# SE33 and PowerPlex ESI 17 Pro Kit

## Concordance Results with NIST U.S. Population Samples

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**Promega Symposium at ISFG 2011**

**Sept 2, 2011 – Vienna, Austria**

NIST

Promega

# Disclaimer

**NIST Funding: Interagency Agreement 2008-DN-R-121 between the National Institute of Justice and NIST Office of Law Enforcement Standards**

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**Points of view are mine** and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

# Presentation Outline

- SE33 background and characteristics
- SE33 allele nomenclature
- Concordance studies
- SE33 differences and kit corrections
- Summary and final thoughts

# History of SE33 Use

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- 1991, 1992 initial NAR articles (primers defined)
- 1993 FSS examination
- 1993-95 FBI and AFDIL exploration
  - found to be too complex and challenging for the DNA separation systems of the time
- 1993-1997 Brinkmann lab
  - Population studies, nomenclature
- 1994 EDNAP study
- 1998 German DNA database adoption
- 2001-2002 STR kits become available
  - PowerPlex ES (Promega), SEfiler (ABI)
- 2009-2011 next generation STR kits
  - PP ESI/ESX 17 (Sept 2009), ESSplex SE (Fall 2010), NGM SElect (early 2011), PP ESI 17 Pro (Fall 2011)

# Polymeropoulos et al. (1992) article

*1432 Nucleic Acids Research, Vol. 20, No. 6*

## Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2)

**Mihael H.Polymeropoulos, Denise S.Rath, Hong Xiao and Carl R.Merril**
National Institute of Mental Health Neuroscience Center, St Elizabeths Hospital, Room 131, 2700 Martin Luther King Avenue, Washington, DC 20032, USA

*Chromosomal Localization*: We have tentatively assigned the human beta-actin related pseudogene H-beta-Ac-psi-2 to chromosome 6 using rodent/human somatic cell hybrids.

**Smaller PCR Product Sizes enabled better resolution of closely spaced alleles**

*Source/Description*: The polymorphic $(AAAG)_n$ repeat begins at base pair 176 of the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2) on chromosome 6 (1). The polymorphism can be typed using the polymerase chain reaction (PCR) as described previously (2). The predicted length of the amplified sequence was 291 bp.

*Primer Sequences*:
AATCTGGGCGACAAGAGTGA (AAAG strand)
ACATCTCCCCTACCGCTATA (TTTC strand)

*Frequency*: Estimated from 78 chromosomes of unrelated individuals. Heterozygosity Index = 93%. PIC = 0.93.

| Allele (bp) | Frequency | Allele (bp) | Frequency |
|---|---|---|---|
| A1 318 | 0.01 | A12 270 | 0.03 |
| A2 314 | 0.04 | A13 266 | 0.01 |
| A3 310 | 0.05 | A14 262 | 0.04 |
| A4 306 | 0.10 | A15 258 | 0.14 |
| A5 302 | 0.09 | A16 254 | 0.06 |
| A6 298 | 0.09 | A17 250 | 0.02 |
| A7 294 | 0.03 | A18 246 | 0.04 |
| A8 290 | 0.04 | A19 242 | 0.05 |
| A9 282 | 0.03 | A20 238 | 0.05 |
| A10 278 | 0.03 | A21 234 | 0.01 |
| A11 274 | 0.04 | | |

**Polymeropoulos primers result in a small sequence length of 291 bp and heterozygosity of 93%**

# Locus Characteristics

# SE33 Locus Characteristics

- **Location:** 6q14 (Chr 6; 89.043 Mb) – beta-actin-related pseudogene
- **Repeat motif**: primarily AAAG (but highly complex patterns)
- **Observed Allele range**: 3 to 49 repeats
- **Heterozygosity**:
  ~ 90-95%
- **Mutation rate**: 0.64%

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT

Forensic Science International 148 (2005) 207–209

Forensic Science International
www.elsevier.com/locate/forsciint

## *ACTBP2* (alias *ACTBP8*) is localized on chromosome 6 (band 6q14)

S. Wenda[a], E.M. Dauber[a], D.W.M. Schwartz[a], C. Jungbauer[b], V. Weirich[c], R. Wegener[c], W.R. Mayr[a,b,*]

[a]*Division of Blood Group Serology, Medical University Vienna, Waehringer Guertel 18-20, A-1090 Wien, Austria*
[b]*Blood Donation Centre, Austrian Red Cross, Wiedner Hauptstrasse 32, A-1040 Wien, Austria*
[c]*Institute of Legal Medicine, University of Rostock, Sankt-Georg-Strasse 108, D-18055 Rostock, Germany*

Received 5 January 2004; received in revised form 19 May 2004; accepted 21 May 2004
Available online 28 July 2004

# 23 STR loci present in STR kits

| STR Locus | Alleles Observed | Genotypes Observed | H(obs) | $P_I$ (all samples) n = 1426 |
|---|---|---|---|---|
| SE33 | 58 | 341 | 0.9383 | 0.0063 |
| Penta E* | 20 | 113 | 0.8779 | 0.0175 |
| D2S1338 | 13 | 73 | 0.8752 | 0.0221 |
| D1S1656 | 17 | 99 | 0.8871 | 0.0229 |
| **D18S51** | 23 | 102 | 0.8696 | 0.0263 |
| D12S391 | 24 | 120 | 0.8654 | 0.0279 |
| **FGA** | 29 | 111 | 0.8702 | 0.0299 |
| Penta D* | 16 | 70 | 0.8733 | 0.0360 |
| **D21S11** | 32 | 98 | 0.8331 | 0.0399 |
| D19S433 | 16 | 83 | 0.8100 | 0.0534 |
| **D8S1179** | 11 | 48 | 0.7966 | 0.0553 |
| **vWA** | 11 | 42 | 0.8000 | 0.0624 |
| **D16S539** | 9 | 30 | 0.7812 | 0.0723 |
| **D13S317** | 9 | 30 | 0.7749 | 0.0724 |
| **D7S820** | 12 | 35 | 0.7826 | 0.0745 |
| **TH01** | 9 | 27 | 0.7518 | 0.0752 |
| D2S441 | 14 | 46 | 0.7777 | 0.0807 |
| D10S1248 | 12 | 41 | 0.7812 | 0.0828 |
| **D3S1358** | 11 | 31 | 0.7489 | 0.0904 |
| D22S1045 | 11 | 45 | 0.7567 | 0.0935 |
| **D5S818** | 9 | 34 | 0.7225 | 0.1057 |
| **CSF1PO** | 10 | 33 | 0.7567 | 0.1071 |
| **TPOX** | 10 | 30 | 0.6830 | 0.1351 |

**Better for mixtures (more alleles seen)**

**Better for kinship (low mutation rate)**

Rank ordered
by their variability

# Allele Nomenclature

# Allele Nomenclature

Int J Legal Med (1997) 110 : 69–72



ORIGINAL ARTICLE

**102 different alleles were observed through sequence analysis**

B. Rolf · M. Schürenkamp · A. Junge · B. Brinkmann

**Sequence polymorphism at the tetranucleotide repeat of the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2) locus**

Int J Legal Med (1998) 111 : 97–100



TECHNICAL NOTE

H. R. Schneider · S. Rand · H. Schmitter
G. Weichhold

**ACTBP2-nomenclature recommendations of GEDNAP**

**Important papers that describe SE33 allele nomenclature**

# 171 Published or Known SE33 Alleles

**John Butler poster at the International Society of Forensic Genetics (ISFG) meeting (Vienna, Austria), August 31-September 2, 2011, "SE33 Variant Alleles: Sequences and Implications“ (P-230)**

| | | | | Repeat Motif Patterns | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Allele (Repeat #)** | **ABI SEfiler** | **Promega ESX 17** | **Promega ESI 17** | AAAG | AG | AAAG | AG | **AAAG** | AAAAAG | AG | AGAAAG | AAAG | AAAAAG | AAAG | G | AAGG | AAAG/ANAG | AG | **Reference** |
| | | | | 5' flanking | | | | **central repeat** | | | | | | | 3' flanking | | | | |
| **3** | 197 bp | 258 bp | 300 bp | | | | | | | | | | | | | | | | STRBase |
| **4.2** | 203 bp | 264 bp | 306 bp | | | | | | | | | | | | | | | | PP-ESI ladder |
| **6.3** | 212 bp | 273 bp | 315 bp | 2 | 1 | 3 | 1 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | Rolf *et al.* (1997) |
| **7** | 213 bp | 274 bp | 316 bp | | | | | | | | | | | | | | | | Lászik *et al.* (2001) |
| **7.3** | 216 bp | 277 bp | 319 bp | 2 | 1 | 3 | 1 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | Dauber *et al.* (2004) |
| **8** | 217 bp | 278 bp | 320 bp | | | | | | | | | | | | | | | | PP-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 | 1 | Dauber *et al.* (2009) |
| **9 (b)** | 221 bp | 282 bp | 324 bp | 2 | 1 | 3 | 1 | 9 | 0 | 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 | | | | | | | | | | | | | | | | PP-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 | | | | | | | | | | | | | | | | Urquhart *et al.* (1993) |
| **11** | 229 bp | 290 bp | 332 bp | | | | | | | | | | | | | | | | PP-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 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 1 | Rolf *et al.* (1997) |
| **13** | 237 bp | 298 bp | 340 bp | | | | | | | | | | | | | | | | PP-ESI ladder |
| **13.2** | 239 bp | 300 bp | 342 bp | 2 | 1 | 3 | 0 | 14 | 0 | 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 | 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 | 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 | 0 | 1 | 0 | 3 | 1 | Kline *et al.* (2010) |

# SE33 Internal Sequence Variation

**Same Length, Different Internal Sequence**

| Allele (Repeat #) | ABI SEfiler | Promega ESX 17 | Promega ESI 17 | Repeat Motif Patterns | | | | | | | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AAAG | AG | AAAG | AG | **AAAG** | AAAAAG | AG | AGAAAG | AAAG | AAAAAG | AAAG | G | AAGG | AAAG/ANAG | AG | |
| | | | | 5' flanking | | | | **central repeat** | | | | | | | 3' flanking | | | | |
| **28.2 (a)** | 299 bp | 360 bp | 402 bp | 2 | 1 | 3 | 1 | 8 | 1 | 0 | 0 | 19 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (b)** | 299 bp | 360 bp | 402 bp | 2 | 1 | 3 | 1 | 9 | 0 | 0 | 0 | 18 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (c)** | 299 bp | 360 bp | 402 bp | 2 | 1 | 3 | 1 | 9 | 0 | 0 | 0 | 15 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (d)** | 299 bp | 360 bp | 402 bp | 2 | 1 | 3 | 1 | 9 | 1 | 0 | 0 | 18 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (e)** | | | | 2 | 1 | 3 | 1 | 10 | 1 | 0 | 0 | 17 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (f)** | | | | 2 | 1 | 3 | 1 | 11 | 1 | 0 | 0 | 16 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (g)** | | | | 2 | 1 | 3 | 1 | 12 | 1 | 0 | 0 | 15 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (h)** | 299 bp | 360 bp | 402 bp | 2 | 1 | 3 | 1 | 13 | 1 | 0 | 0 | 14 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (i)** | 299 bp | 360 bp | 402 bp | 2 | 1 | 3 | 1 | 14 | 1 | 0 | 0 | 13 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.2 (j)** | 299 bp | 360 bp | 402 bp | 2 | 1 | 3 | 1 | 14 | 1 | 0 | 0 | 13 | 0 | 0 | 1 | 3 | 0 | 1 | Rolf *et al.* (1997) |
| **28.2 (k)** | 299 bp | 360 bp | 402 bp | 2 | 1 | 3 | 1 | 16 | 1 | 0 | 0 | 11 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **28.3** | 300 bp | 361 bp | 403 bp | 2 | 1 | 3 | 1 | 10 | 1 | 0 | 0 | 12 | +A | 4 | 1 | 1 | 2 | 1 | Dauber *et al.* (2009) |
| **29** | 301 bp | 362 bp | 404 bp | 2 | 1 | 0 | 0 | 15 | 1 | 0 | 0 | 16 | 0 | 0 | 1 | 1 | 2 | 1 | Dauber *et al.* (2009) |
| **29.2 (a)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 8 | 1 | 0 | 0 | 20 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (b)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 9 | 0 | 0 | 1 | 19 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (c)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 9 | 1 | 0 | 0 | 19 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (d)** | 303 bp | 364 bp | 406 bp | 1 | 1 | 3 | 1 | 10 | 1 | 0 | 0 | 19 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (e)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 11 | 0 | 5 | 0 | 16 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (f)** | | | | 1 | 1 | 3 | 1 | 11 | 1 | 0 | 0 | 18 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (g)** | | | | 2 | 1 | 3 | 1 | 11 | 1 | 0 | 0 | 17 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (h)** | | | | 2 | 1 | 3 | 1 | 12 | 1 | 0 | 0 | 16 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (i)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 13 | 0 | 0 | 1 | 15 | 0 | 0 | 1 | 3 | 0 | 1 | Rolf *et al.* (1997) |
| **29.2 (j)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 13 | 1 | 0 | 0 | 15 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (k)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 14 | 1 | 0 | 0 | 14 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (l)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 16 | 1 | 0 | 0 | 12 | 0 | 0 | 1 | 1 | 2 | 1 | Rolf *et al.* (1997) |
| **29.2 (m)** | 303 bp | 364 bp | 406 bp | 2 | 1 | 3 | 1 | 11 | 1 | 0 | 0 | 17 | 0 | 0 | 1 | 1 | 2 | 1 | D41-TTG-deletion -- Kline *et al.* (2010) |

**Allele 28.2 (11 sequences)**

**Allele 29.2 (13 sequences)**

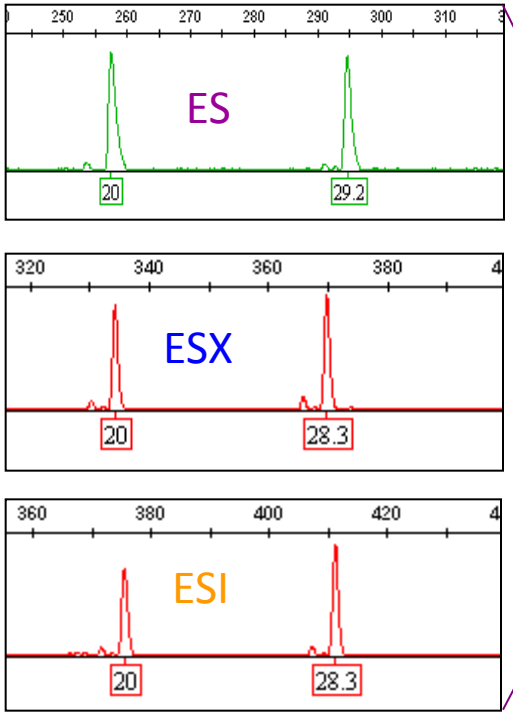
# Concordance Studies

# Concordance Studies

- Concordance studies are valuable because different primer sets are available
- SE33 primer changes were not an issue really until recently because ABI and Promega used 1992 published primers
- For more information on concordance studies, see Hill *et al* (2010) and ISFG poster P-110

**Hill, C.R., Kline, M.C., Duewer, D.L., Butler, J.M. (2010) Strategies for concordance testing. *Profiles in DNA (Promega)*, 13(1).**

# NIST Concordance Results




| ES Primers | ESX Primers | ESI Primers |
|---|---|---|
| 26.2, 26.2 | 26.2, 27.2 | 26.2, 27.2 |
| 20,29.2 | 20,28.3 | 20,28.3 |
| 28.2,28.2 | 24.2,28.2 | 24.2,28.2 |
| 21.2,21.2 | 21.2,26.2 | 21.2,26.2 |
| 24.2,24.2 | 24.2,25.2 | 24.2,25.2 |
| 19,25.2 | 19,19 | 19,25.2 |

## Only Six Discordant Results Were Observed

2886 alleles x 3 primer sets = 8658 comparisons

6/8658 = 0.07% discordance

# Sequence Reasons for Primer Discordance

Sequence Reason
C→T 110 bp upstream (impacts ES-F primer)
3 bp deletion (TTG) 28 bp downstream (outside ES-R primer)
C→T 110 bp upstream (impacts ES-F primer)
C→T 110 bp upstream (impacts ES-F primer)
C→T 110 bp upstream (impacts ES-F primer)
C→T 60 bp downstream (impacts ESX-R primer annealing)

# SE33 Differences

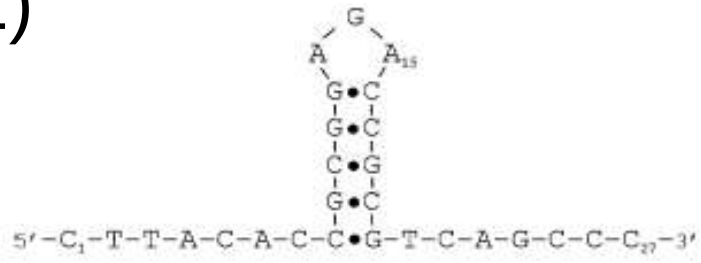
NGM Select/PP ESX 17 vs

PP ESI 17/ESSplex SE vs

PP ESI 17 Pro

# Discordance between kits

- 1 bp migration differences were observed between PP ESX 17/NGM SElect and PP ESI 17 amplicons
  - “x.3” or OL allele calls were reported as opposed to the correct “x.2” allele call
- This is due to repeat flanking region variation impacting the secondary structure in a PCR product, impacting how the amplicon migrates during CE (Wang et al. 2011)




Hairpin secondary structure proposed by Wang et al. (2011) in normal SE33 allele containing a G 68 bp downstream of the repeat region

# ABI NGM SElect Relative Primer Positions

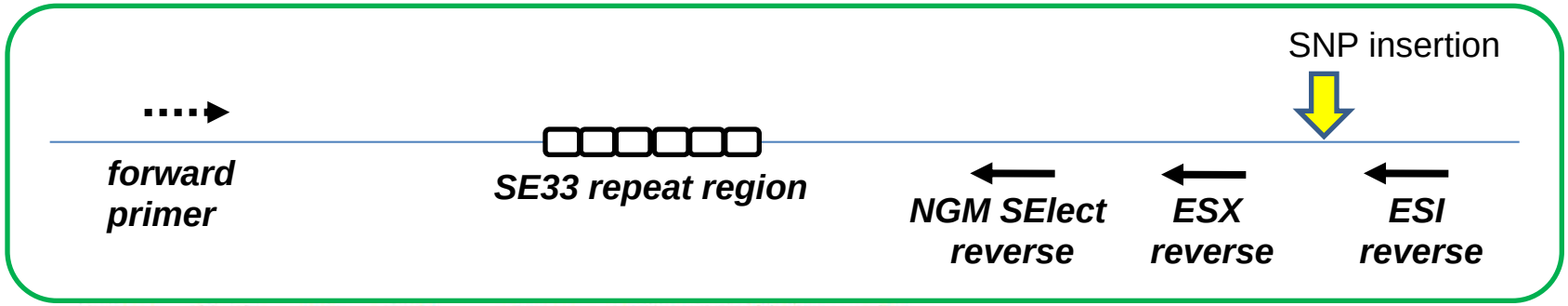
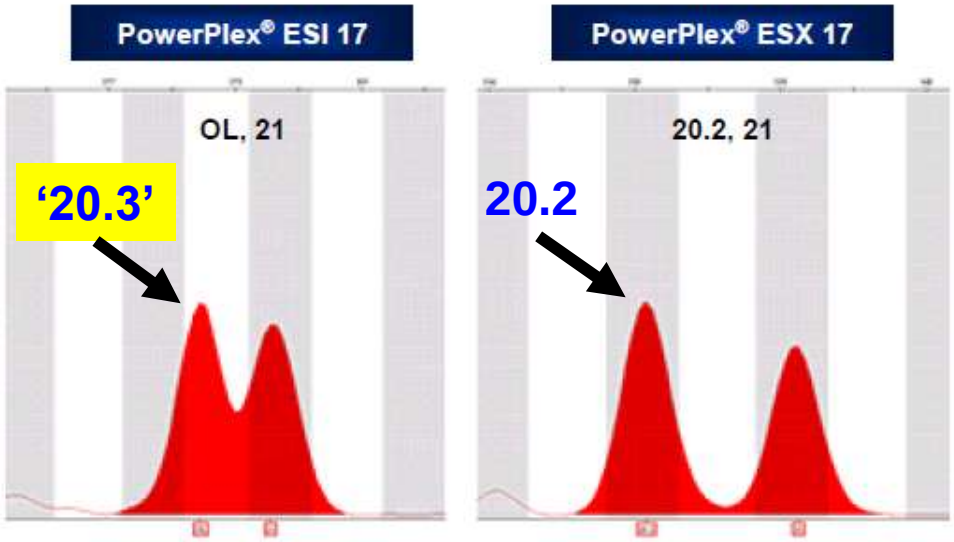


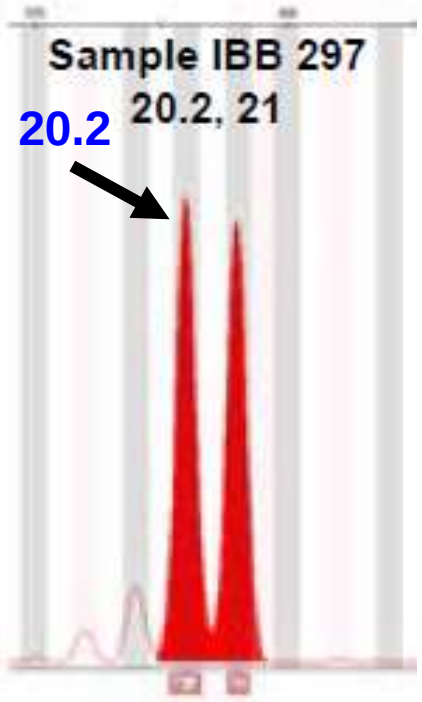

**Figure 3. Example of discordance at the SE33 locus for sample IBB 297 between PowerPlex® ESI 17 and ESX 17 results**




Each sample which exhibited discordance using the SE33 prototype primers also showed the same discordance when amplified with the ESI kit.

Sequence investigations revealed a SNP-containing region within the prototype SE33 amplicon which, when a SNP occurs, affects the mobility of the amplicon on the capillary electrophoresis platform.

## NGM SElect

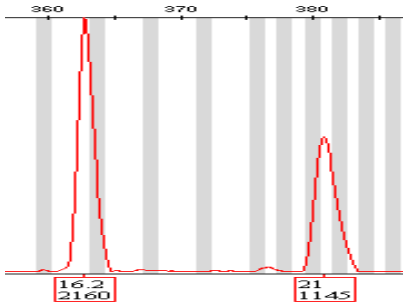



http://www3.appliedbiosystems.com/cms/groups/applied_markets_marketing/documents/generaldocuments/cms_088773.pdf

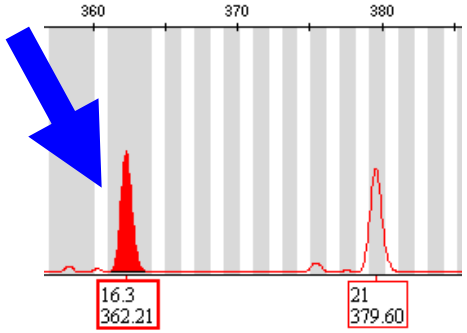
# Why were these not initially observed in the NIST concordance study?

- In the original NIST concordance study with PP ESX 17 and PP ESI 17, the 1 bp shift was not observed
- This was due to poor resolution with our 3130xl
  - Broad peaks, peak tailing, shifting of peaks, poor allelic ladder resolution
- Our 3130xl has been completely refurbished and upon re-run of the samples, differences were discovered

# Review of Our SE33 Data

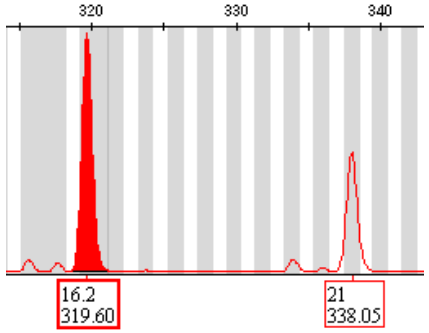



**Original ESI 17 data – incorrectly designated “16.2, 21”**
**(broad peaks due to poor 3130xl resolution)**



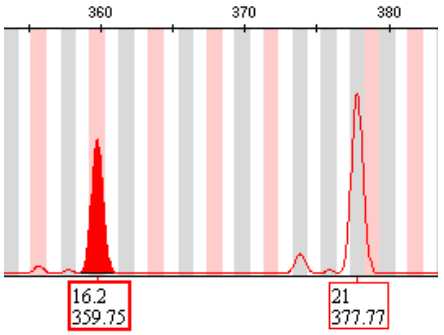

## PowerPlex ESI 17 (30 cycles)

**“16.3”,21**




## PowerPlex ESX 17 (30 cycles)

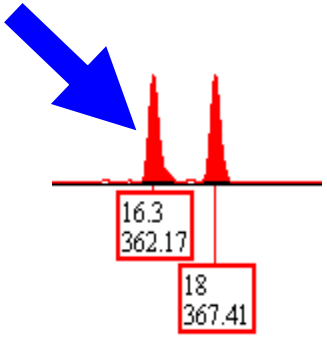
**16.2,21**




## NGM SElect (29 cycles)

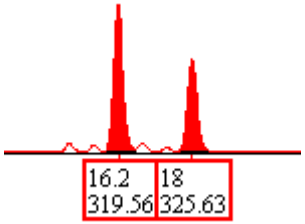
**16.2,21**

# Impact of SE33 Primer Positions



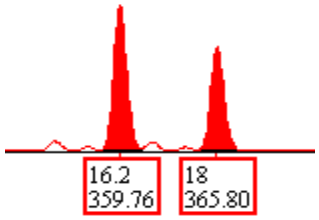

**PowerPlex ESI 17** (30 cycles)

**"16.3",18**



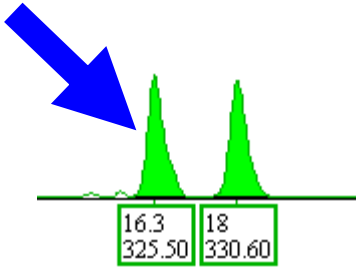

**PowerPlex ESX 17** (30 cycles)

**16.2,18**




**NGM SElect** (29 cycles)

**16.2,18**




**ESSplex SE** (30 cycles)

**"16.3",18**

# SE33 Seq Differences

- Total African American samples tested:
  - 46 Blood samples
  - 258 Population samples
  - 190 Father/Son samples

**494 AA samples total**

- 12 seq variations found out of 494 samples
  - **9 from earlier ESI/ESX data (not detected previously due to poor resolution of SE33 alleles)**

**2.43% NIST AA samples exhibit ESI difference**

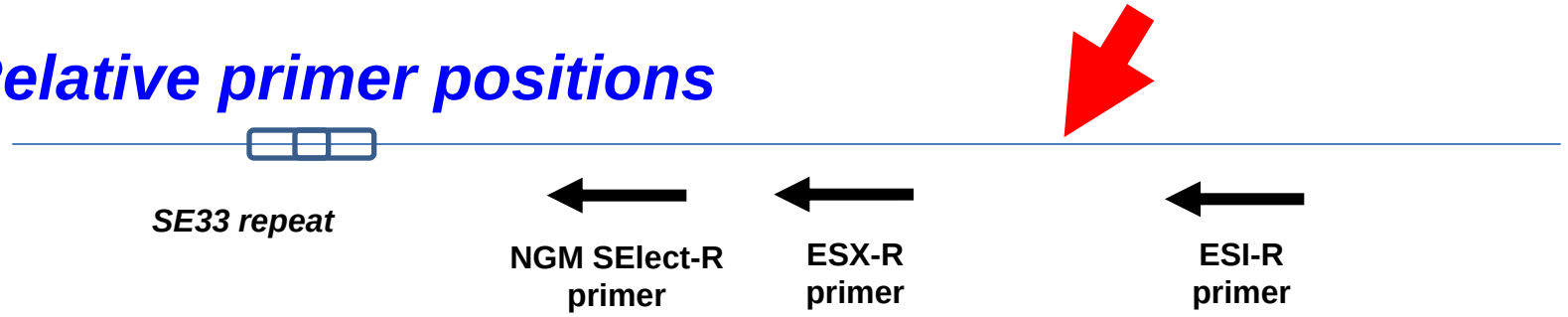
# SE33 Sequence Reason for Migration Shift

**NIST Sequencing Results from SE33 Alleles 14.2, 18** (LKA MV003)

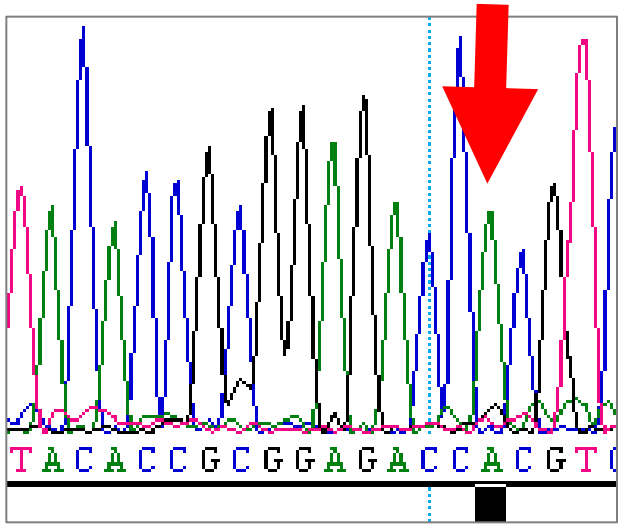

**Normal SE33 allele flanking region (57-68 bases downstream of repeat)**

**Mutant SE33 Allele G → A 68 bp downstream of SE33 repeat** (no length difference)

## *Relative primer positions*

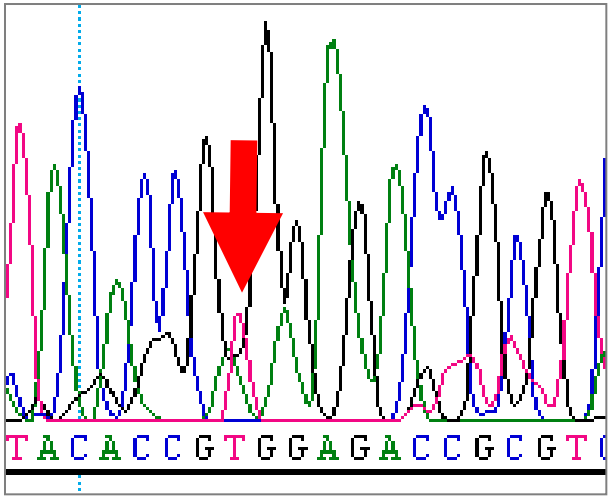


# SE33 Sequence Reason for Migration Shift




**G → A 68 bp downstream of SE33 repeat**

***Observed in >11 samples so far…***




**C → T 60 bp downstream of SE33 repeat**

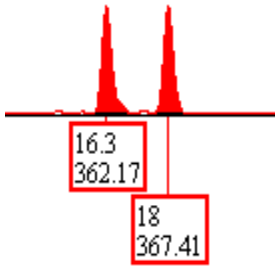
***Observed once***

# PP ESI 17 Pro

- The SE33 reverse primer was redesigned in the **PP ESI 17 Pro** kit to allow for the correct genotype
- All African American samples were rerun with the **PP ESI 17 Pro kit** (with excellent 3130xl data resolution) and there were no discordant results.
- The 1 bp shift for SE33 has been corrected with the new reverse primer redesign

**PP ESI 17**

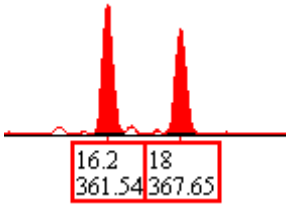
**5.24 bp**




**“16.3”,18**

**PP ESI 17 Pro**

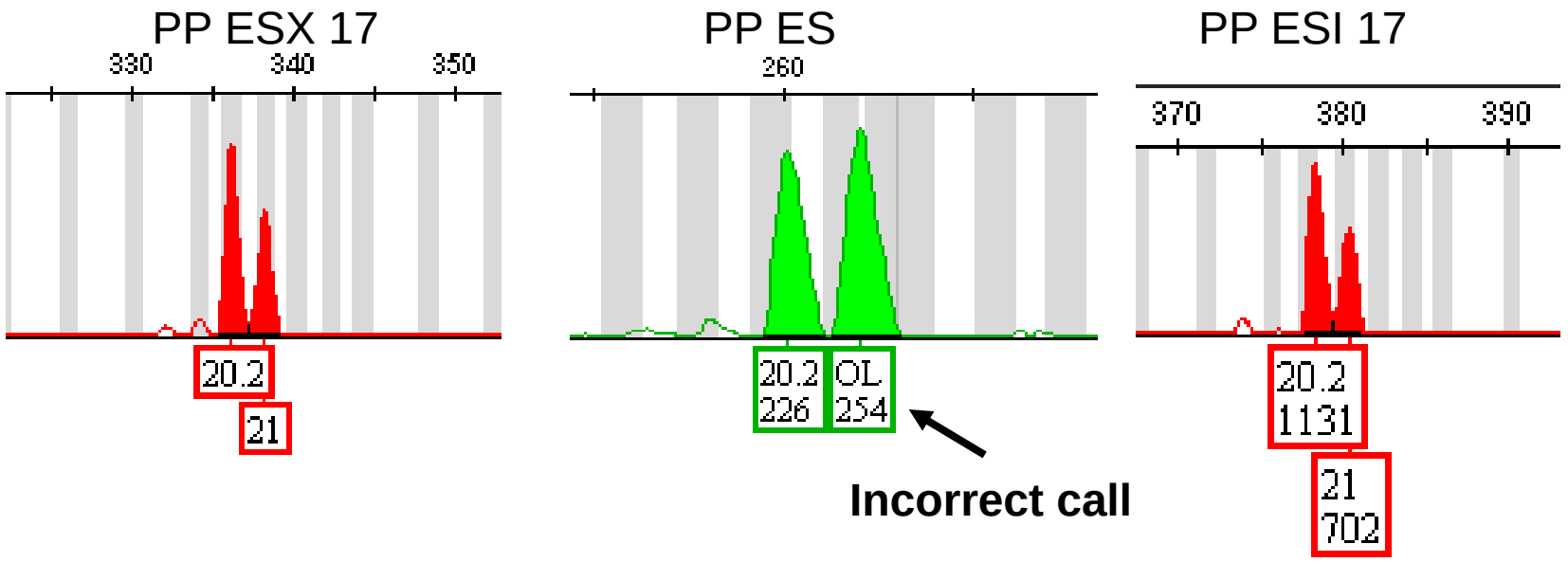
**6.11 bp**

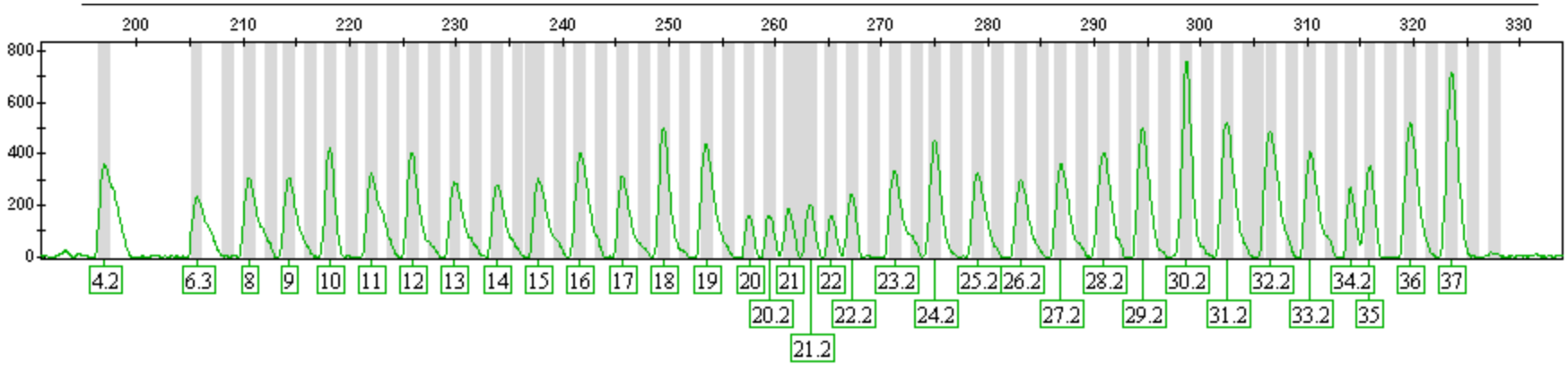



**16.2,18**

# Importance of CE Resolution

# Differences in CE Resolution Impact Allele Calls




**SE33 (PP ES) Ladder with bad resolution**

# Summary

- SE33 is a complex marker that requires excellent CE resolution for genotypes to be called correctly
- Between PP ESX 17/NGM SElect and PP ESI 17, we observed 12 SE33 discordant calls due to a SNP prior to the PP ESI 17 reverse primer – this has been corrected with a reverse primer redesign in PP ESI 17 Pro
- The 1 bp shift for SE33 is no longer an issue and all of these samples are now concordant with PP ESX 17 and NGM SElect
- No primer sets are completely immune from the possibility of primer binding site mutations

# SRM 2391c
# PCR-based DNA Profiling Standard

- 6 components
  - 4 genomic DNA (one mixture)
  - 2 cell lines (903 and FTA paper)

- The genotypes for all 6 components have been certified for SE33
  - Genotyped with several STR multiplex kits
  - Each component has been DNA sequenced

- Now available for purchase (replaces SRM 2391b)
  - See poster **P-348** for more details

# SRM 2391c – SE33 Allele Sequencing

| Component | Genotype | Allele Sequence |
|---|---|---|
| A | 16,18 | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{16}G[AAAG]_{\mathbf{3}}AG,$ |
| | | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{18}G[AAAG]_{\mathbf{3}}AG$ |
| B | 17,18 | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{17}G[AAAG]_{\mathbf{3}}AG,$ |
| | | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{18}G[AAAG]_{\mathbf{3}}AG$ |
| C | 28.2,31.2 | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{10}AAAAAG[AAAG]_{17}G\ AAGG[AAAG]_2AG,$ |
| | | $[AAAG]_2AG[AAAG]_3AG[AAAG]_9AAAAAG[AAAG]_{21}G\ AAGG[AAAG]_2AG$ |
| E | 22,30.2 | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{21}G\ AAGG[AAAG]_{\mathbf{3}}AG,$ |
| | | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{12}AAAAAG\ [AAAG]_{17}\ G\ AAGG[AAAG]_{\mathbf{2}}AG$ |
| F | 12,21 | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{12}G[AAAG]_3AG$ |
| | | $[AAAG]_2AG[AAAG]_3AG[AAAG]_{21}G[AAAG]_3AG$ |

**All SE33 alleles have been certified with allele sequencing**

# Acknowledgments


**NIST Team for This Work**

**John Butler**
**(help w/ slides)**

**Dave Duewer**
**(data crunching)**

**Margaret Kline**
**(allele sequencing)**

**A special thanks to Promega for providing the kits used in this study**

**Promega R&D Scientists**

**Bob McLaren**
Cindy Sprecher
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Ben Krenke
Doug Storts



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