 2	1 1	3 3	1 1	12 12 13	1 0	0 0	0 1	15 13	0 0	0 0	1 1 1	1 3	2 0	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
27.2 (i) 27.2 (j)	295 bp 295 bp	356 bp 356 bp	398 bp 398 bp	2 2	1 1	3 3	1 1	13 15	1 1	0 0	0 0	13 11	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
27.2 (h) 27.3 28	295 bp 296 bp 297 bp	356 bp 357 bp 358 bp	398 bp 399 bp 400 bp	2	1	3	1	14	0	9	0	15 16	0	0	1	1	3	1 U134C>1 Kline <i>et al.</i> (2010) Hill <i>et al.</i> (2010) 1 Dauber <i>et al.</i> (2009)
28.2 (a) 28.2 (b)	299 bp 299 bp	360 bp 360 bp	402 bp 402 bp	2 2	1 1	3 3	1 1	8 9	1 0	0 0	0 0	19 18	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
28.2 (c) 28.2 (d)	299 bp 299 bp	360 bp 360 bp	402 bp 402 bp	2 2 2	1 1	3 3 2	1	9 9 10	0 1	0 0	0 0	15 18	0 0	0 0	1	1 1	2 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
28.2 (e) 28.2 (f) 28.2 (q)	299 bp 299 bp 299 bp	360 bp 360 bp 360 bp	402 bp 402 bp 402 bp	2 2 2	1 1	3 3 3	1 1	10 11 12	1 1	0 0	0 0	17 16 15	0	0 0 0	1 1 1	1 1 1	2 2 2	I Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
28.2 (h) 28.2 (i)	299 bp 299 bp	360 bp 360 bp	402 bp 402 bp	2 2	1 1	3 3	1 1	13 14	1 1	0 0	0 0	14 13	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
28.2 (j) 28.2 (k) 28.3	299 bp 299 bp 300 bp	360 bp 360 bp 361 bp	402 bp 402 bp 403 bp	2 2 2	1 1 1	3 3	1 1 1	14 16 10	1 1	0 0	0 0	13 11 12	0	0 0 1	1 1 1	3 1 1	0 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Dauber et al. (2009)
29 29.2 (a)	301 bp 303 bp	362 bp 364 bp	404 bp 406 bp	2 2 2	1 1	0	0	15 8	1	0	0	16 20	0	0	1 1	1 1	2	1 Dauber et al. (2009) 1 Rolf et al. (1997)
29.2 (b) 29.2 (c)	303 bp 303 bp	364 bp 364 bp	406 bp 406 bp	2 2	1 1	3 3	1 1	9 9	0 1	0 0	1 0	19 19	0 0	0 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
29.2 (d) 29.2 (e) 29.2 (f)	303 bp 303 bp 303 bp	364 bp 364 bp 364 bp	406 bp 406 bp 406 bp	1 2 1	1 1 1	3 3 3	1 1 1	10 11 11	1 0 1	0 5 0	0 0	19 16 18	0 0	0 0	1 1 1	1 1 1	2 2 2	1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997)
29.2 (g) 29.2 (h)	303 bp 303 bp	364 bp 364 bp	406 bp 406 bp 406 bp	2 2	1 1	3 3	1 1	11 12	1 1	0 0	0	17 16	0 0	0 0	1 1	1 1	2 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
29.2 (i) 29.2 (j)	303 bp 303 bp	364 bp 364 bp	406 bp 406 bp	2 2	1 1	3 3	1 1	13 13	0 1	0 0	1 0	15 15	0 0	0 0	1 1	3 1	0 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
29.2 (k) 29.2 (l) 29 2 (m)	303 bp 303 bp 303 bp	364 bp 364 bp 364 bp	406 bp 406 bp 406 bp	2 2 2	1 1 1	3 3 3	1 1 1	14 16 11	1 1 1	0 0 0	0 0 0	14 12 17	0 0 0	0 0 0	1 1 1	1 1 1	2 2 2	1 Rolf <i>et al.</i> (1997) 1 Rolf <i>et al.</i> (1997) 1 D41-TTG-deletion Kline <i>et al.</i> (2010)
29.3 30	304 bp 305 bp	365 bp 366 bp	407 bp 408 bp	_		U					U		U					Hill <i>et al.</i> (2010) Lászik <i>et al.</i> (2001)
30.2 (a) 30.2 (b)	307 bp 307 bp	368 bp 368 bp	410 bp 410 bp	2	1 1	3 3 2	1	11 12	1 1	0 0	0 0	18 17	0 0	0 0	1	1 1	2 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
30.2 (C) 30.2 (d) 30.2 (e)	307 bp 307 bp 307 bp	368 bp 368 bp 368 bp	410 bp 410 bp 410 bp	1 2 2	1 1	3 3 3	1 1	12 13 14	1 1	0 0	0 0	16 16 15	0	0 0 0	1 1 1	1 1 1	2 2 2	I Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
30.2 (f) 31	307 bp 309 bp	368 bp 370 bp	410 bp 412 bp	2	1	3	1	15	1	0	0	14	0	0	1	1	2	1 Rolf <i>et al.</i> (1997) Lászik <i>et al.</i> (2001)
31.2 (a) 31.2 (b) 31.2 (c)	311 bp 311 bp	372 bp 372 bp 372 bp	414 bp 414 bp 414 bp	1 1 2	1 1 1	3 3 2	1 1 1	9 10 12	1 1	0 0	0 0	22 21	0 0	0 0	1 1 1	1 1 1	2 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
31.2 (d) 31.2 (e)	311 bp 311 bp	372 bp 372 bp 372 bp	414 bp 414 bp	2 2 2	' 1 1	3 3	1 1	12 13 14	1 1	0 0 0	0 0 0	17 16	0 0 0	0 0 0	1 1	1 1	2 2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
32 32.1	313 bp 314 bp	374 bp 375 bp	416 bp 417 bp	0	4	0	4	40	4	0	0	10	0	0	4	4	0	Lászik <i>et al.</i> (2001)
32.2 (a) 32.2 (b) 33 (a)	315 bp 315 bp 317 bp	376 bp 376 bp 378 bp	418 bp 418 bp 420 bp	2 2 2	1 1 1	3 3 2	1 1 1	13 14 10	1 1 1	0 0 0	0 0 0	18 17 12	0 0 1	0 0 9	1 1 1	1 1 1	2 2 2	Rolf et al. (1997) 1 Rolf et al. (1997) 1 Rolf et al. (1997)
33 (b) 33.2 (a)	317 bp 319 bp	378 bp 380 bp	420 bp 422 bp	2 1	1 1	3 3	1 1	10 10	1 1	0 0	0 0	11 23	1 0	9 0	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Rolf et al. (1997)
33.2 (b) 34 34 2	319 bp 321 bp	380 bp 382 bp	422 bp 424 bp 426 bp	2 2 2	1 1 1	3 3 3	1 1 1	12 9 12	1 1 1	0 0	0 0	20 13 20	0 1 0	0 9 0	1 1 1	1 1 1	2 2 2	Dauber et al. (2004) 1 Rolf et al. (1997) 1 Dauber et al. (2004)
34.∠ 35 35.2	325 bp 327 bp	386 bp 388 bp	428 bp 430 bp	∠ 1	1	ა 3	1	13	1	0	0	20 22	0	0	1	1	∠ 2	PP-ESI ladder 1 Rolf et al. (1997)
36 (a) 36 (b)	329 bp 329 bp	390 bp 390 bp	432 bp 432 bp	2 2	1 1	3 3	1 1	10 12	1 1	0 0	0 0	14 10	1 1	9 11	1 1	1 1	2 2	1 Rolf et al. (1997) 1 Dauber et al. (2009)
36.2 37 38	331 bp 333 bp 337 bp	392 bp 394 bp 398 bp	434 bp 436 bp 440 bp	2	1	3	1	9	1	0	0	16	1	9	1	1	2	Lászik <i>et al.</i> (2001) 1 Rolf <i>et al.</i> (1997)
39 39.2	341 bp 343 bp	402 bp 404 bp	444 bp 446 bp															PP-ESI ladder Lászik <i>et al.</i> (2001)
41 42	349 bp 353 bp	410 bp 414 bp	452 bp 456 bp															PP-ESI ladder
40	291 bp	τ∠∠ υμ 112 hn	-τυ τι υμ 191 bp	2	4	0	4		4	0	0	10		40	4	4	0	$\frac{1}{2} = \frac{1}{2} \left(\frac{1}{2} - \frac{1}{2} \right) \left(\frac{1}{2}$

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Reverse Primer SNP Region SEfiler reverse primer ESX 17 reverse primer Polymeropoulos et al. (1992) = NGM SElect reverse primer (relative position only) Wang et al. (2011)

TGTCCTTGCCGCTGCGCCTTGCGTCCGCACCCGCCGCCAGCTCACCATGGATGATGCTATCACCGCGCTC ****

Sequencing Reverse

ESI 17 reverse primer

(relative position only)

Hering et al. (2002) and Heinrich et al. (2004) reported primer binding site mutations (boxed in orange) for the widely used Polymeropoulos et al. (1992) primers (orange arrows), which are used in the AmpFISTR SEfiler and PowerPlex ES kits. NGM SElect uses the Polymeropoulos reverse primer with a new forward primer and creates a 396 bp amplicon with SE33 allele 25.2. We have observed two flanking region mutations (boxed in red) in the Wang et al. (2011) "SNP Region" that impact migration of amplicons created with primers such as ESI 17 that include this SNP region. A 4 bp deletion has been reported by Rolf et al. (2011) and also observed during NIST allele sequencing that will cause STR typing results to differ from the repeat sequence by 4 bp or a single repeat unit. A TTG deletion just downstream of the Polymeropoulos reverse primer was also observed. The sequencing primers used in this study are indicated by the purple arrows and generate a 520 bp product with allele 25.2.

SE33 Flanking Region Mutations that Impact Migration and/or Primer Annealing

Mutations in primer binding regions can create allele dropout. Flanking region variation may also impact local secondary structure in a PCR product and impact how the amplicon migrates during capillary electrophoresis (Wang et al. 2011).









A total of 12 out of 494 African American samples (2.4 %) tested from the NIST data set (involving 46 new blood samples, 258 population samples, and 190 father/son samples) contained either the C \rightarrow T SNP (observed once) 60 bp downstream of the repeat or the G \rightarrow A SNP (observed 11 times) 68 bp downstream of the repeat. Nine other samples provided to NIST by collaborators all contained the $G \rightarrow A$ SNP.

The +1 base migration differences between ESX 17 and ESI 17 amplicons were missed in our initial study (Hill et al. 2011) because of poor resolution at the time these samples were run on the NIST ABI 3130xl Genetic Analyzer. Hence, it is important to have good resolution and precise sizing in order to detect potential SE33 variant alleles. However, allele sequencing is still the best way to observe the full extent of variation at the highly polymorphic SE33 locus due to many alleles having internal sequence variation (see listing to the right).

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Poster available for download from STRBase: http://www.cstl.nist.gov/strbase/pub_pres/ButlerISFG2011poster.pdf

More than 170 alleles are listed here and others continue to be identified... (we plan to add further information to STRBase to reflect these new alleles as they are discovered)