Concordance Testing Comparing STR Multiplex Kits with a Standard Data Set

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Poster available for download from STRBase: http://www.cstl.nist.gov/strbase/pub_pres/Hill-ISHI2011-Concordance.pdf

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 [1]. 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 allele dropout may occur due to primer binding site mutations that affect one set of primers but not another [2]. These null alleles become evident only when data sets are compared. Null alleles are a concern because they can result in a false-negative or incorrect exclusion of samples that come from a common source (if the samples have been typed with kits using different PCR primers). A base pair change in the DNA template at the PCR primer binding region can disrupt primer hybridization and result in a failure to amplify and detect an existing allele [2].

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 including Applied Biosystems Identifiler, MiniFiler, NGM, NGM SElect, SGM Plus, and Profiler Plus kits, Promega PowerPlex 16, ESX 17 and ESI 17 Systems, and Qiagen ESSplex, ESSplex SE, IDplex, and Hexaplex kits [3-8]. 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 web page of STRBase [9]. A summary of the results of our concordance studies will be shown in order to help assess 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 allele 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.

(a) Samples and multiplex kits tested

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 AmpFℓSTR Kits	Promega PowerPlex Systems	Qiagen Investigator HID Kits	In-House NIST Assays
Identifiler [3]	PowerPlex 16	ESSplex	miniSTRs [6,7]
MiniFiler [4]	PowerPlex ESX 17 [5]	ESSplex SE	26plex [8]
Profiler Plus	PowerPlex ESI 17 [5]	Hexaplex ESS	
SGM Plus	*PowerPlex 18D	IDplex	
NGM			
NGM SElect			

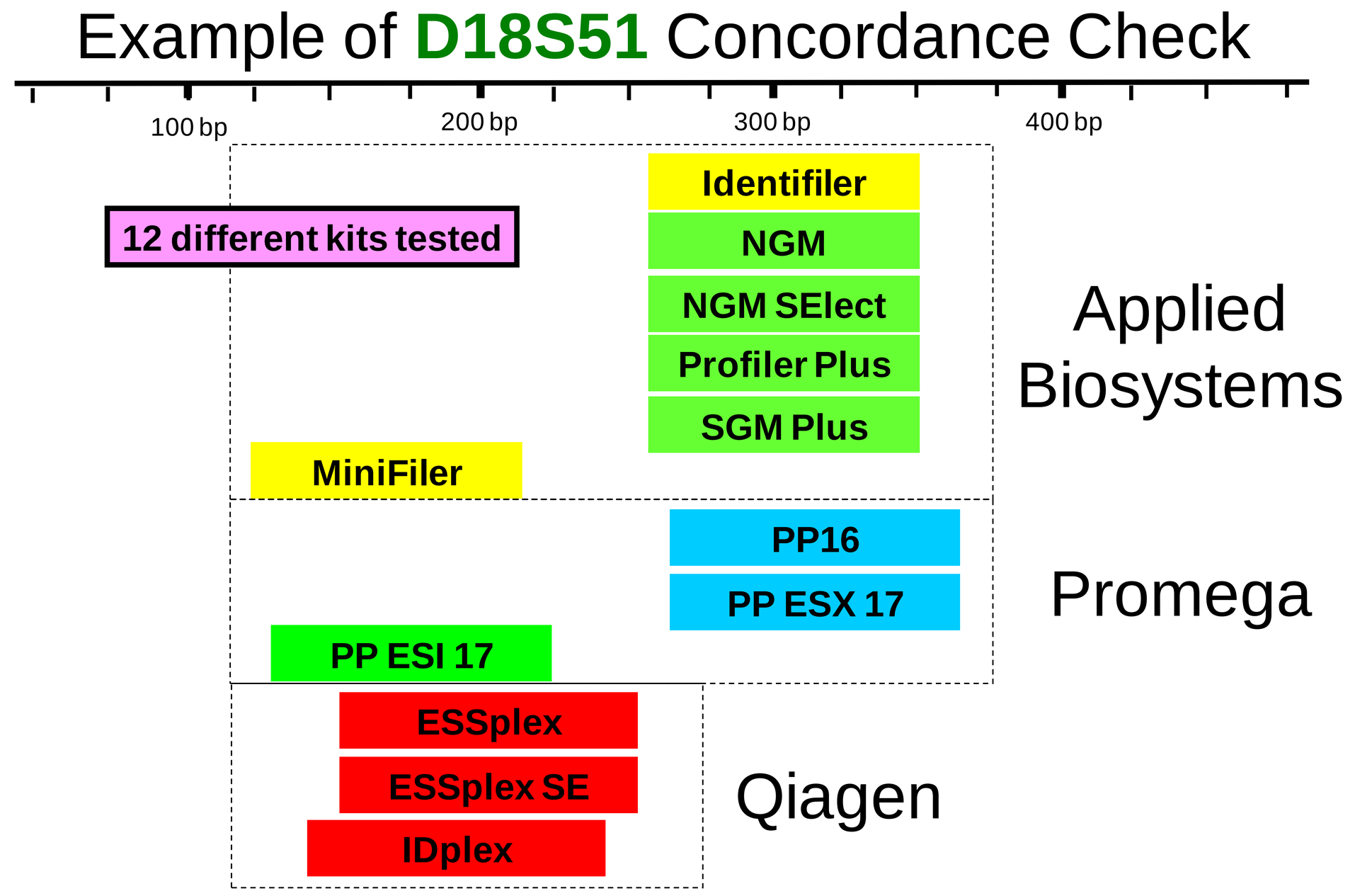
*PowerPlex 18D studies were performed by Erica Butts and Peter Vallone at NIST

(b) Software used to perform concordance checks

Numbers of Primer Sets Compared for Each Marker

Marker	# of Sets	Marker	# of Sets
Amelogenin	13	D2S441	9
D18S51	12	D19S433	9
D21S11	12	D1S1656	7
FGA	12	D12S391	7
D3S1358	11	SE33	5
TH01	11	D5S818	4
D16S539	11	D7S820	4
vWA	11	D13S317	4
D8S1179	11	TPOX	3
D2S1338	10	CSF1PO	4
D10S1248	9	Penta D	1
D22S1045	9	Penta E	1

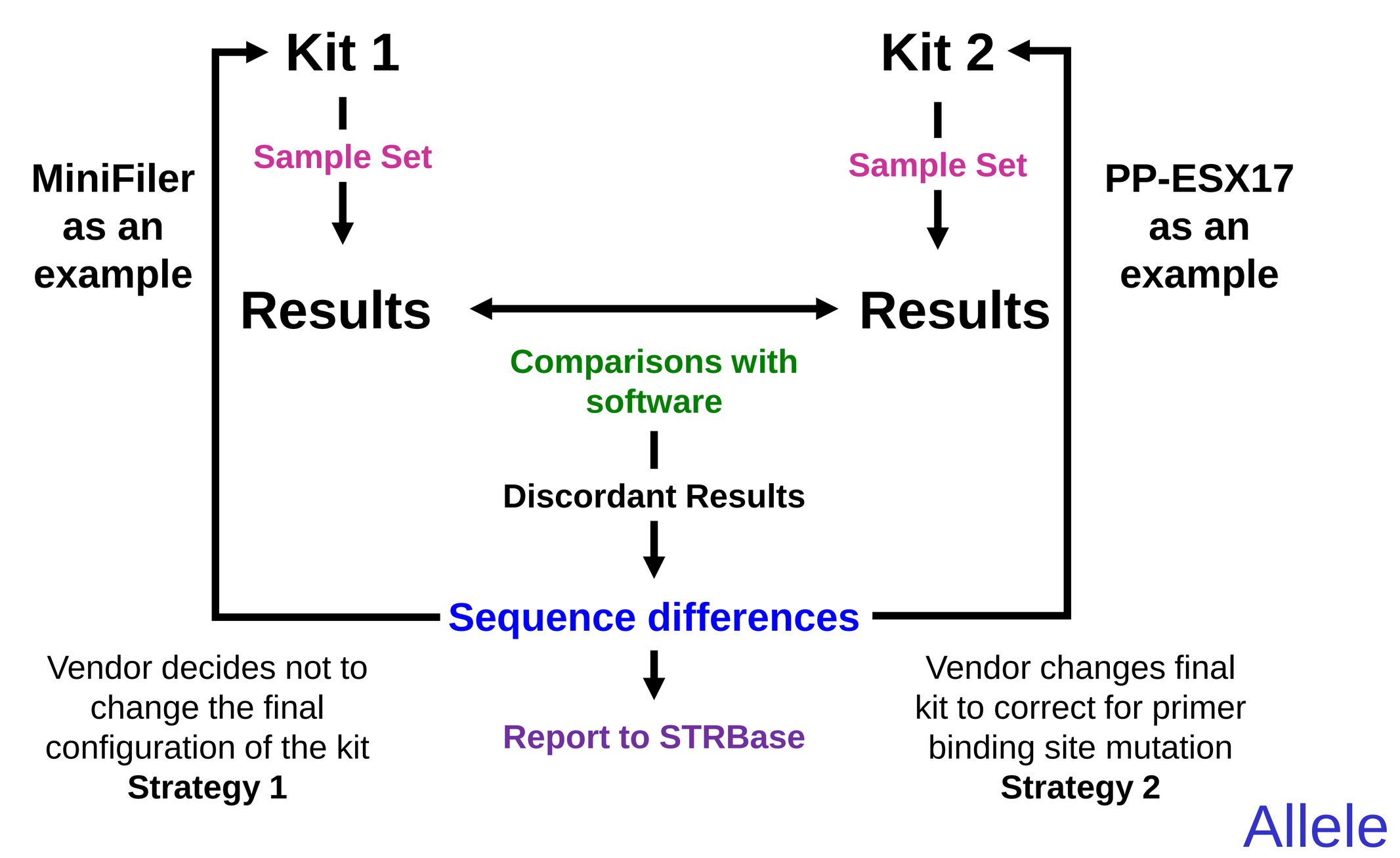
Example of D18S51 Concordance Check



http://www.cstl.nist.gov/strbase/software.htm (STR_MatchSamples)

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]



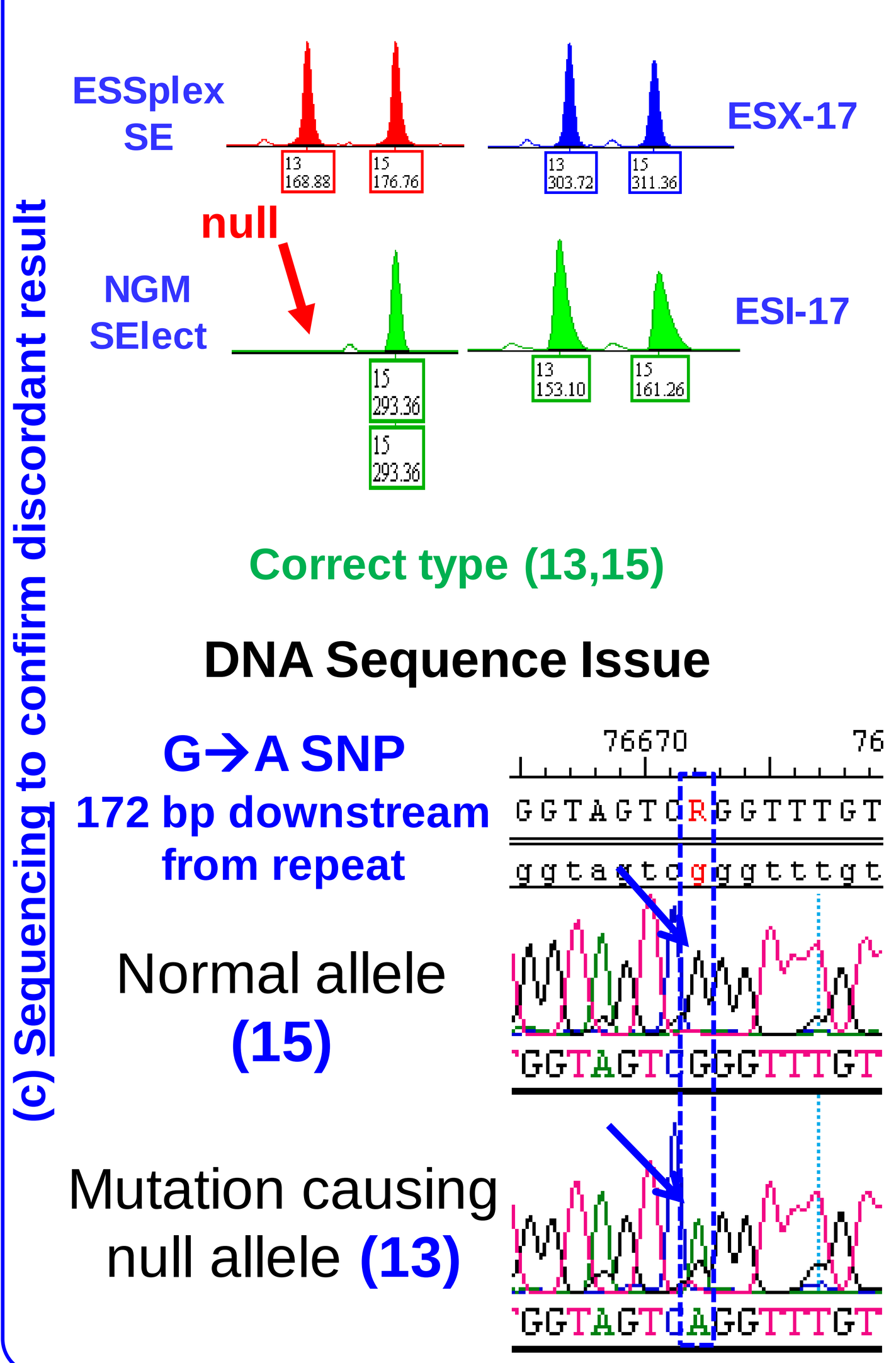
Final Concordance %

948,301 allele comparisons
1,109 total differences
99.88% concordance

(c) Sequencing to confirm discordant result

Example Discordant Profiles

D18S51 Null Allele



(d) STRBase reports results

Null alleles and discordant results are reported to the forensic community on STRBase:

http://www.cstl.nist.gov/strbase/NullAlleles.htm

They are also reported to collaborating commercial vendors
- Several null alleles have been remedied prior to the final release of new STR multiplex kits

Alleles Impacted by Insertions, Deletions, or Primer Binding Site Mutations

Marker	Impacted Alleles
Amel	X
CSF1PO	11.1
CSF1PO	9
D10S1248	16
D13S317	8,9,10
D16S539	13
D16S539	9
D16S539	11,12
D18S51	13
D19S433	13
D1S1656	14
D22S1045	15
D2S1338	17
D2S441	9.1
D3S1358	17
D7S820	9.3 or 11
D8S1179	15
SE33	24.2,25.2,26.2,27.2
SE33	28.3 or 29.2
SE33	13.2,15.2,16.2,23.2,25.2
SE33	13,15,16,17
TH01	7
vWA	12,13,14,15

Allele Comparisons Performed

Kits compared	Samples	Loci Compared	Comparisons	# Differences	Concordance (%)
ID-SGM+	1424	11	15,664	1	99.994
ID-Pro+	1415	10	14,150	1	99.993
ID-IDplex	1426	16	22,816	29	99.873
ID-PP16	662	14	9,268	4	99.957
ID-MiniFiler	1137	9	10,233	26	99.746
ID-NGM	1437	11	15,807	3	99.981
ID-NGMs	663	11	7,293	0	100.000
ID-ESX17	1443	11	15,873	5	99.968
ID-ESI17	1443	11	15,873	4	99.975
ID-ESSplex	1433	11	15,763	28	99.822
ID-ESSplexSE	662	11	7,282	17	99.767
ID-Hexaplex	653	2	1,306	1	99.923
PP16-SGM+	651	9	5,859	1	99.983
PP16-Pro+	647	10	6,470	2	99.969
PP16-IDplex	657	14	9,198	3	99.967
PP16-MiniFiler	656	8	5,248	14	99.733
PP16-NGM	657	9	5,913	3	99.949
PP16-NGMs	662	9	5,958	1	99.983
PP16-ESX17	662	9	5,958	1	99.983
PP16-ESI17	662	9	5,958	0	100.000
PP16-ESSplex	653	9	5,877	16	99.728
PP16-ESSplexSE	662	9	5,958	16	99.731
PP16-Hexaplex	653	2	1,306	1	99.923
SGM+-Pro+	1415	7	9,905	0	100.000
SGM+-IDplex	1424	11	15,664	5	99.968
SGM+-MiniFiler	1137	6	6,822	10	99.853
SGM+-NGM	1424	11	15,664	4	99.974
SGM+-NGMs	651	11	7,161	0	100.000
SGM+-ESX17	1424	11	15,664	6	99.962
SGM+-ESI17	1424	11	15,664	5	99.968
SGM+-ESS	1424	11	15,664	5	99.968
SGM+-ESSplexSE	651	11	7,161	5	99.930
SGM+-Hexaplex	651	2	1,302	1	99.923
Pro+-IDplex	1415	10	14,150	5	99.965
Pro+-MiniFiler	1137	6	6,822	16	99.765
Pro+-NGM	1415	7	9,905	4	99.960
Pro+-NGMs	647	7	4,529	0	100.000
Pro+-ESX17	1415	7	9,905	4	99.960
Pro+-ESI17	1415	7	9,905	3	99.970
Pro+-ESS	1415	7	9,905	4	99.960
Pro+-ESSplexSE	647	7	4,529	4	99.912
Pro+-Hexaplex	647	1	647	1	99.845
IDplex-MiniFiler	1137	9	10,233	48	99.531
IDplex-NGM	1426	11	15,686	30	99.809
IDplex-NGMs	657	11	7,227	17	99.765
IDplex-ESX17	1426	11	15,686	28	99.821
IDplex-ESI17	1426	11	15,686	27	99.828
IDplex-ESS	1426	11	15,686	1	99.994
IDplex-ESSplexSE	657	11	7,227	1	99.986
IDplex-Hexaplex	653	2	1,306	1	99.923
MiniFiler-NGM	1137	6	6,822	13	99.809
MiniFiler-NGMs	656	6	3,936	10	99.746
MiniFiler-ESX17	1137	6	6,822	10	99.853
MiniFiler-ESI17	1137	6	6,822	9	99.868
MiniFiler-ESS	1137	6	6,822	35	99.487
MiniFiler-ESSplexSE	656	6	3,936	35	99.111
MiniFiler-Hexaplex	653	1	653	1	99.847
NGM-NGMs	657	16	10,512	14	99.867
NGM-ESX17	1437	16	22,992	16	99.930
NGM-ESI17	1437	16	22,992	18	99.922
NGM-ESS	1433	16	22,928	42	99.817
NGM-ESSplexSE	657	16	10,512	22	99.791
NGM-Hexaplex	653	7	4,571	9	99.803
NGMs-ESX17	662	17	11,254	4	99.964
NGMs-ESI17	662	17	11,254	14	99.876
NGMs-ESS	653	16	10,448	17	99.837
NGMs-ESSplexSE	662	17	11,254	34	99.698
NGMs_Hexaplex	653	7	4,571	3	99.934
ESX17-ESI17	1443	17	24,531	19	99.923
ESX17-ESS	653	16	10,448	34	99.675
ESX17-ESSplexSE	662	17	11,254	25	99.778
ESX17-Hexaplex	657	7	4,599	6	99.870
ESI17-ESS	653	16	10,448	28	99.732
ESI17-ESSplexSE	662	17	11,254	30	99.733
ESI17-Hexaplex	657	7	4,599	3	99.935
ESS-ESSplexSE	653	16	10,448	0	100.000
ESS-Hexaplex	653	7	4,571	3	99.934
ESSplexSE-Hexaplex	653	7	4,571	3	99.934
SE33-ESX17	1443	1	1,443	6	99.584
SE33-ESI17	1443	1	1,443	17	98.822
SE33-NGMs	663	1	663	4	99.397
SE33-ESSplexSE	662	1	662	21	96.828
ESI17p-ESX17	477	17	8,109	7	99.914
ESI17p-NGMs	477	17	8,109	2	99.975
ESI17p-ESSplexSE	477	17	8,109	42	99.482
ESI17p-SE33	477	1	477	4	99.161
PP18D-ID	50	16	800	2	99.750
PP18D-PP16	703	16	11,248	1	99.991
ESX17*/ESX17	1443	17	24531	4	99.984
ESX17*/ESI17p	477	17	8109	3	99.963
ESX17*/NGM	1437	16	22992	22	99.904
ESX17*/NGMs	663	17	11271	4	99.965
ESX17*/ESS	1433	16	22928	30	99.869
ESX17*/ESSplexSE	662	17	11254	44	99.609
ESX17*/Hexaplex	653	7	4571	2	99.956
26plex/ESX17	1443	3	4329	4	99.908
26plex/ESI17	1443	3	4329	0	100.000
26plex/NGM	1437	3	4311	11	99.745
26plex/NGMs	663	3	1989	0	100.000
26plex/ESS	1433	3	4299	0	100.000
26plex/ESSplexSE	662	3	1986	0	100.000
26plex/Hexaplex	653	3	1959	2	99.898
26plex/ESX17*	663	3	1989	0	100.000
miniSTRs/ESX17	663	3	1989	3	99.849
miniSTRs/ESI17	663	3	1989	0	100.000
miniSTRs/NGM	657	3	1971	3	99.848
miniSTRs/NGMs	663	3	1989	0	100.000
miniSTRs/ESS	653	3	1959	0	100.000
miniSTRs/ESSplexSE	662	3	1986	0	100.000
miniSTRs/Hexaplex	653	3	1959	2	99.898
miniSTRs/ESX17*	663	3	1989	0	100.000
Totals	102345	1021	948301	1109	99.883

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	ID/ESX17/ESI17/Pro+/SGM+/ESS/IDplex	8	X,Y	NGM*	Y,Y	780	yet to be determined
Amel	ESS/ESSplexSE/IDplex/Hexaplex call	13	X,Y	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+/PP18D	Y,Y	653	yet to be determined
CSF1PO	PP16/MiniFiler/IDplex/PP18D	5	11,11	ID call	11,11.1	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, 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/IDplex	4	9,10	MiniFiler	10,10	481	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro+/ID/IDplex	4	10,11	MiniFiler	11,11	481	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro+/ID/IDplex	4	8,10	MiniFiler	8,8	481	4 bp del in the rev MiniFiler primer binding site [2]
D13S317	Pro+/ID/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/ESX17/PP16/NGM/NGMs/SGM+/IDplex	11	9,11	ESS/ESSplexSE	11,11	653	C/G SNP 10 bp us from repeat
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	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/NGM/SGM+/ESS/IDplex	8	11,14	MiniFiler	14,14	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/NGM/SGM+/ESS/IDplex	8	9,11	MiniFiler	9,9	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/NGM/SGM+/ESS/IDplex	8	11,13	MiniFiler	13,13	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/NGM/SGM+/ESS/IDplex	8	11,12	MiniFiler	12,12	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/NGM/SGM+/ESS/IDplex	8	11,12	MiniFiler	12,12	481	A/G SNP in MiniFiler primer binding site [2]
D16S539	ID/ESX17/ESI17/NGM/SGM+/ESS/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
D19S433	ESX17/ESI17	7	13,14	ID/NGM/SGM+/ESS/IDplex	14,14	785	G/A SNP 32 bp ds from repeat
D19S433	ESX17/ESI17	7	13,14.2	ID/NGM/SGM+/ESS/IDplex	14.2,14.2	785	G/A SNP 32 bp ds from repeat
D1S1656	ESI17/NGM/ESSplex	4	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	23plex/ESI17/NGMs/ESS	6	15,16	ESX17*/NGM*	16,16	789	G/T SNP 15 bp us from repeat
D2S1338	PP18D	2	17,23	ID	23,23	50	G/A SNP 174 bp ds from repeat
D2S441	ESX17/ESI17/23plex/ESS/NGMs	6	9.1,11	NGM*	11,11	780	A ins adjacent to the 5' end of repeat
D2S441	ESX17/ESI17/23plex/ESS/NGMs	6	9.1,12	NGM*	12,12	780	A ins adjacent to the 5' end of repeat
D2S441	ESX17/ESI17/23plex/ESS/NGMs	6	9.1,10	NGM*	10,10	780	A ins adjacent to the 5' end of repeat
D2S441	ESX17/ESI17/23plex/ESS/NGMs	6	9.1,11	NGM*	11,11	780	A ins adjacent to the 5' end of repeat
D2S441	ESX17/ESI17/23plex/ESS/NGMs	6	9.1,11	NGM*	11,11	780	A ins adjacent to the 5' end of repeat
D2S441	ESX17/ESI17/23plex/ESS/NGMs	6	9.1,10	NGM*	10,10	780	A ins adjacent to the 5' end of repeat
D2S441	ESX17/ESI17/23plex/ESS/NGMs	6	9.1,11	NGM*	11,11	780	A ins adjacent to the 5' end of repeat
D3S1358	ESX17/ESI17/ESS/IDplex	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/NGM/ESS/IDplex	8	14,15	Pro+/SGM+	14,14	773	A/G SNP 15 bp us from repeat
SE33	ESX17/ESI17/NGMs/ESSplexSE	5	26.2,27.2	SE33 Monoplex (SE33)	26.2,26.2	663	C/T SNP 110 bp us from repeat
SE33	ESX17/ESI17/NGMs/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/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/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	20,29.2	ESX17/ESI17/ESSplexSE	20,28.3	663	TTG del 28 bp ds from repeat
SE33	SE33/NGMs	3	19,25.2	ESX17	19,19	663	C/T SNP 60 bp ds from repeat
SE33	SE33/NGMs	4	19,25.2	ESI17*/ESSplexSE	19,25.3	663	C/T SNP 60 bp ds from repeat
SE33	SE33/ESX17/NGMs	5	13.2,18	ESI17*/ESSplexSE	13.3,18	50	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	15.2,19	ESI17*/ESSplexSE	15.3,19	50	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	15.2,28.2	ESI17*/ESSplexSE	15.3,28.2	50	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	19,23.2	ESI17*/ESSplexSE	19,23.3	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	16.2,18	ESI17*/ESSplexSE	16.3,18	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	12.2,27.2	ESI17*/ESSplexSE	12.3,27.2	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	16.2,21	ESI17*/ESSplexSE	16.3,21	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	16.2,28.2	ESI17*/ESSplexSE	16.3,28.2	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	13.2,19	ESI17*/ESSplexSE	13.3,19	663	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	13.2,20	ESI17*/ESSplexSE	13.3,20	789	G/A SNP 68 bp ds from the repeat
SE33	SE33/ESX17/NGMs	5	15.2,27.2	ESI17*/ESSplexSE	15.3,27.2	789	G/A SNP 68 bp ds from the repeat
SE33	ESX17/ESI17/SE33/NGMs	5	13,18	ESSplexSE	18,18	662	AAAA del 85 bp us of the repeat
SE33	ESX17/ESI17/SE33/NGMs	5	15,17	ESSplexSE	15,15	662	AAAA del 85 bp us of the repeat
SE33	ESX17/ESI17/SE33/NGMs	5	16,17	ESSplexSE	17,17	662	AAAA del 85 bp us of the repeat
SE33	ESX17/ESI17/SE33/NGMs	5	15,19	ESSplexSE	19,19	662	AAAA del 85 bp us of the repeat
TH01	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+/Hexaplex	12	6,7	ESSplex/ESSplexSE/IDplex	6,6	653	G/A SNP 37 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	12,16	ESS/ESSplexSE/IDplex	16,16	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	13,18	ESS/ESSplexSE/IDplex	18,18	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,15	ESS/ESSplexSE/IDplex	15,15	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,15	ESS/ESSplexSE/IDplex	15,15	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,16	ESS/ESSplexSE/IDplex	16,16	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,17	ESS/ESSplexSE/IDplex	17,17	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,17	ESS/ESSplexSE/IDplex	17,17	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,17	ESS/ESSplexSE/IDplex	17,17	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,19	ESS/ESSplexSE/IDplex	19,19	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,19	ESS/ESSplexSE/IDplex	19,19	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	14,19	ESS/ESSplexSE/IDplex	19,19	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	15,16	ESS/ESSplexSE/IDplex	16,16	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/PP16/NGM/NGMs/SGM+	11	15,17	ESS/ESSplexSE/IDplex	17,17	653	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	13,15	ESS/IDplex	15,15	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	13,15	ESS/IDplex	15,15	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	13,16	ESS/IDplex	16,16	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	13,17	ESS/IDplex	17,17	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	13,19	ESS/IDplex	19,19	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	14,16	ESS/IDplex	16,16	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	14,17	ESS/IDplex	17,17	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	14,17	ESS/IDplex	17,17	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	14,18	ESS/IDplex	18,18	780	ATCCATCC del 4 bp ds from repeat
vWA	ID/MiniFiler/ESI17/ESX17/NGM/SGM+	8	14,18	ESS/IDplex	18,18	780	ATCCATCC del 4 bp ds from repeat

*Primer sequences were added or changed to correct the sequence issue and now result in the correct genotype: NGM and NGM SElect (D2S441, D22S1045, Amelogenin), PP ESX 17 (D22S1045), PP ESI 17 Pro (SE33)

Total Number of Samples

Total number of samples is different for each comparison because samples exhibiting full locus dropout were removed from the sample set. Also, some kits have been analyzed with the full sample set (population samples *and* father/son samples) while other kits were only tested with the population samples. Total samples are listed as either total amount of population (~665) or father/son samples (~802). The final total number of samples listed in the table is based on the concordance evaluation in which the null allele/incorrect allele call was discovered.

Abbreviations	
bp	base pair
SNP	single nucleotide polymorphism
ins	insertion
del	deletion
us	upstream
ds	downstream
ID	Identifiler
Pro+	Profiler Plus
SGM+	SGM Plus
NGMs	NGM SElect
ESS	ESSplex
ESX17	PowerPlex ESX 17
ESI17	PowerPlex ESI 17
PP16	PowerPlex 16
PP18D	PowerPlex 18D
Amel	Amelogenin

SRM 2391c Concordance

- Every next-generation STR multiplex kit is tested with NIST SRM 2391c, the new PCR-based Profiling Standard (**see Poster #41**)
- All tested kits are fully concordant with the certified values of all markers for each component

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