

Concordance Testing Comparing STR Multiplex Kits with a Standard Data Set



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Concordance evaluations are important to detect allelic dropout or "null alleles" present in a data set. These studies are performed because there are a variety of commercial STR multiplex kits with different configurations of STR markers available to the forensic community. The electrophoretic mobility of the markers can vary between kits because the primer sequences were designed to amplify different polymerase chain reaction (PCR) product sizes. When multiple primer sets are used, there is concern that allelic dropout may occur due to primer binding site mutations that affect one set of primers but not another. These null alleles become evident only when data sets are compared. Null alleles are a concern because this could result in a false-negative or incorrect exclusion of two samples that come from a common source (only if different PCR primers are used). Multiple concordance studies have been performed at NIST with a standard sample set (~1450 in-house U.S. population samples) using various STR multiplex kits from Applied Biosystems, Promega, and Qiagen, including many of the next-generation European kits. Various discordant results have been identified using concordance software developed at NIST, confirmed by DNA sequencing, and reported to the forensic community on the null allele section of STRBase. A summary of the results, including discordance and sequencing results, are shown here in order to help demonstrate the benefits of performing concordance testing using a standard data set with STR multiplex kits that have different primer sequences for the same markers.

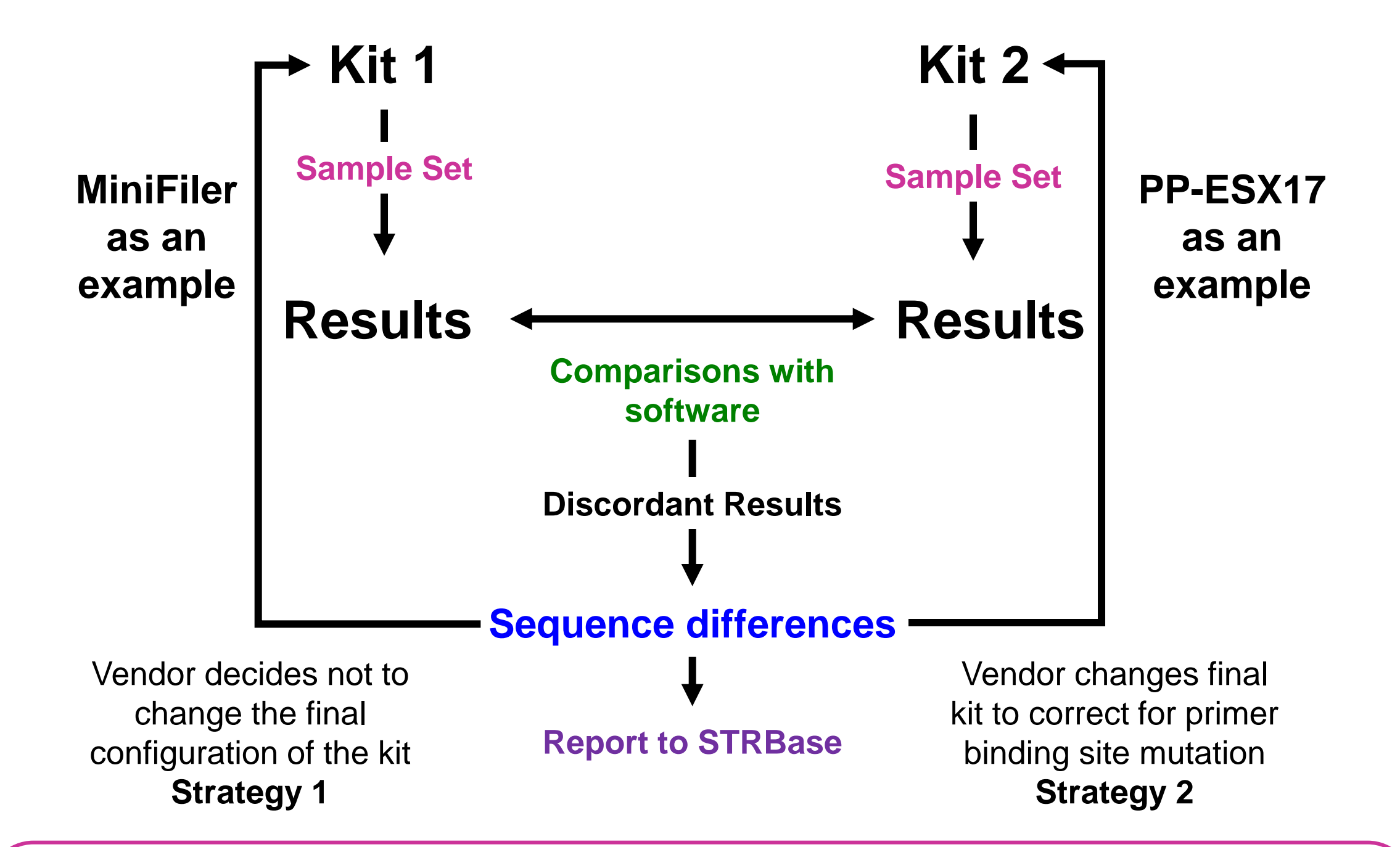
Why Concordance Testing is Important

Concordance studies are necessary when comparing genotypes of samples that have been tested with STR multiplex kits that have varying configurations of the same loci. These markers are able to be in different positions because they have alternative primer sequences from one another. There is the potential for allelic dropout or a "null allele" if a primer binding site mutation impacts one of the primer pairs. The use of non-overlapping primers permits the detection of allelic dropout. A base pair change in the DNA template in the PCR primer binding region can disrupt the hybridization of the primer and result in a failure to amplify and detect an existing allele. Null alleles are a concern because discordant results can impact DNA databases. Therefore, when discordant results are discovered, it is important to confirm the result with DNA sequencing to fully understand and characterize the direct cause of the mutation. At NIST we also establish concordance with standard reference materials (SRM 2391c: PCR-based Profiling Standard) and all next-generation STR typing kits.

Discordant Results Summary from NIST Studies

Marker	Kits with Correct Genotype	# Kits Compared	Correct Type	Kits with Null Allele/Discordant Genotype	Incorrect Type	Total Samples	Sequence Issue
Amel	ID/ESX17/ESI17/PP16/MiniFiler/Pro/SGM+/ESS/ESSplexSE/IDplex/NGMs/Hexaplex	13	X,Y	NGM*	Y,Y	657	yet to be determined
Amel	ID/ESX17/ESI17/PP16/MiniFiler/Pro/SGM+/ESS/ESSplexSE/IDplex/NGMs/Hexaplex	13	X,Y	NGM*	Y,Y	657	yet to be determined
Amel	ESS/ESSplexSE/IDplex/Hexaplex call	13	X,Y	ID/MiniFiler/ESI17/ESI17/PP16/NGM/NGMs/SGM+/PP18D	Y,Y	653	yet to be determined
CSF1PO	PP16/MiniFiler/IDplex/PP18D	5	11,11	ID call	11,11	656	1 bp ins in ID amplicon outside of PP16 and MiniFiler primers [2]
CSF1PO	ID/MiniFiler/IDplex/PP18D	5	9,12	PP16 call	12,12	662	C/T SNP 16 bp ds from repeat
D10S1248	ESX17/ESI17/NGM/NGMs/ESS/ESSplexSE	7	14,16	Hexaplex	14,14	653	G/T SNP 24 bp ds from repeat
D10S1248	ESX17/ESI17/NGM/NGMs/ESS/ESSplexSE	7	14,16	Hexaplex	14,14	653	G/T SNP 24 bp ds from repeat
D13S317	Pro/ID/PP16/IDplex	5	9,11	MiniFiler	11,11	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	5	9,13	MiniFiler	13,13	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	5	9,12	MiniFiler	12,12	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	5	8,12	MiniFiler	12,12	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	5	8,11	MiniFiler	11,11	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	5	10,13	MiniFiler	13,13	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	5	9,11	MiniFiler	11,11	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	5	9,12	MiniFiler	12,12	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	5	9,14	MiniFiler	14,14	656	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	4	20,22	MiniFiler	10,10	481	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	4	10,11	MiniFiler	11,11	481	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	4	8,10	MiniFiler	8,8	481	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro/ID/PP16/IDplex	4	10,12	MiniFiler	12,12	481	4 bp del in the rev MiniFiler primer binding site [2]
D16S539	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	11	12,13	ESX17	12,12	660	yet to be determined
D16S539	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	11	9,11	ESS/ESSplexSE	11,11	653	G/G SNP 10 bp us from repeat
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	11	9,11	MiniFiler	11,11	656	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	11	11,12	MiniFiler	12,12	656	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	11	9,11	MiniFiler	9,9	656	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	8	11,14	MiniFiler	14,14	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	8	9,11	MiniFiler	9,9	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	8	11,13	MiniFiler	13,13	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	8	11,12	MiniFiler	12,12	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	8	11,12	MiniFiler	12,12	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	8	9,12	MiniFiler	9,9	481	A/G SNP in MiniFiler primer binding site [2]
D18S51	ESX17/ESI17/PP16/MiniFiler/ESS/ESSplexSE/IDplex	12	13,15	ID/NGM/NGMs/Pro/SGM+	15,15	660	G/A SNP 172 bp ds from repeat
D18S51	ESX17/ESI17/PP16/MiniFiler/ESS/ESSplexSE/IDplex	7	13,14	ID/NGM/NGMs/Pro/SGM+	14,14	773	G/A SNP 32 bp ds from repeat
D19S433	ESX17/ESI17/PP16/MiniFiler/ESS/ESSplexSE/IDplex	7	13,14,2	ID/NGM/NGMs/ESS/IDplex	14,2,14,2	785	G/A SNP 32 bp ds from repeat
D19S433	ESX17/ESI17/PP16/MiniFiler/ESS/ESSplexSE/IDplex	7	14,15,3	ESX17	15,3,15,3	786	C/T SNP 30 bp us from repeat
D22S1045	miniSTR/23plex/NGMs/ESI17/ESS/ESSplexSE/Hexaplex	8	15,17	ESX17/NGM*	17,17	663	G/T SNP 15 bp us from repeat
D22S1045	miniSTR/23plex/NGMs/ESI17/ESS/ESSplexSE/Hexaplex	8	15,17	ESX17/NGM*	17,17	663	G/T SNP 15 bp us from repeat
D22S1045	miniSTR/23plex/NGMs/ESI17/ESS/ESSplexSE/Hexaplex	8	15,17	ESX17/NGM*	17,17	663	G/T SNP 15 bp us from repeat
D22S1045	miniSTR/23plex/NGMs/ESI17/ESS/ESSplexSE/Hexaplex	6	15,18	ESX17/NGM*	18,18	786	G/T SNP 15 bp us from repeat
D251338	PP18D	2	17,23	ID	23,23	50	G/A SNP 174 bp ds from repeat
D25441	ESX17/ESI17/23plex/ESS/NGMs	6	9,11,11	NGM*	11,11	780	A ins adjacent to the 5' end of repeat
D25441	ESX17/ESI17/23plex/ESS/NGMs	6	9,12,12	NGM*	12,12	780	A ins adjacent to the 5' end of repeat
D25441	ESX17/ESI17/23plex/ESS/NGMs	6	9,10,10	NGM*	10,10	780	A ins adjacent to the 5' end of repeat
D25441	ESX17/ESI17/23plex/ESS/NGMs	6	9,11,11	NGM*	11,11	780	A ins adjacent to the 5' end of repeat
D25441	ESX17/ESI17/23plex/ESS/NGMs	6	9,11,11	NGM*	11,11	780	A ins adjacent to the 5' end of repeat
D25441	ESX17/ESI17/23plex/ESS/NGMs	6	9,10,10	NGM*	10,10	780	A ins adjacent to the 5' end of repeat
D25441	ESX17/ESI17/23plex/ESS/NGMs	6	9,11,11	NGM*	11,11	780	A ins adjacent to the 5' end of repeat
D31S158	ESX17/ESI17/23plex/ESS/NGMs	8	14,17	ID/NGM/Pro/SGM+	14,14	785	G/C SNP 12 bp us from repeat
D7S820	Pro/ID	5	8,9,3	PP16/MiniFiler/IDplex	8,11	656	5 bp del 114 bp ds from repeat
D8S1179	ID/ESX17/ESI17/PP16/NGM/NGMs/SGM+/ESS/ESSplexSE/IDplex	8	14,15	Pro/SGM+	14,14	654	A/G SNP 15 bp us from repeat
SE33	ESX17/ESI17/NGMs/ESS/ESSplexSE	5	24,2,27,2	SE33 Monoplex (SE33)	26,2,26,2	663	C/T SNP 110 bp us from repeat
SE33	ESX17/ESI17/NGMs/ESS/ESSplexSE	5	24,2,28,2	SE33 Monoplex (SE33)	28,2,28,2	663	C/T SNP 110 bp us from repeat
SE33	ESX17/ESI17/NGMs/ESS/ESSplexSE	5	21,2,26,2	SE33 Monoplex (SE33)	21,2,21,2	663	C/T SNP 110 bp us from repeat
SE33	ESX17/ESI17/NGMs/ESS/ESSplexSE	5	24,2,25,2	SE33 Monoplex (SE33)	24,2,24,2	663	C/T SNP 110 bp us from repeat
SE33	SE33/NGMs	5	20,28,2	ESX17/ESS/ESSplexSE	20,28,2	663	T/G SNP 28 bp ds from repeat
SE33	SE33/NGMs	3	19,25,2	ESX17/ESS/ESSplexSE	19,19	663	C/T SNP 60 bp ds from repeat
SE33	SE33/NGMs	4	19,25,2	ESX17/ESS/ESSplexSE	19,25,3	663	C/T SNP 60 bp ds from repeat
SE33	SE33/ESX17/NGMs	5	13,2,18	ESX17/ESS/ESSplexSE	13,3,18	50	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	15,2,19	ESX17/ESS/ESSplexSE	15,3,19	50	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	15,2,28,2	ESX17/ESS/ESSplexSE	15,3,28,2	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	19,2,2,2	ESX17/ESS/ESSplexSE	19,2,3	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	16,2,18	ESX17/ESS/ESSplexSE	16,3,18	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	12,2,27,2	ESX17/ESS/ESSplexSE	12,3,27,2	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	16,2,21	ESX17/ESS/ESSplexSE	16,3,21	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	16,2,28,2	ESX17/ESS/ESSplexSE	16,3,28,2	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	13,2,19	ESX17/ESS/ESSplexSE	13,3,19	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	13,2,20	ESX17/ESS/ESSplexSE	13,3,20	789	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	15,2,27,2	ESX17/ESS/ESSplexSE	15,3,27,2	789	G/A SNP 68 bp ds from the repeat
SE33	ESX17/ESI17/SE33/NGMs	5	13,18	ESS/ESSplexSE	18,18	662	AAA del 85 bp us of the repeat
SE33	ESX17/ESI17/SE33/NGMs	5	15,17	ESS/ESSplexSE	15,15	662	AAA del 85 bp us of the repeat
SE33	ESX17/ESI17/SE33/NGMs	5	16,17	ESS/ESSplexSE	17,17	662	AAA del 85 bp us of the repeat
SE33	ESX17/ESI17/SE33/NGMs	5	15,19	ESS/ESSplexSE	15,19	662	AAA del 85 bp us of the repeat
TH01	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+/Hexaplex	12	6,7	ESS/ESSplexSE/IDplex	6,6	653	G/A SNP 37 bp ds from the repeat
vWA	ESS/ESSplexSE/IDplex	11	12,16	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	16,16	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	13,18	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	18,18	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	14,15	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	15,15	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	14,15	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	15,15	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	14,16	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	16,16	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	14,17	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	17,17	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	14,17	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	17,17	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	14,19	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	19,19	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	14,19	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	19,19	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	14,19	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	19,19	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	15,16	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	16,16	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	11	15,17	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	17,17	653	ATCCATCC del 4 bp ds from repeat
vWA	ESS/ESSplexSE/IDplex	8	13,15	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	15,15	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	13,15	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	15,15	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	13,16	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	16,16	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	13,17	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	17,17	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	13,19	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	19,19	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	14,16	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	16,16	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	14,17	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	17,17	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	14,17	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	17,17	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	14,18	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	18,18	780	ATCCATCC del 4 bp ds from repeat
vWA	ESS/IDplex	8	14,18	ID/MiniFiler/ESI17/PP16/NGM/NGMs/SGM+	18,18	780	ATCCATCC del 4 bp ds from repeat

NIST Strategies for Concordance Testing
NIST Four "S's" of Concordance: (a) NIST in-house samples are run with multiple kits/in-house assays to compare genotyping results; (b) concordance software is used to detect the differences between the results; (c) DNA sequencing is performed to confirm the results and determine the cause of the null allele; and (d) the final discordant results and verified null alleles are reported to the forensic community on STRBase: <http://www.cstl.nist.gov/strbase/NullAlleles.htm> [1]



NIST Samples Tested (~1450 Samples)
NIST U.S. population samples
• 260 African American, 262 Caucasian, 140 Hispanic, 3 Asian
U.S. father/son paired samples
• 200 African American, 200 Caucasian, 200 Hispanic, 202 Asian
50 anonymous blood samples
• 46 African American, 4 Caucasian
NIST SRM 2391c, PCR-based DNA Profiling Standard
• 4 genomic samples (with one mixture)
• 2 cell line samples (FTA and 903 paper)

Commercial and In-house STR Multiplex Kits Used in NIST Concordance Studies

Applied Biosystems AmpFSTR Kits	Promega PowerPlex Systems	Qiagen Investigator HID Kits	In-House NIST Assays
Identifiler	PowerPlex 16	ESSplex	26plex [4]
MiniFiler [2]	PowerPlex ESX 17 [3]	ESSplex SE	miniSTRs [5,6]
Profiler Plus	PowerPlex ESI 17 [3]	Hexaplex ESS	
SGM Plus	*PowerPlex 18D	IDplex	
NGM			
NGM SSelect			