NIST Concordance Evaluations to Assist in the Improvement of Commercial STR Multiplex Kits

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National Institute of Standards and Technology

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Outline of Topics to Discuss

- Introduction and importance of concordance testing
- NIST role in concordance testing
- Commercial STR multiplex kits examined
- Concordance results with various STR multiplex kits
- Summary and conclusions

Why are concordance studies important?

Importance of Concordance Testing

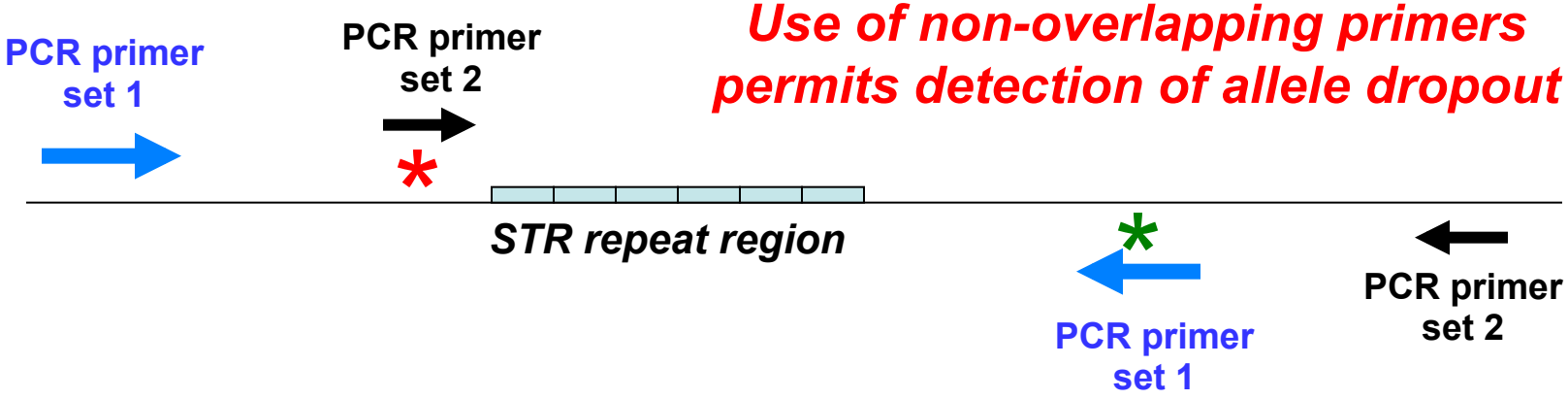
- There are a variety of commercial STR multiplex kits with different configurations of STR markers
  - Different primer sequences are used to amplify the same markers
  - Discordant results can impact DNA databases
- Detection of primer binding site mutations that cause **null alleles**, or allele drop-out
  - Can only be determined with concordance testing and DNA sequencing
- Concordance with NIST reference materials
  - Important to test with all new STR typing kits

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

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

* represents potential mutations impacting primer annealing

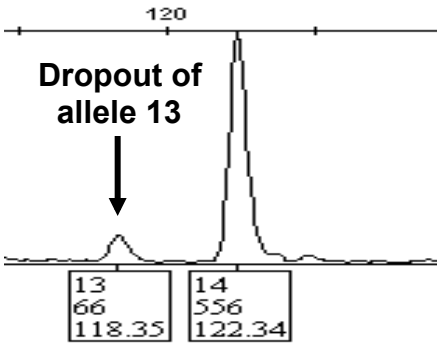


If no primer binding site mutations

If a primer binding site mutation exists

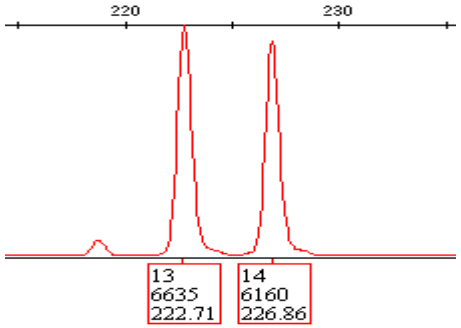
Example Primer Binding Site Mutation that Causes a Null Allele

Identifiler = 14,14



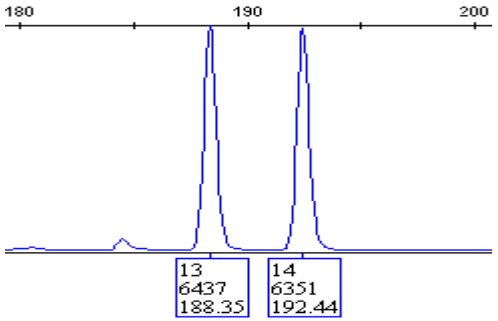
PHR = 11.9%

PP ESX 17 = 13,14



PHR = 92.8%

PP ESI 17 = 13,14



PHR = 98.7%

G → A SNP

D19S433 repeat region

ggaaagaaggtaggaaggaaggaaggaagtaagtaaggaaggaaggaaggaaggaaggaaggagagaggaagaaagagagaagatttttattcgggtaat

X

This region could potentially represent where the reverse primer is located to include the primer binding site mutation

*Applied Biosystems does not publish their primer sequences

To Avoid Overlapping PCR Product Size Ranges with STR Loci in the Same Dye Channel

- Life Technologies (Strategy 1)
  - **Maintains primer sequences** (except MiniFiler & NGM kits)
  - Utilizes mobility modifiers or additional dyes, no primer redesign is necessary
  - Enables comparison to legacy data with earlier kits but null alleles may go undetected with the potential for incorrect genotypes within data sets

- Promega Corporation (Strategy 2)
  - Moves primer sequences to change PCR product size ranges
  - Primer redesign can be difficult, but can be moved from primer-binding-site mutations
  - **Requires concordance studies to check for potential allele dropout**

Why is NIST involved in concordance studies?

Purpose of Concordance Studies

1. To test SRM 2391c (PCR-based DNA Profiling Standard) components with all new STR multiplex kits and verify results against certified reference values

2. To gain a better understanding of primer binding site mutations that cause null alleles

What are the NIST strategies for concordance testing?

STR Kit Concordance Testing

Profiles in DNA Article Published April 2010

Article Type: Feature

Volume 13 No. 1, April 2010

Strategies for Concordance Testing

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Concordance evaluations are important to conduct to determine if there are any allelic dropout or "null alleles" present in a data set. These studies are performed because there are a variety of commercial short tandem repeat (STR) multiplex kits with different configurations of STR markers available to the forensic community. The placement 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.

http://www.promega.com/profiles/1301/1301_08.html

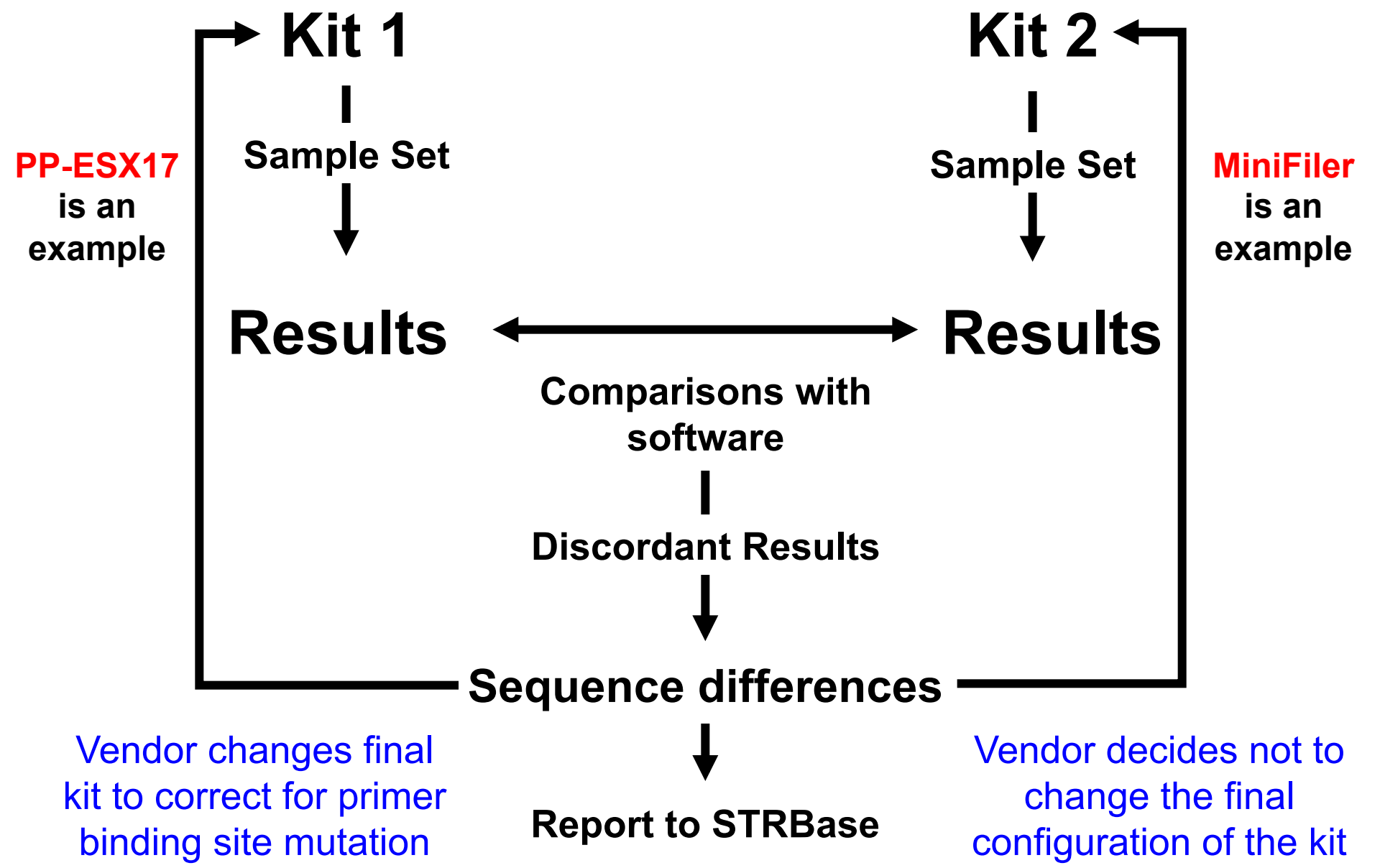
The 4 “S’s” of Concordance

- NIST Standard Samples
  - Run same samples with multiple kits to compare results
- Concordance Software
  - Allows comparison of data sets using NIST developed software

    http://www.cstl.nist.gov/biotech/strbase/software.htm
- DNA Sequencing
  - To validate and determine the exact cause for the null allele
- STRBase website
  - To report verified null alleles and discordant results to the forensic community

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

NIST Concordance Testing Steps



What concordance studies have been completed thus far?

Life Technologies AmpFℓSTR Kits

- Identifiler
- **MiniFiler**
- Profiler Plus
- SGM Plus
- NGM
- NGM SElect
- Yfiler Plus

Hill, C.R., Kline, M.C., Mulero, J.J., Lagace, R.E., Chang, C.-W., Hennessy, L.K., Butler, J.M. (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci.* 52(4): 870-873.

Promega PowerPlex Systems

- PowerPlex 16/16HS
- **PowerPlex ESX 17** (& Fast)
- **PowerPlex ESI 17** (& Fast)
- PowerPlex ESI 17 Pro
- PowerPlex 18D (rapid and direct kit)
- PowerPlex 21
- PowerPlex Fusion
- PowerPlex Fusion 6C
- PowerPlex Y23

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems

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Qiagen Investigator HID Kits

- ESSplex
- ESSplex Plus
- ESSplex SE
- ESSplex SE Plus
- Hexaplex ESS
- IDplex
- IDplex Plus
- 24plex
- 24plex GO!

What samples are used at NIST to perform concordance testing?

NIST Sample Set (>1450 Samples)

- **NIST U.S. population samples**
  - 260 African American, 260 Caucasian, 140 Hispanic, 3 Asian
- **U.S. father/son paired samples**
  - **~100 fathers/100 sons for each group**: 200 African American, 200 Caucasian, 200 Hispanic, 200 Asian
- **NIST SRM 2391b**, PCR-based DNA Profiling Standard (highly characterized)
  - 10 genomic DNA samples, 2 cell line samples
  - Includes 9947A and 9948
- **NIST SRM 2391c**, PCR-based DNA Profiling Standard
  - 4 genomic DNA (one mixture)
  - 2 cell lines (903 and FTA paper)

Publications using NIST Population Samples

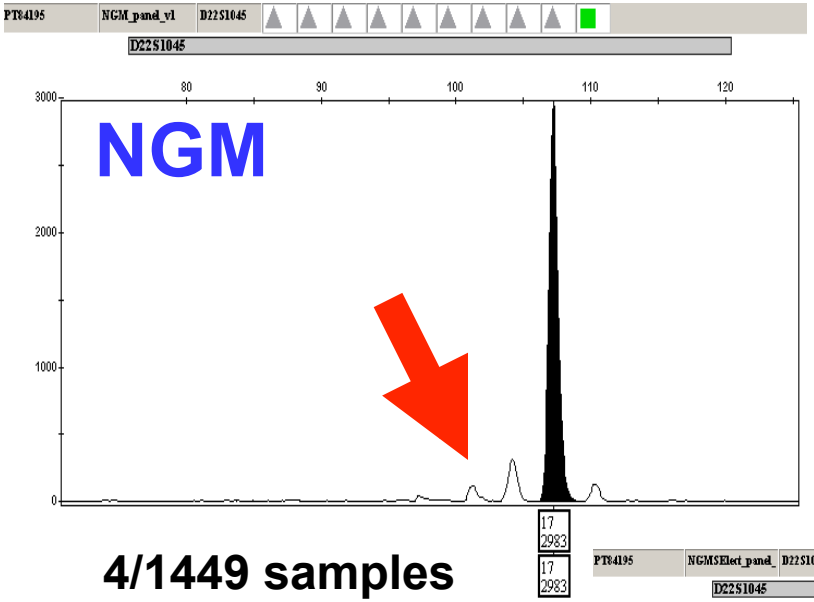
Data available at
http://www.cstl.nist.gov/strbase/NISTpop.htm

1. Butler et al. (2003) *J. Forensic Sci.* – Identifiler allele frequencies
2. Butler et al. (2003) *J. Forensic Sci.* – miniSTR assay development
3. Drabek et al. (2004) *J. Forensic Sci.* – miniSTR concordance
4. Schoske et al. (2004) *Forensic Sci. Int.* – Y-STR 20plex & 11plex
5. Vallone et al. (2004) *J. Forensic Sci.* – 50 Y-SNPs
6. Coble & Butler (2005) *J. Forensic Sci.* – NC01 & NC02 assay development
7. Butler et al. (2005) *J. Forensic Sci.* – PowerPlex Y with Y-STR duplications & triplications
8. Vallone et al. (2005) *Forensic Sci. Int.* – 70 autosomal SNPs
9. Butler et al. (2006) *Forensic Sci. Int.* – 27 Y-STR additional loci
10. Hill et al. (2007) *J. Forensic Sci.* – MiniFiler concordance
11. Decker et al. (2008) *FSI Genetics* - Yfiler mutation rates
12. Saunier et al. (2008) *FSI Genetics* – mtDNA control region sequencing (AFDIL)
13. Just et al. (2008) *FSI Genetics* – mtGenome analysis (AFDIL)
14. Hill et al. (2008) *J. Forensic Sci.* – NC01-NC09 miniSTR loci
15. Diegoli et al. (2009) *FSI Genetics* – mtDNA control region sequencing (AFDIL)
16. Hill et al. (2009) *J. Forensic Sci.* – NIST 26plex
17. Lao et al. (2010) *Human Mutation* – 24 ancestry SNPs, Y-SNPs, mtDNA
18. Hill et al. (2011) *FSI Genetics* – ESI 17 & ESX 17 concordance
19. Diegoli et al. (2011) *FSI Genetics Suppl. Ser.* – Argus X-12 X-STR loci
20. Fondevila et al. (2012) *Int. J. Legal Med*. – 68 InDel loci
21. Fondevila et al. (2012) *FSI Genetics* – 34 ancestry SNPs
22. Butler et al. (2012) *Profiles in DNA* – introduces NIST 1036 data set
23. Hill et al. (2013) *FSI Genetics* – 29 autosomal STRs in PowerPlex CS7 and other kits
24. Coble et al. (2013) *FSI Genetics (in press)* – 23 Y-STRs in PowerPlex Y23

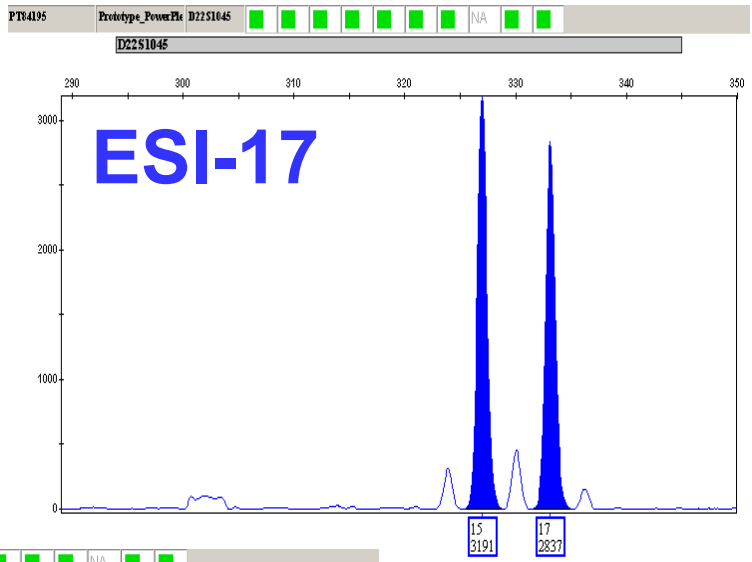
Testing also completed with 16 X-STR loci and 14 rapidly mutating (RM) Y-STRs

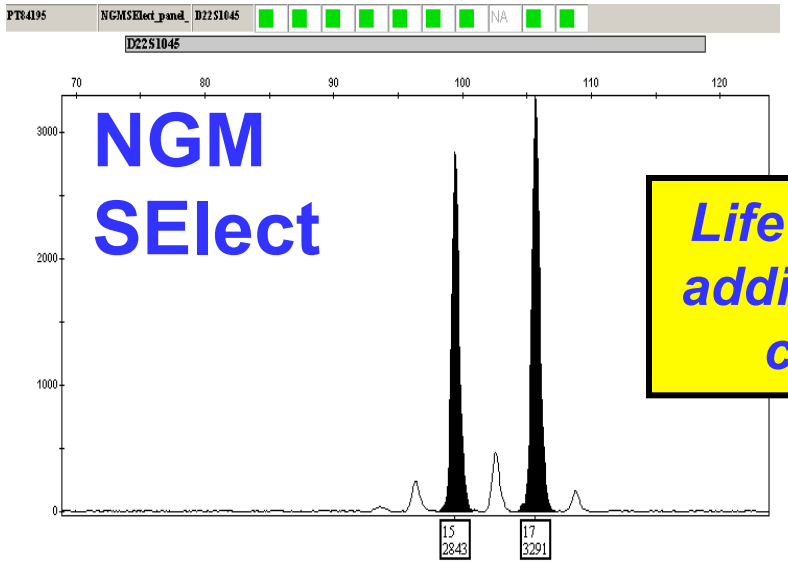
What are the results from the completed concordance studies?

D22S1045 Null Allele



4/1449 samples



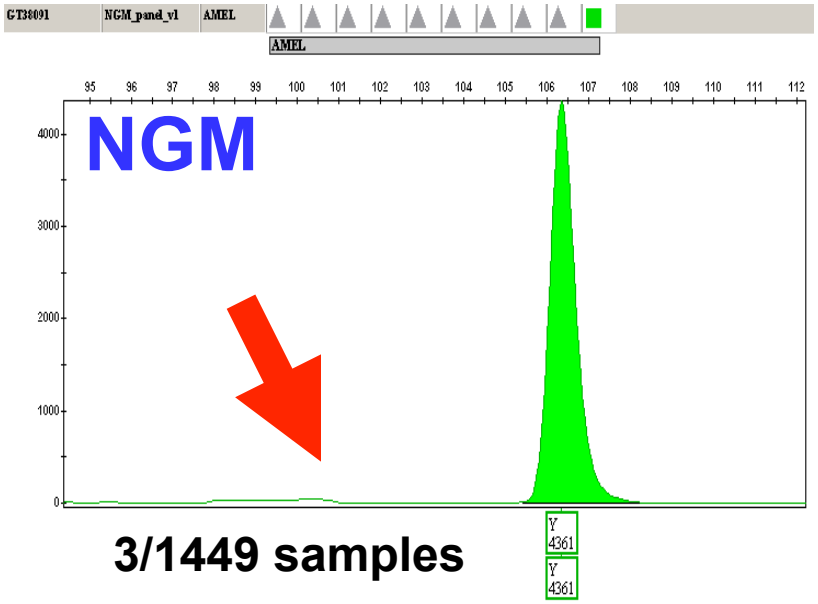


Correct type (15,17)

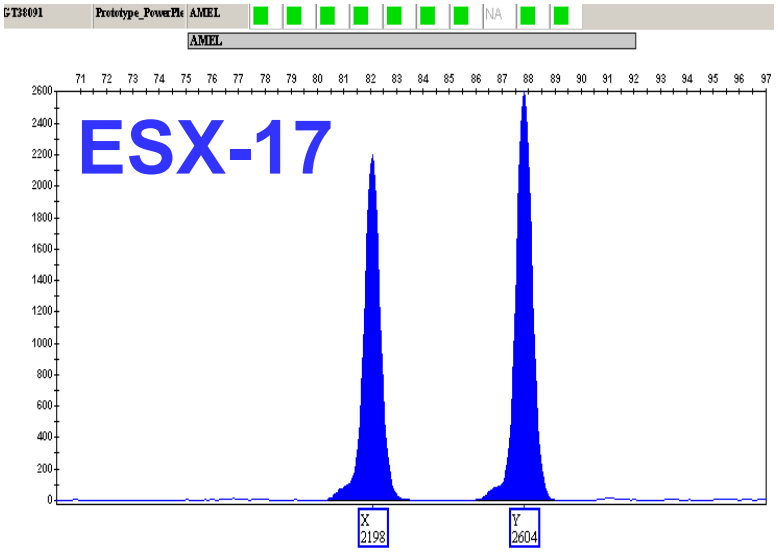
Life Tech added an additional primer to correct issue

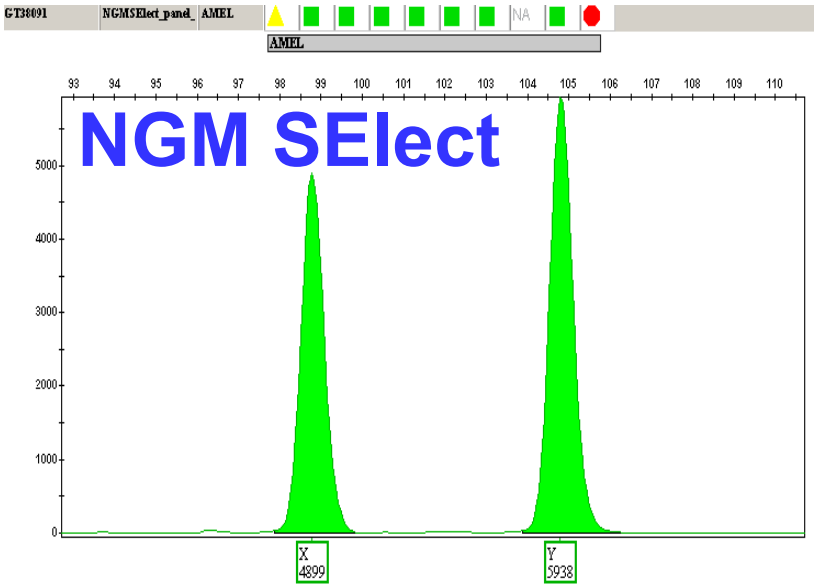
G→T SNP 15 bp upstream impacting forward primer binding with NGM

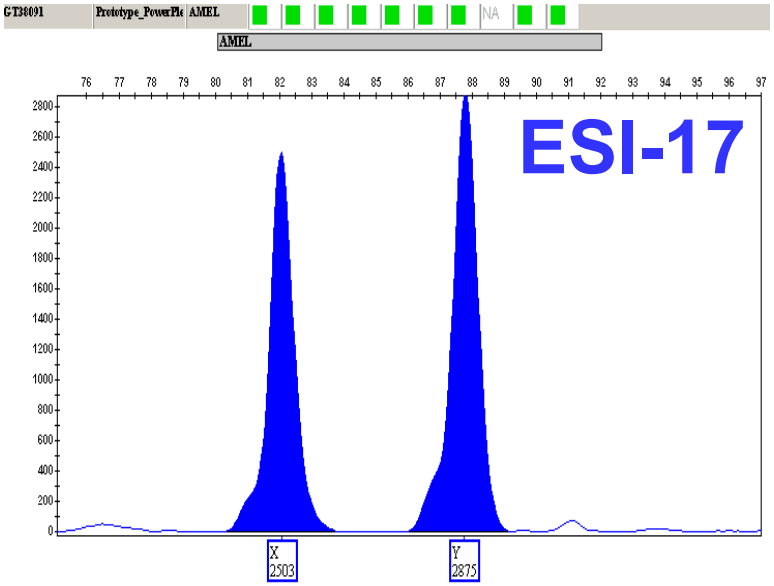
Amelogenin X Null Allele



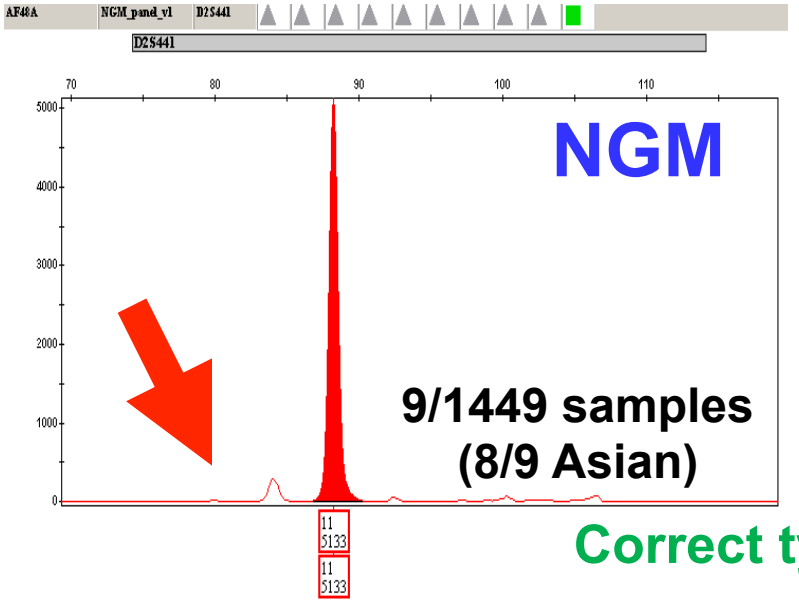
3/1449 samples

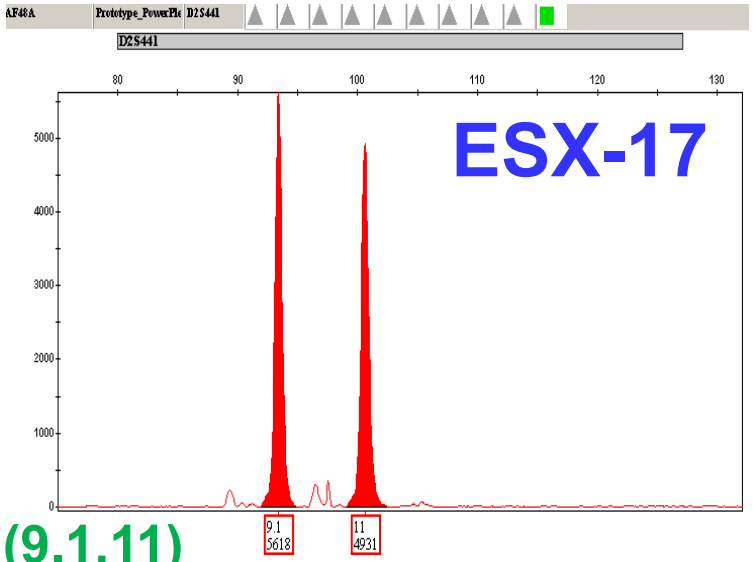




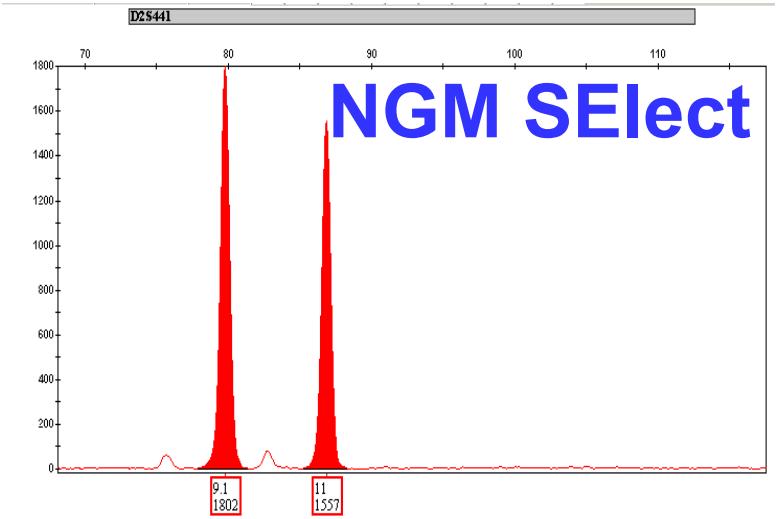


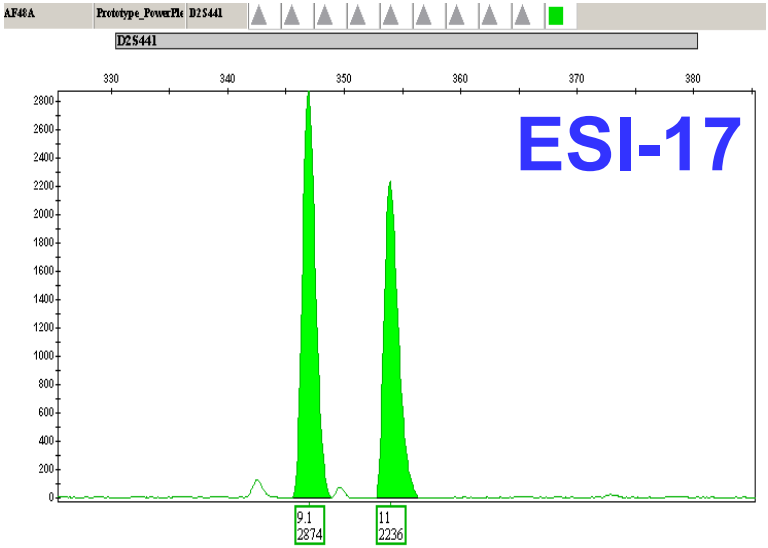
D2S441 Null Allele





Correct type (9.1,11)

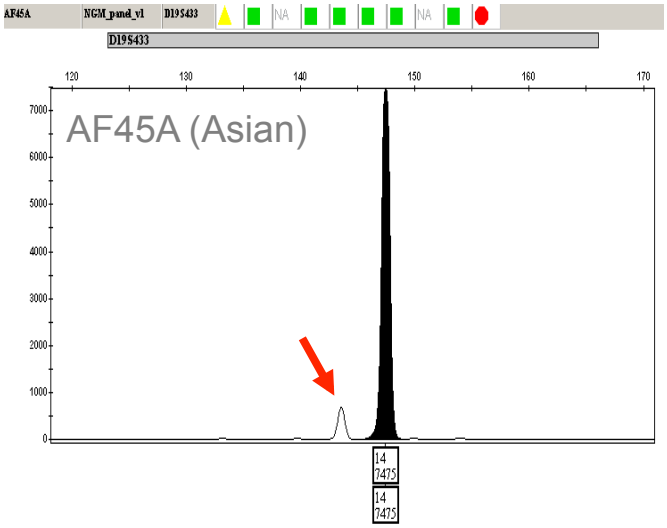




G→A SNP 26 bp upstream impacting forward primer binding with NGM

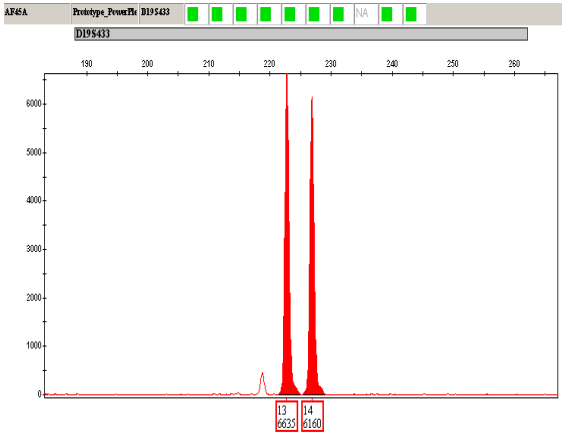
D19S433 Discordance

Identifiler & NGM = 14,14

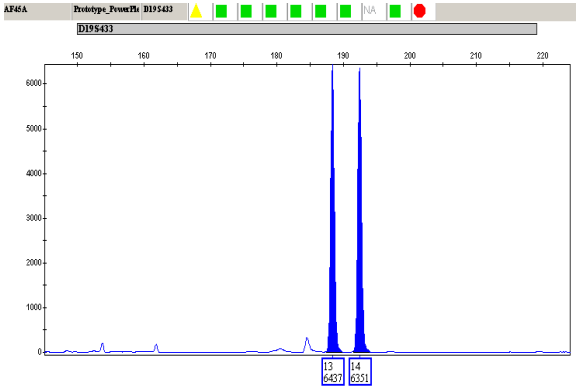


Allele 13 was missing in **two different Asian samples** with ABI primers = 2/2886 = **0.07% discordance**

ESX 17 = 13,14



ESI 17 = 13,14



Frequencies [for] the silent allele were determined to be 0.0114 in 176 people from Shizuoka (Honshu) and 0.0128 in 156 people from Okinawa

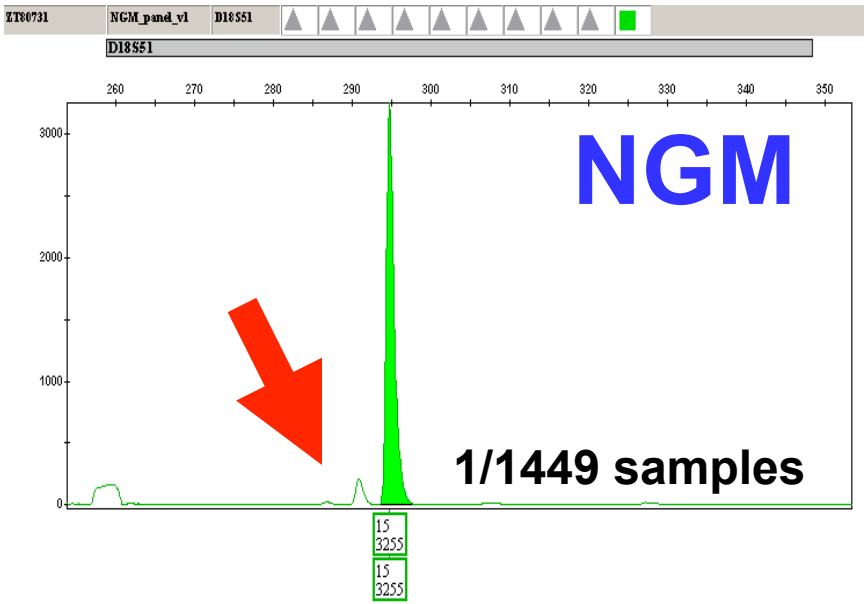
J Forensic Sci, September 2008, Vol. 53, No. 5
doi: 10.1111/j.1556-4029.2008.00806.x
Available online at: www.blackwell-synergy.com

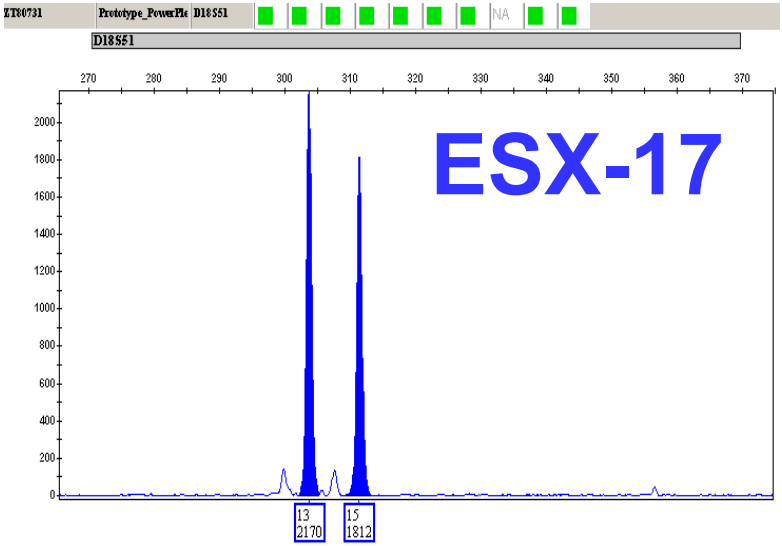
Natsuko Mizuno,[1] D.V.M.; Tetsushi Kitayama,[1] M.Sc.; Koji Fujii,[1] Ph.D.; Hiroaki Nakahara,[1] D.V.M.; Kanako Yoshida,[1] Ph.D.; Kazumasa Sekiguchi,[1] Ph.D.; Naoto Yonezawa,[2] Ph.D.; Minoru Nakano,[2] Ph.D.; and Kentaro Kasai,[1] Ph.D.

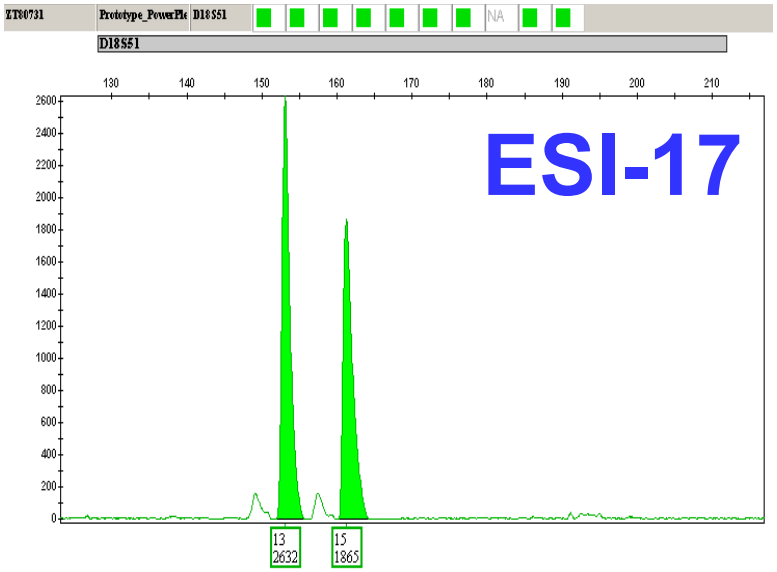
A D19S433 Primer Binding Site Mutation and the Frequency in Japanese of the Silent Allele It Causes

T→A SNP 8 bp downstream impacting reverse primer binding with Identifiler (and thus SGM Plus & NGM)

D18S51 Null Allele



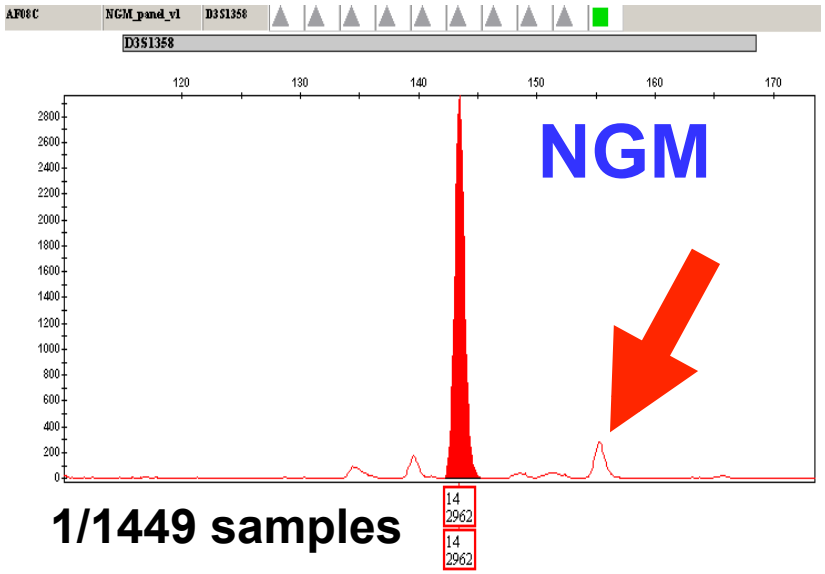




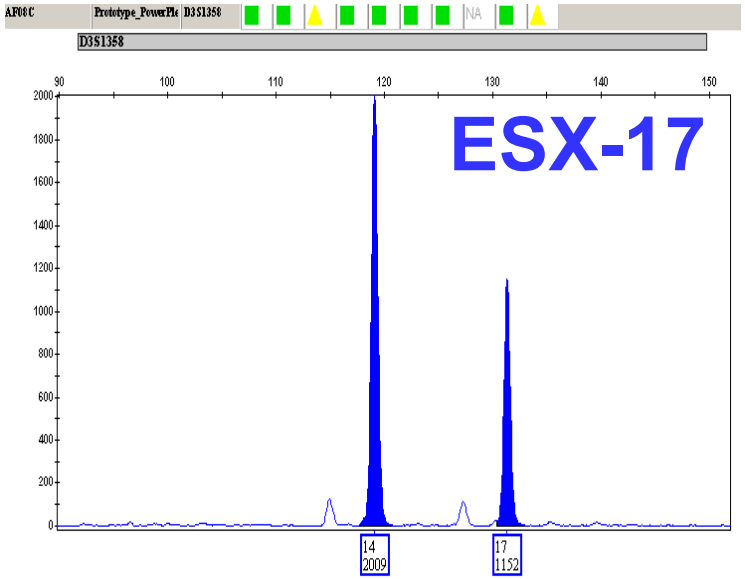
Correct type (13,15)

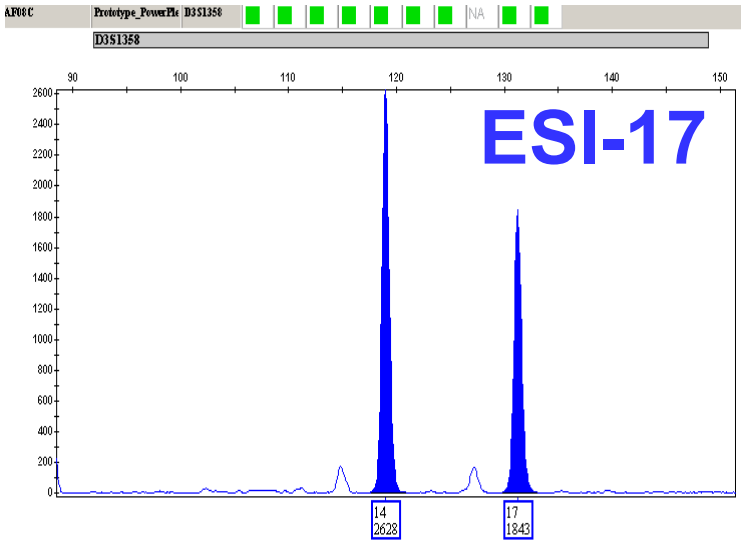
C→T SNP 172 bp downstream impacting forward primer binding with NGM

D3S1358 Null Allele



1/1449 samples





Correct type (14,17)

G→C SNP 11 bp downstream impacting forward primer binding with NGM

Completed Concordance Studies

Kits compared	Samples	Loci Compared	Comparisons	# Differences	Concordance (%)
IDIGS/I+	1424	11	15,664	1	99.994
IDIPro+	1415	10	14,150	1	99.993
IDIDplex+	1426	16	22,816	29	99.873
IDIPP16	662	14	9,268	4	99.957
IDIMiniFiler	1137	9	10,233	26	99.746
IDINGM	1437	11	15,807	3	99.981
IDINGMs	883	11	7,293	0	100.000
IDIESX17	1443	11	15,873	5	99.968
IDIESI17	1443	11	15,873	4	99.975
IDIESSplex	1433	11	15,763	28	99.822
IDIESSplexSE	662	11	7,282	17	99.767
ID/Hexaplex	653	2	1,306	1	99.923
PP16IGS/I+	651	9	5,859	1	99.983
PP16IPro+	647	10	6,470	2	99.969
PP16IDplex	657	14	9,198	3	99.967
PP16IMiniFiler	656	8	5,248	14	99.733
PP16INGM	657	9	5,913	3	99.949
PP16INGMs	662	9	5,958	1	99.983
PP16IESX17	662	9	5,958	1	99.983
PP16IESI17	662	9	5,958	0	100.000
PP16IESSplex	653	9	5,877	16	99.728
PP16IESSplexSE	662	9	5,958	16	99.731
PP16IHexaplex	653	2	1,306	1	99.923
SGMI+Pro+	1415	7	9,905	0	100.000
SGMI+IDplex	1424	11	15,664	5	99.968
SGMI+MiniFiler	1137	6	6,822	10	99.853
SGMI+NGM	1424	11	15,664	4	99.974
SGMI+NGMs	651	11	7,161	0	100.000
SGMI+ESX17	1424	11	15,664	6	99.962
SGMI+ESI17	1424	11	15,664	5	99.968
SGMI+ESS	1424	11	15,664	5	99.968
SGMI+ESSplexSE	651	11	7,161	5	99.930
SGMI+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
IDplexMiniFiler	1137	9	10,233	48	99.531
IDplexNGM	1426	11	15,686	30	99.809
IDplexNGMs	657	11	7,227	17	99.765
IDplexESX17	1426	11	15,686	28	99.821
IDplexESI17	1426	11	15,686	27	99.828
IDplex-ESS	1426	11	15,686	1	99.994
IDplexESSplexSE	657	11	7,227	1	99.986
IDplexHexaplex	653	2	1,306	1	99.923
MiniFilerNGM	1137	6	6,822	13	99.809
MiniFilerNGMs	656	6	3,936	10	99.746
MiniFilerESX17	1137	6	6,822	10	99.853
MiniFilerESI17	1137	6	6,822	9	99.868
MiniFilerESS	1137	6	6,822	35	99.487
MiniFilerESSplexSE	656	6	3,936	35	99.111
MiniFilerHexaplex	653	1	653	1	99.847
NGMNGMs	657	16	10,512	14	99.867
NGMESX17	1437	16	22,992	16	99.930
NGMESI17	1437	16	22,992	18	99.922
NGMESS	1433	16	22,928	42	99.817
NGMESSplexSE	657	16	10,512	22	99.791
NGMHexaplex	653	7	4,571	9	99.803
NGMsESX17	662	17	11,254	4	99.964
NGMsESI17	662	17	11,254	14	99.876
NGMsESS	653	16	10,448	17	99.837
NGMsESSplexSE	662	17	11,254	34	99.698
NGMsHexaplex	653	7	4,571	3	99.934
ESX17ESI17	1443	17	24,531	19	99.923
ESX17ESS	653	16	10,448	34	99.675
ESX17ESSplexSE	662	17	11,254	25	99.778
ESX17Hexaplex	657	7	4,599	6	99.870
ESI17ESS	653	16	10,448	28	99.732
ESI17ESSplexSE	662	17	11,254	30	99.733
ESI17Hexaplex	657	7	4,599	3	99.935
ESS/ESSplexSE	653	16	10,448	0	100.000
ESSHexaplex	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
ESI17plESX17	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
PP16D/D	50	16	800	2	99.750
PP16D/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
PP21/Identifiler	761	16	12176	6	99.951
PP21/PP16	761	16	12176	3	99.975
PP21/SGM+	761	11	8371	4	99.952
PP21/Pro+	761	10	7610	2	99.974
PP21/*Dplex	761	16	12176	20	99.836
PP21/MiniFiler	761	9	6849	14	99.796
PP21/ESX17	761	13	9893	1	99.990
PP21/ESI17	761	13	9893	0	100.000
PP21/NGM	761	13	9893	5	99.949
PP21/NGMs	568	13	7384	1	99.986
PP21/ESS	761	13	9893	18	99.818
PP21/ESSplexSE	568	13	7384	16	99.783
PP21/Hexaplex	568	4	2272	2	99.912
PP21/Smifiler	568	16	9088	1	99.989
PP21/Identifiler	639	16	10224	4	99.961
PP21/PP16	639	16	10224	1	99.990
PP21/IDplex	639	16	10224	18	99.824
PP16/Identifiler	652	16	10432	2	99.981
PP16/PP16	652	16	10432	2	99.981
PP16/SGM+	651	11	7161	1	99.986
PP16/Pro+	647	10	6470	1	99.985
PP16/IDplex	652	16	10432	16	99.847
PP16/MiniFiler	652	9	5868	14	99.761
PP16/ESX17	652	17	11084	1	99.991
PP16/ESI17	652	17	11084	0	100.000
PP16/NGM	652	16	10432	1	99.990
PP16/NGMs	652	17	11084	1	99.991
PP16/ESS	652	16	10432	16	99.847
PP16/ESSplexSE	652	17	11084	16	99.856
PP16/Hexaplex	652	8	3912	2	99.949
PP16/Smifiler	568	16	9088	1	99.989
PP16/PP21mk	639	21	13419	0	100.000
PP16/PP23	652	1	652	0	100.000
PP16/Y16	652	1	652	0	100.000
PP16/PP23	652	1	652	0	100.000
24plex/Identifiler	650	16	10400	2	99.981
24plex/PP16	650	16	10400	2	99.981
24plex/SGM+	650	11	7150	1	99.986
24plex/Pro+	647	10	6470	1	99.985
24plex/IDplex	650	16	10400	16	99.846
24plex/MiniFiler	650	9	5850	14	99.761
24plex/ESX17	650	17	11050	1	99.991
24plex/ESI17	650	17	11050	0	100.000
24plex/NGM	650	16	10400	1	99.990
24plex/NGMs	650	17	11050	1	99.991
24plex/ESS	650	16	10400	16	99.846
24plex/ESSplexSE	650	17	11050	16	99.855
24plex/Hexaplex	650	6	3900	2	99.949
24plex/Smifiler	568	16	9088	1	99.989
24plex/PP21mk	639	19	12141	0	100.000
24plex/PPF	650	21	13650	0	100.000
24plex/PP23	650	1	650	0	100.000
24plex/Y7	650	1	650	0	100.000

Totals	138031	1712	1,404,800	1373	99.902

1,404,800 allele comparisons

1,373 total differences

99.9% concordance

*Kits (except Identifiler) were kindly provided by **Promega, Qiagen and Life Technologies** for concordance testing performed at NIST*

Final Concordance Results

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 the 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

- All up-to-date results can be found on STRBase:
  - ISFG poster (Vienna, Austria), 8/31-9/2, 2011, "Concordance Testing Comparing STR Multiplex Kits with a Standard Data Set"
  - Promega ISHI (National Harbor, MD), 10/4-10/5, 2011, "Concordance Testing Comparing STR Multiplex Kits with a Standard Data Set"

Was there complete concordance with SRM 2391c?

SRM 2391c
PCR-Based Profiling Standard

- The first set of samples run with new STR multiplex kits is SRM 2391c

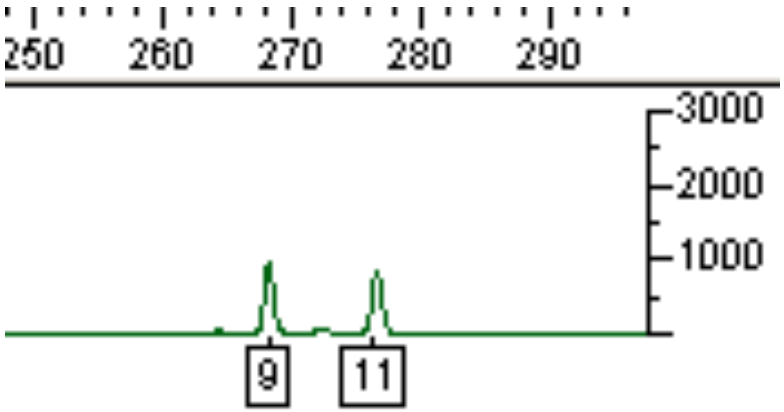
- All new kits tested have been completely concordant with the certified values of all markers for each component for SRM 2391c

- One exception for SRM 2391b: MiniFiler
  - Genomic 8 with D16S539

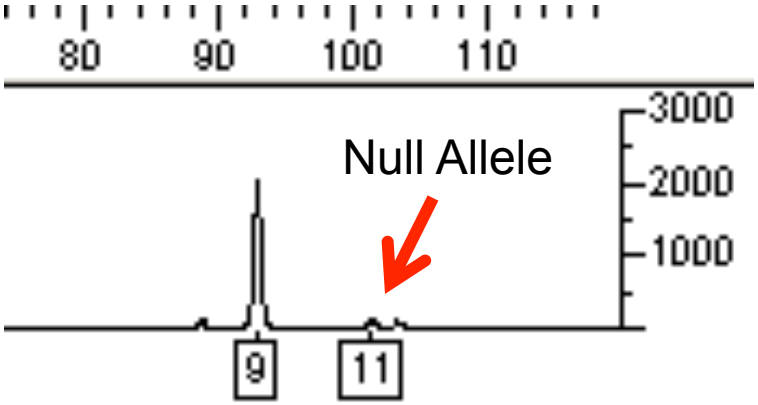
SRM 2391b Genomic 8 with D16S539

All allele calls with MiniFiler for CSF1PO, D7S820, D13S317, D18S51, D21S11, FGA, and D16S539 (with the exception noted below) ***match previously certified values.***

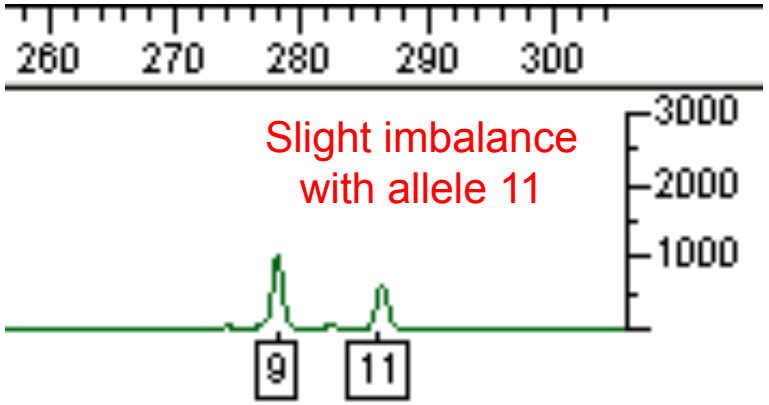
Identifiler



MiniFiler



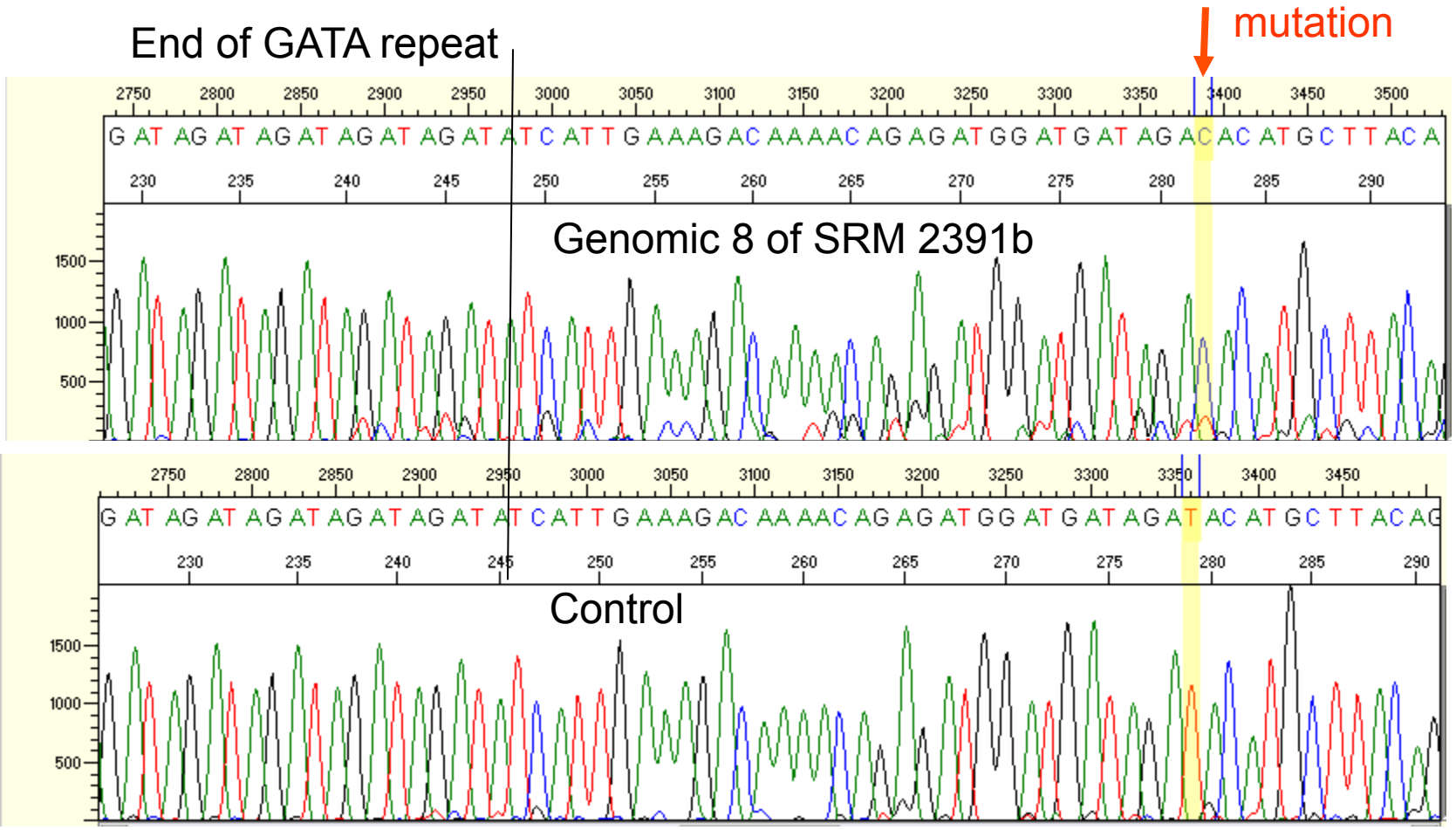
PowerPlex 16



***Due to primer binding site mutation**

D16S539 SRM 2391b Genomic 8

T→C mutation 34 bp downstream of the repeat



Position of the T→C probably affects the reverse primer of Minifiler and is the 3rd base found the 5'end of the Reverse PP16 primer. This could explain the imbalance of the allele seen when using PP16.

Summary & Final Thoughts

Conclusions

- Concordance testing is valuable when different sets of primers are used to amplify the same markers
- Null alleles and discordant results are reported on STRBase:

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

- NIST plays an important role in concordance testing to aid the community
  - SRM 2391c concordance
  - Several null alleles have been fixed before the final release of new STR multiplex kits

Acknowledgments

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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.

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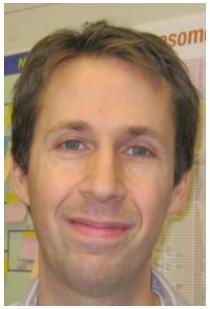

Pete Vallone

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