http://www.cstl.nist.gov/biotech/strbase
http://www.dna.gov/research/nist

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Concordance Testing with New STR Kits

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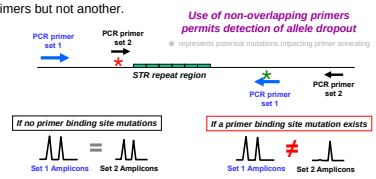
Promega has developed several new STR kits to address recommendations of additional loci requested by the European community [1,2]. These kits include D10S1248, D2S441, D22S1045, D12S391, D1S1656, and SE33 as well as the 10 STRs and the sex-typing marker amelogenin present in the Applied Biosystems' SGM Plus™ kit. In order to evaluate the performance of new PCR primer sets compared with ones currently in use, concordance testing was performed on over 1400 samples from U.S. population groups. Comparisons were made with previous genotyping results from commonly used STR kits including PowerPlex® 16, Identifiler™, and MiniFiler™ as well as in-house assays. From almost 100,000 alleles compared between the PowerPlex® ESX and ESI Systems (2 kits x 17 loci x 2 alleles/locus x 1443 samples), a total of 7 differences were observed. Additional allele differences were observed when comparisons were made to currently available kits with many of the dropouts coming from the present commercial kits or published primer sets. Sequence analysis was performed on all discordant samples to ascertain the primer binding site mutation. An additional primer was added in the final PowerPlex ESX 16 and 17 Systems to correct for allele dropout with D22S1045. Our results indicate that these new kits enable reliable STR typing with sensitive DNA detection and high powers of discrimination. With an overall concordance of greater than 99.9% to STR loci typed with currently available kits, these new kits should permit reliable extensions of DNA database and forensic casework efforts.

[1] Gill, P., Fereday, L., Morling, N., Schneider, P.M. (2006) The evolution of DNA databases--recommendations for new European loci. *Forensic Sci. Int.* 156:242-244.
[2] Gill, P., Fereday, L., Morling, N., Schneider, P.M. (2006) Letter to editor -- New multiplexes for Europe-amendments and clarification of strategic development. *Forensic Sci Int.* 163:155-157.

Purpose of Concordance Studies

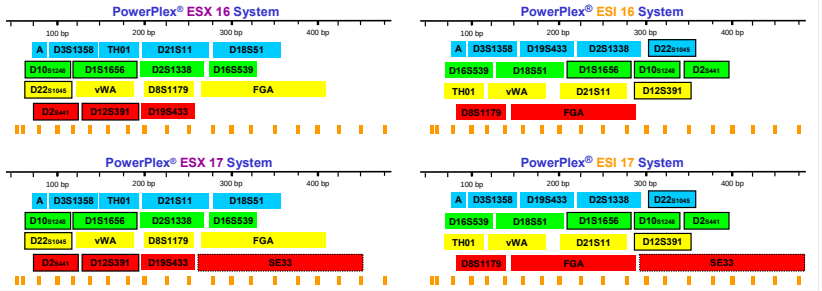
When different primer sets are utilized, there is a concern that allele dropout may occur due to primer binding site mutations that impact one set of primers but not another.



Applied Biosystems has taken the strategy of not changing primer sequences with equivalent STR loci between their kits (except with MiniFiler) and uses mobility modifiers to adjust spacing between loci in the same dye channel where needed. Promega has moved primer positions in order to optimize spacing between STR allele ranges with new kits—thus, necessitating concordance studies to check for potential allele dropout due to primer binding site mutations. Published differences between STR kits due to allele dropout have been noted on the NIST STRBase website at http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm.

PCR Product Size Ranges and Dye Labels for STR Loci in New Promega Kits

Five new European loci (boxed) are placed at the upper end with ESI vs ESX systems.
ESX 16 and ESX 17 (as well as ESI 16 vs ESI 17) systems only differ by the addition of SE33 (German-recommended locus)



Total Number of Samples Attempted = 1461
1443 with complete profiles

U.S. Population Samples (663 samples)

- Previously studied with Identifiler™, MiniFiler™, Yfiler™, PP16, miniSTRs, and many additional assays *(>200,000 allele calls)*
- 260 African Americans, 260 Caucasians, 140 Hispanics, and 3 Asians See http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm

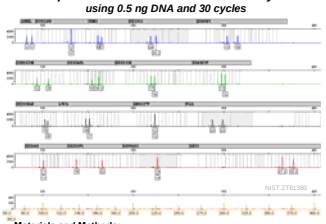
U.S. Father/Son pairs (786 samples)

- Previously studied with Identifiler, MiniFiler, Yfiler, 23plex
- **~100 fathers/100 sons for each group**: African Americans, Caucasians, Hispanics, and Asians

NIST SRM 2391b PCR DNA Profiling Standard **(12 samples)**

- Genomic Components 1-10 (includes 9947A and 9948)
- ABI 007 and K562

Example Data from PowerPlex ESX 17 System
using 0.5 ng DNA and 30 cycles



Materials and Methods:
DNA template: 0.5-1 ng input DNA
PCR (GeneAmp 9700): manufacturer recommended protocols with some half reactions
ABI 3130xl: 36cm array, POP-4, 10s@3kV

Amplicon Size Differences (bp) for STR Loci from Several Kits Relative to ESX 17

STR Locus	ESI 17	PP16	Identifiler
D1S1656	87	--	--
D2S441	260	--	--
D2S1338	26	--	74
D3S1358	0	0	-1
D8S1179	-128	0	-78
D10S1248	200	--	--
D12S391	164	--	--
D16S539	-187	-11	-42
D18S51	-151	0	-19
D19S433	-30	--	-100
D21S11	0	0	-17
D22S1045	225	--	--
FGA	-118	55	-56
TH01	-85	0	11
vWA	0	0	28
SE33	42	--	--
Amelogenin X	0	19	19
Amelogenin Y	0	19	19

Key:

Where "0" is indicated (e.g., D3S1358), there is no difference noted between kits and the primers positions are likely the same.

Where " --" is indicated (e.g, D1S1656), the locus is question is not present in the kit represented in that column.

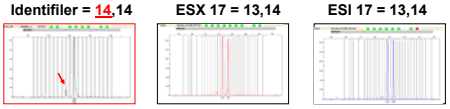
A positive number (e.g., "87") means that the PCR product for the kit in that column is larger than the ESX 17 kit by that number of base pairs.

Comparisons for Each Locus between Various Kits and In-House Assays

As many as five different PCR primer sets from various kits and assays have been compared to one another

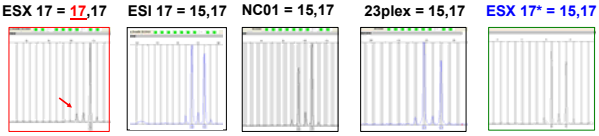
Locus	Kit/Assay 1	Kit/Assay 2	Kit/Assay 3	Kit/Assay 4	Kit/Assay 5
Amelogenin	PP-ESX17	PP-ESI17	Identifiler	NIST 23plex	MiniFiler
D3S1358	PP-ESX17	PP-ESI17	Identifiler	PP16	
TH01	PP-ESX17	PP-ESI17	Identifiler	PP16	
D21S11	PP-ESX17	PP-ESI17	Identifiler	PP16	MiniFiler
D18S51	PP-ESX17	PP-ESI17	Identifiler	PP16	MiniFiler
D10S1248	PP-ESX17	PP-ESI17	NIST 23plex	NIST NC01	
D1S1656	PP-ESX17	PP-ESI17			
D2S1338	PP-ESX17	PP-ESI17	Identifiler	MiniFiler	
D16S539	PP-ESX17	PP-ESI17	Identifiler	PP16	MiniFiler
D22S1045	PP-ESX17	PP-ESI17	NIST 23plex	NIST NC01	
vWA	PP-ESX17	PP-ESI17	Identifiler	PP16	
D8S1179	PP-ESX17	PP-ESI17	Identifiler	PP16	
FGA	PP-ESX17	PP-ESI17	Identifiler	PP16	MiniFiler
D2S441	PP-ESX17	PP-ESI17	NIST 23plex	NIST NC02	
D12S391	PP-ESX17	PP-ESI17			
D19S433	PP-ESX17	PP-ESI17	Identifiler		
SE33	PP-ESX17	PP-ESI17	SE33 monoplex		

D19S433 Discordance
AF45A (Asian)



T→A 8 bp downstream impacting reverse primer binding with Identifiler (and thus other ABI kits)

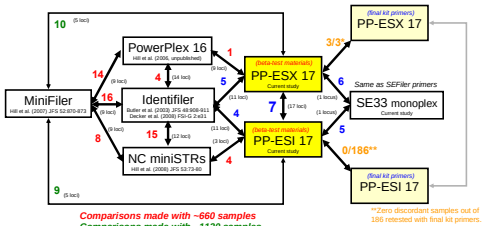
D22S1045 Discordance
NIST PT84195



G→T 15 bp upstream impacting forward primer binding with ESX17
Promega added additional primer to correct issue

Summary of Allele Discordance Observed

Number of Discordant Results Observed between Various STR Kits/Assays



Comparisons made with ~660 samples
Comparisons made with ~1120 samples
Comparisons made with ~1440 samples

7 differences between ESX 17 and ESI 17 beta-test materials

98,124 allele comparisons (1,443 samples x 17 loci x 2 alleles/loci x 2 kits)

0.0071% discordance (primarily D22S1045, which has been corrected with an additional T-variant primer)
0.0031% after correction

Details Regarding the 25 Discordant Results Observed (from >100,000 allele comparisons)

Locus	ESX 17	ESI 17	Identifiler	MiniFiler	PP16	NC01	23plex	Sequence Reason for Discordance
D16S539	12,12	12,13	12,13	12,13	12,13	--	--	yet to be determined
D3S1358	14,17	14,17	14,14	--	14,17	--	--	G>C SNP 11 bp downstream - Identifiler reverse primer
D19S433	13,14	13,14	14,14	--	--	--	--	T>A SNP 8 bp downstream - Identifiler reverse primer
D19S433	13,14.2	13,14.2	14.2,14.2	--	--	--	--	T>A SNP 8 bp downstream - Identifiler reverse primer
D22S1045	17,17	15,17	--	--	--	15,17	15,17	G>T SNP 15 bp upstream - ESX forward primer (corrected)
D22S1045	17,17	15,17	--	--	--	15,17	15,17	G>T SNP 15 bp upstream - ESX forward primer (corrected)
D22S1045	17,17	15,17	--	--	--	15,17	15,17	G>T SNP 15 bp upstream - ESX forward primer (corrected)
D22S1045	15,15	15,16	--	--	--	15,16	15,16	G>T SNP 15 bp upstream - ESX forward primer (corrected)
D1S1656	15.3,15.3	14,15.3	--	--	--	--	--	C>T SNP 30 bp upstream - ESX forward primer
SE33	26.2,27.2	26.2,27.2	26.2,26.2	--	--	--	--	C>T SNP 134 bp upstream - monoplex/SEFiler forward primer
SE33	20,28.3	20,28.3	20,29.2	--	--	--	--	3 bp deletion 41bp downstream – outside monoplex/SEFiler
SE33	24.2,28.2	24.2,28.2	28.2,28.2	--	--	--	--	C>T SNP 134 bp upstream - monoplex/SEFiler forward primer
SE33	21.2,26.2	21.2,26.2	21.2,21.2	--	--	--	--	C>T SNP 134 bp upstream - monoplex/SEFiler forward primer
SE33	24.2,25.2	24.2,25.2	24.2,24.2	--	--	--	--	C>T SNP 134 bp upstream - monoplex/SEFiler forward primer
SE33	19,19	19,25.2	19,25.2	--	--	--	--	C>T SNP 75 bp downstream - ESX reverse primer
D16S539	9,11	9,11	9,11	9,9	9,11	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D16S539	11,12	11,12	11,12	12,12	11,12	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D16S539	9,11	9,11	9,11	9,9	9,11	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D16S539	11,14	11,14	11,14	14,14	11,14	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D16S539	9,11	9,11	9,11	9,9	9,11	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D16S539	11,13	11,13	11,13	13,13	11,13	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D16S539	11,12	11,12	11,12	12,12	11,12	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D16S539	9,12	9,12	9,12	9,9	9,12	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D16S539	11,12	11,12	11,12	12,12	11,12	--	--	T>C SNP 34 bp downstream - MiniFiler reverse primer
D18S51	13,15	13,15	15,15	13,15	13,15	--	--	C/T SNP 172 bp downstream - Identifiler reverse primer

See also http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm

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Poster available for download from STRBase: http://www.cstl.nist.gov/biotech/strbase/pub_pres/Butler-ISFG2009-Concordance.pdf