ISHI Workshop on New Loci and Kits
October 2, 2014 (Phoenix, AZ)
New Autosomal and Y-STR Loci and Kits: Making Data Driven Decisions

NIST Studies:

Kit Concordance and U.S. Population Data

Carolyn R. (Becky) Hill
NIST Applied Genetics Group

Presentation given by Mike Coble

Product Disclaimer

- **I will mention commercial STR kit names and information, but I am in no way attempting to endorse any specific products.**

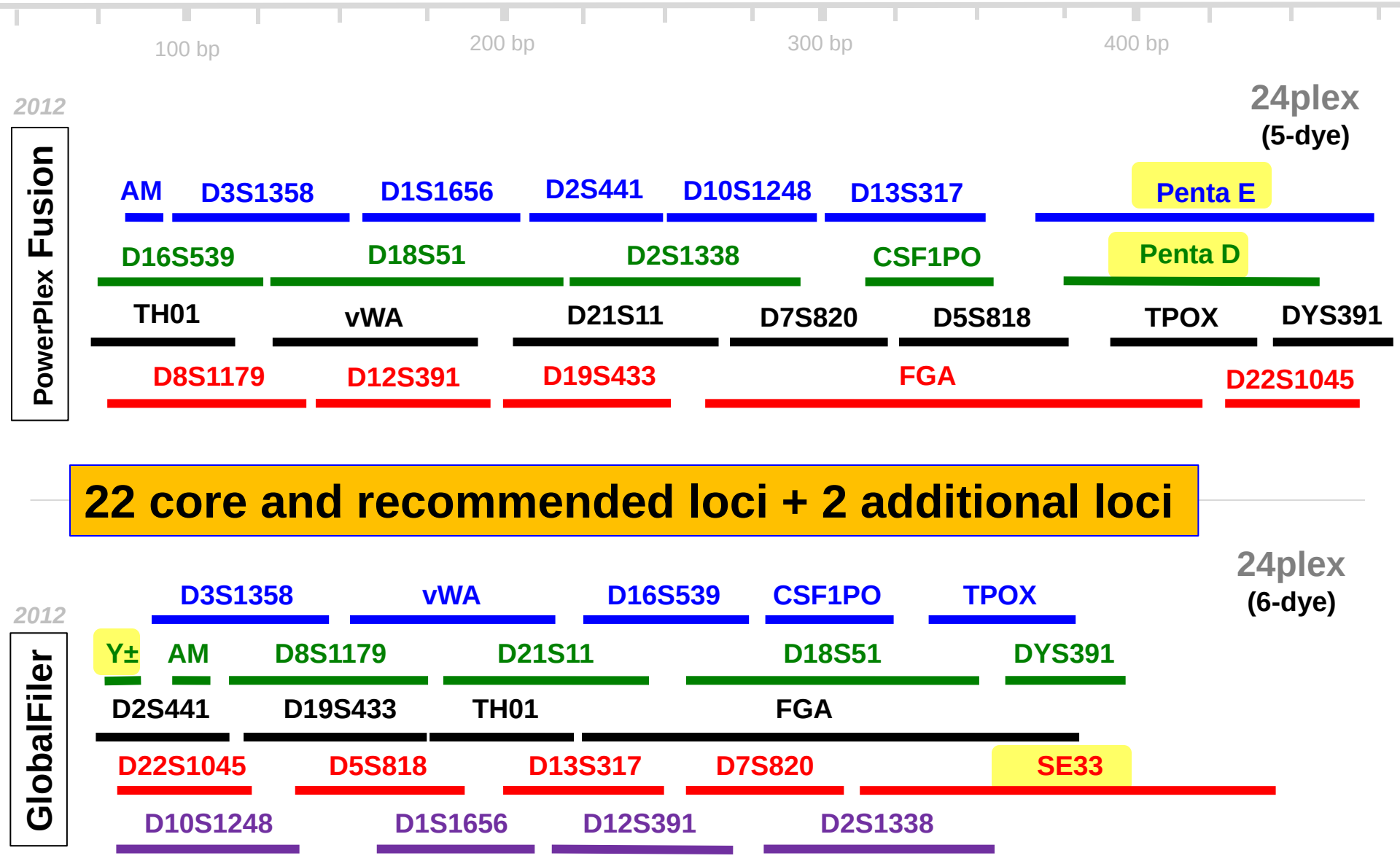
- **NIST Disclaimer**: Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

- **Points of view are mine** and do not necessarily represent the official position of the National Institute of Standards and Technology or the U.S. Department of Justice. **Our group receives or has received funding from the FBI Laboratory and the National Institute of Justice.**

Presentation Outline

- STR kits (including Fusion and GlobalFiler)
- NIST U.S. population samples
- Kit concordance study design
- Concordance study results
- U.S. allele frequencies – *FSI Genetics* article

STR Marker Layouts for New U.S. Kits



GlobalFiler STR Kit

Launched Friday, September 14, 2012

Human Identification

- GlobalFiler™ Kit
- Go Faster
- Go Further
- Go Global
- Powered by 6-Dye™
- Resources
- Human Identification Home
- GlobalFiler™ Express Kit
- GlobalFiler™ Kit

Introducing the world's most powerful STR kits

Around the world, forensic labs are being asked to do more with less. That's why GlobalFiler® STR Kits combine reduced amplification time with maximum data recovery power. As part of the only fully integrated and validated forensic workflow, this breakthrough 6-dye, 24-locus technology is designed to deliver unprecedented lab performance. And it's backed by best-in-class Life Technologies™ training, service, and support.

NDIS Approved

Both the GlobalFiler® and GlobalFiler® Express kits are approved for use by laboratories generating DNA profiles for inclusion in the US National DNA Index System (NDIS) CODIS database.

Order now

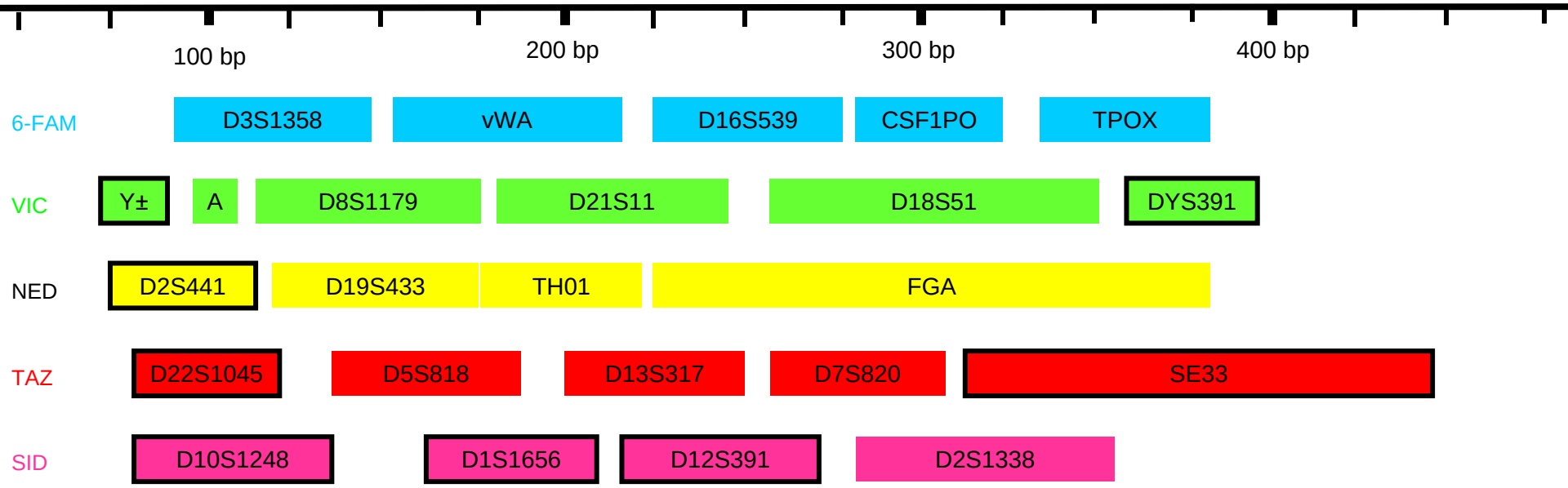
Request a demo

Go Faster | Go Further | Go Global | Powered by 6-Dye™

http://www.lifetechnologies.com/us/en/home/industrial/human-identification/globalfiler-str-kit.html

Life Technologies **GlobalFiler**

24plex



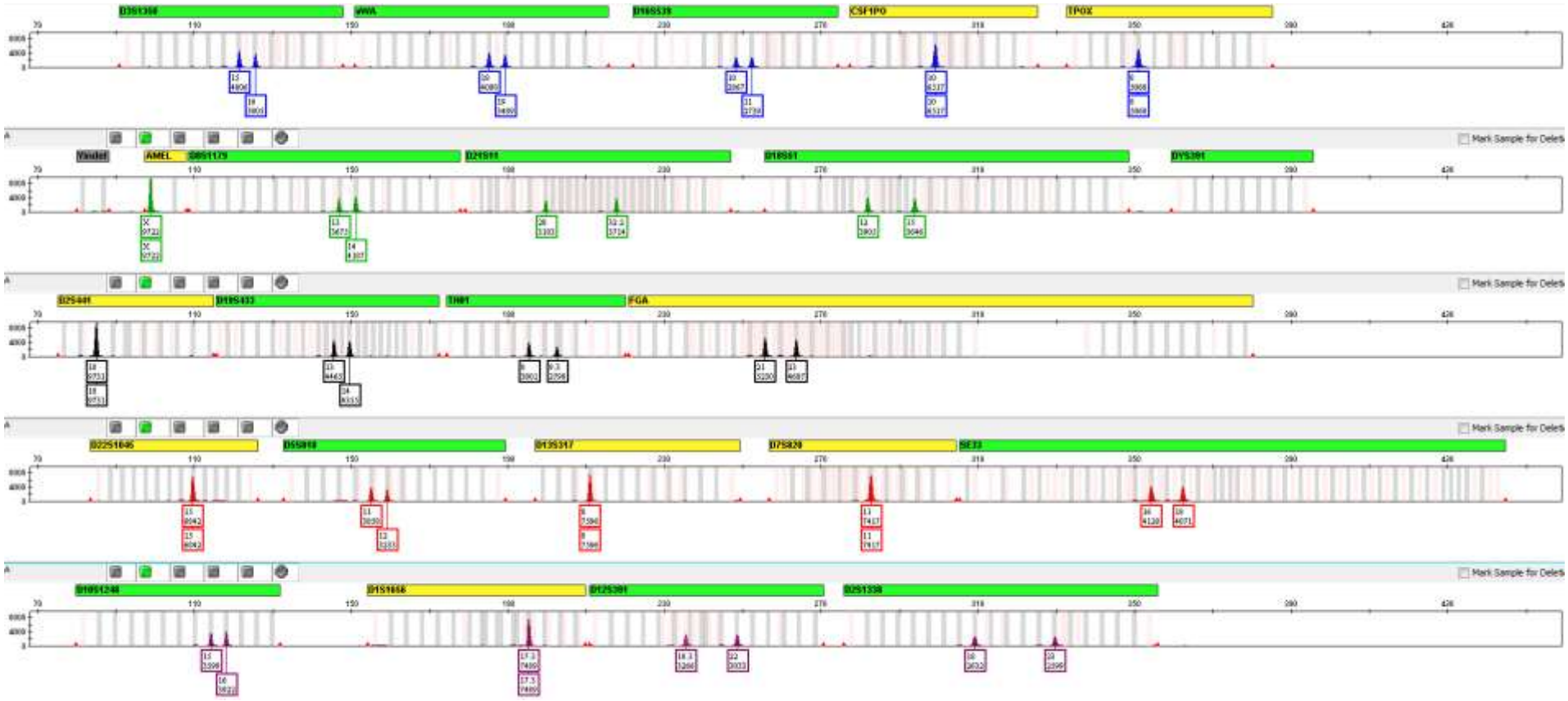
- 24 STR loci in 6 dyes (3500 use or 3130 upgrade required)
  - Includes SE33 and a Y-indel
- GlobalFiler Express: direct amplification capabilities
  - Single source samples: 40 min amplification
- GlobalFiler Casework
  - Casework samples: 80 min amplification

Two separate kits

- GlobalFiler gives ~12 orders of magnitude improvement using the NIST 1036 data set

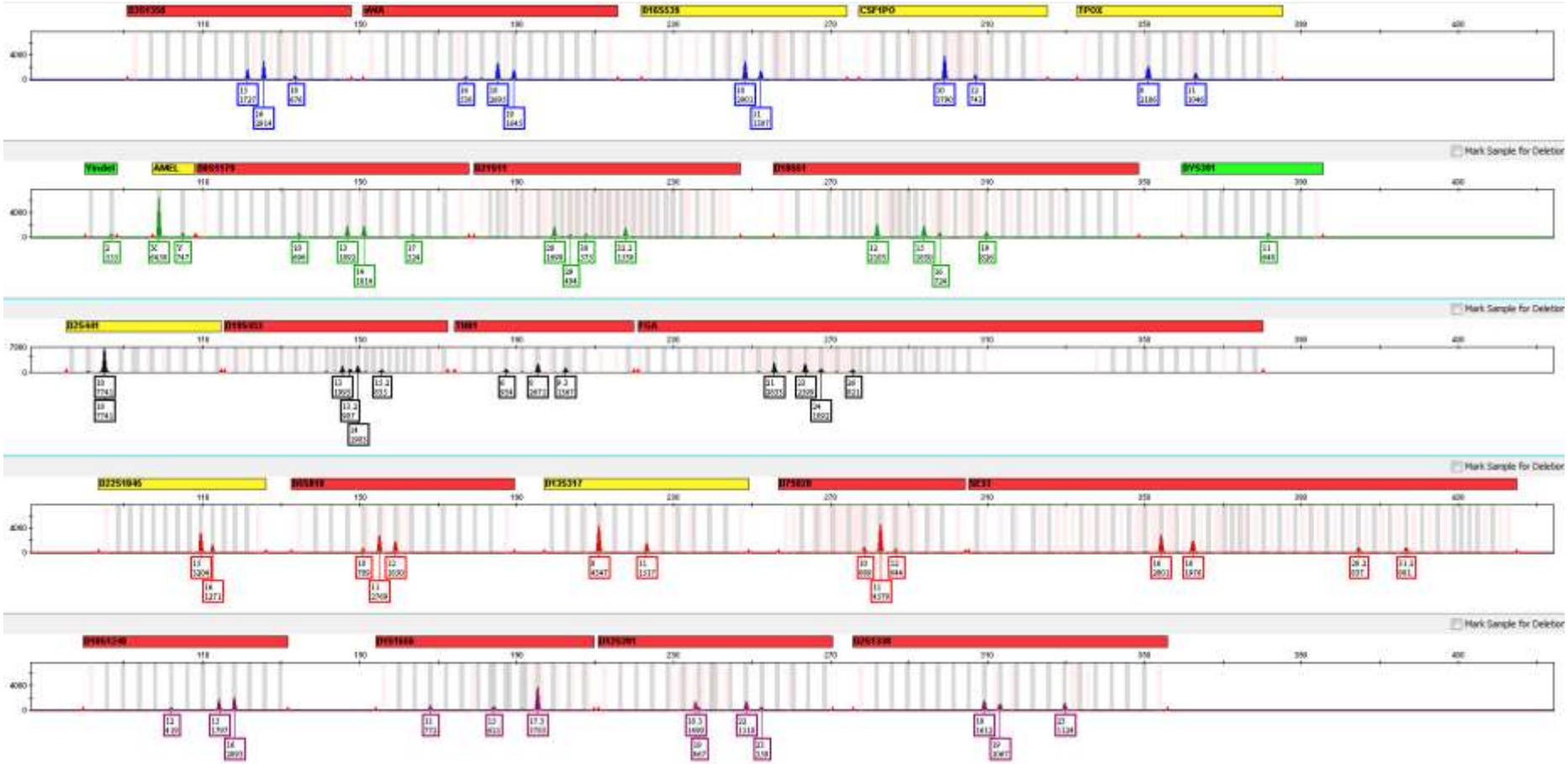
http://www.lifetechnologies.com/us/en/home/industrial/human-identification/globalfiler-str-kit.html

SRM 2391c is **fully concordant** at all loci for GlobalFiler kit – **Component A Profile**



1 ng, 29 cycles, 3500xl

SRM 2391c Mixture Component D



1 ng DNA, 29 cycles, 3500xl

PowerPlex Fusion

Launched Friday, September 14, 2012

Designed to meet CODIS and European standards, the PowerPlex® Fusion System enables laboratories to:

- Achieve the most inter database compatibility and highest discrimination of any autosomal STR kit.
- Improve laboratory efficiencies with rapid cycling and direct amplification protocols.
- Obtain a higher success rate with difficult casework samples due to robustness and sensitivity.
- Simplify validation and QC efforts by using one kit for both casework and databasing sections.

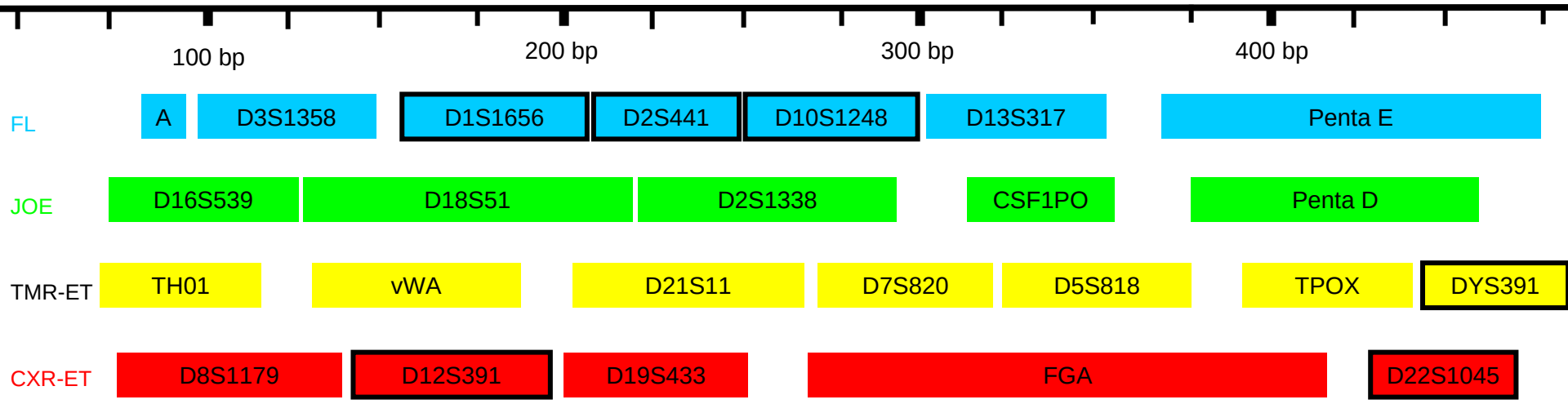
The PowerPlex® Fusion System provides all of the materials needed for co-amplification and five-color fluorescent detection of 24 loci (23 STR loci and Amelogenin), including the CODIS core loci and the European Standard Set (ESS) loci. With 24 loci, the system offers the most STR loci and highest discrimination from a single reaction and delivers more information in demanding forensic, paternity and relationship testing cases. Utilizing proven STR chemistries on existing instrument platforms and software, the PowerPlex® Fusion System requires no software or instrument upgrades.

Figure 1. Configuration of the PowerPlex® Fusion System. The PowerPlex® Fusion System allows co-amplification and four-color detection of 24 loci, including all CODIS and ESS loci: D3S1358, D1S1656, D2S441, D10S1248, D13S317, D16S539, D18S51, D2S1338, CSF1PO, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA, D22S1045, plus Penta E, Penta D, DYS391 and Amelogenin.

http://www.promega.com/products/pm/genetic-identity/powerplex-fusion/

Promega PowerPlex **FUSION**

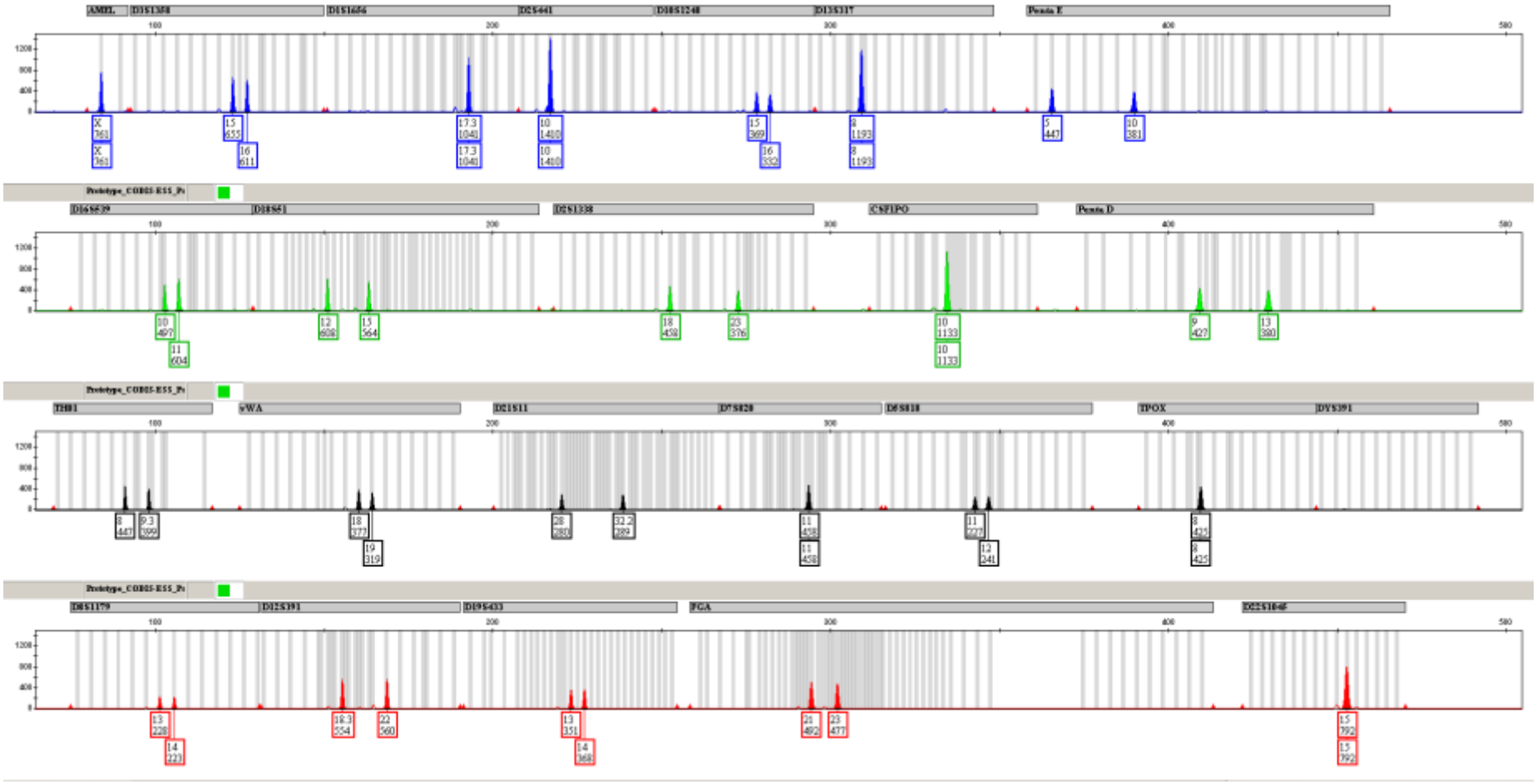
24plex



- 24 STR loci in 5 dyes (3130 and 3500 instrument use)
  - Includes Penta D and E
- Direct amplification and casework capabilities: 85 min amp for both (one kit)
- PowerPlex Fusion gives ~13 orders of magnitude improvement using the NIST 1036 data set

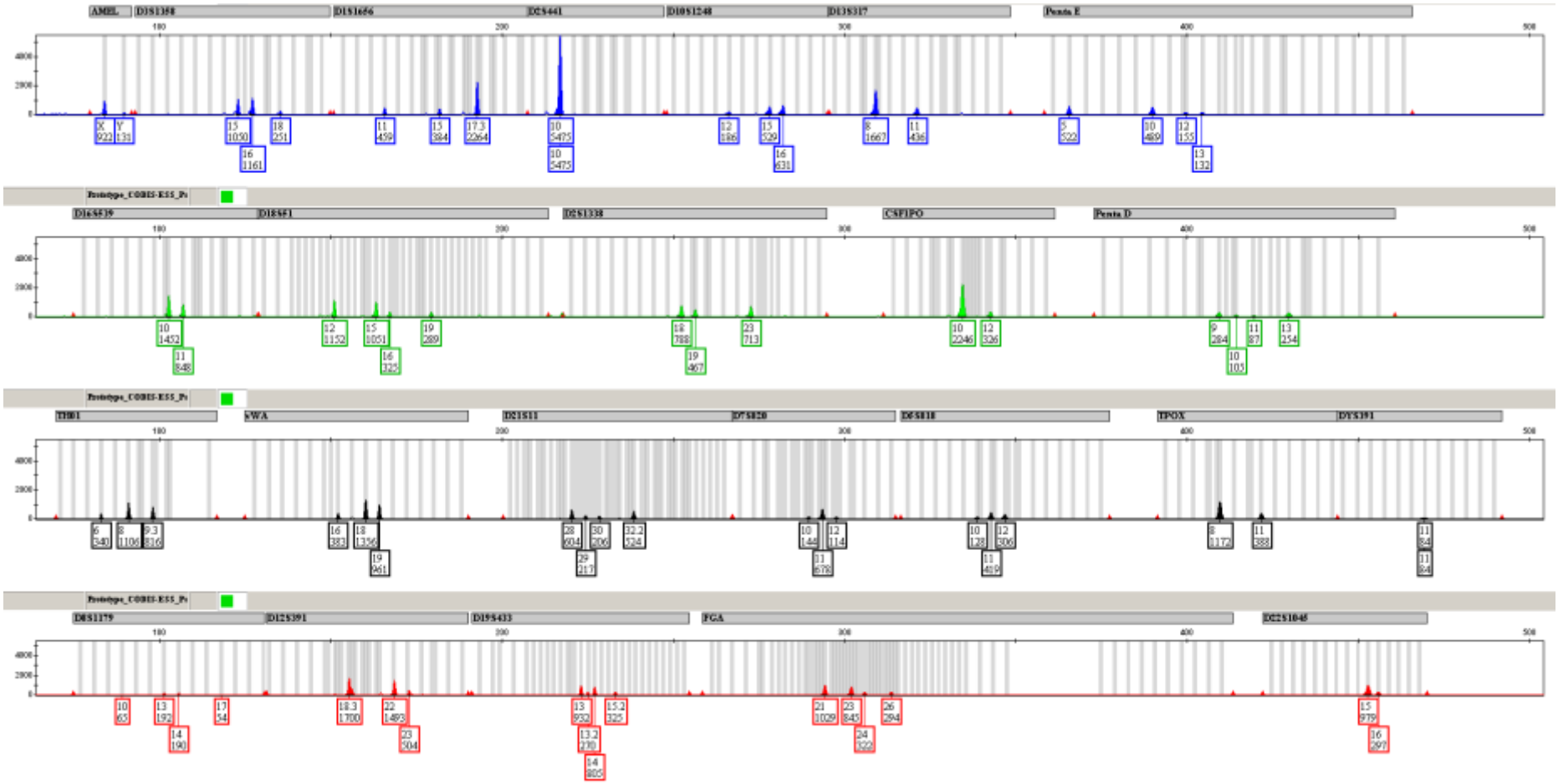
http://www.promega.com/products/pm/genetic-identity/powerplex-fusion/

SRM 2391c is **fully concordant** at all loci for PP Fusion kit – **Component A Profile**



1 ng DNA, 30 cycles, 3130xl

SRM 2391c Mixture Component D



1 ng DNA, 30 cycles, 3130xl

Qiagen Investigator 24plex

Available worldwide from Oct 2014 and not before June 2015 in the U.S.

There's More to a Sample than a Profile — Global STR Analysis Including Quality Control

Print Bookmark Share

The FBI CODIS Core Loci Working Group have published the recommendation that the CODIS core loci should be expanded, and a combination of STR markers from the Combined DNA Index System (CODIS), the European Network of Forensic Science Institutes (ENFSI), and the European DNA Profiling Group (EDNAP) should be used to improve the accuaracy of forensic testing.

QIAGEN has developed new kits — Investigator 24plex Kits — that coamplify all 23 recommended markers. The kits use novel 6-dye technology to keep the amplicon length of markers short while avoiding overlapping of markers. Kits are available for purified DNA from casework and for reference samples.

This webinar will outline the benefits of the Investigator 24plex Kits and highlight the improved workflow, which saves unnecessary re-runs of a sample by use of an internal "Quality Sensor" control.

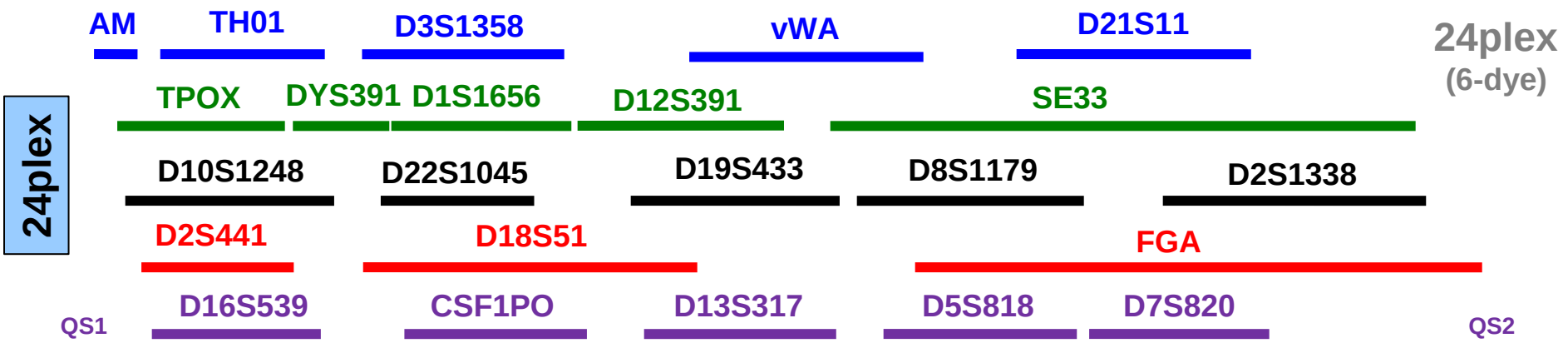
This unique Quality Sensor can distinguish:

- Successful amplification
- Degraded DNA
- Inhibited DNA
- No DNA
- Failed PCR amplification

Join us to learn how to improve results and streamline your forensic STR analysis with the new Investigator 24plex Kits.

http://www.qiagen.com/resources/e-learning/webinars/human-identity-and-forensics/multiplex-pcr-global-str/

Qiagen Investigator **24plex**



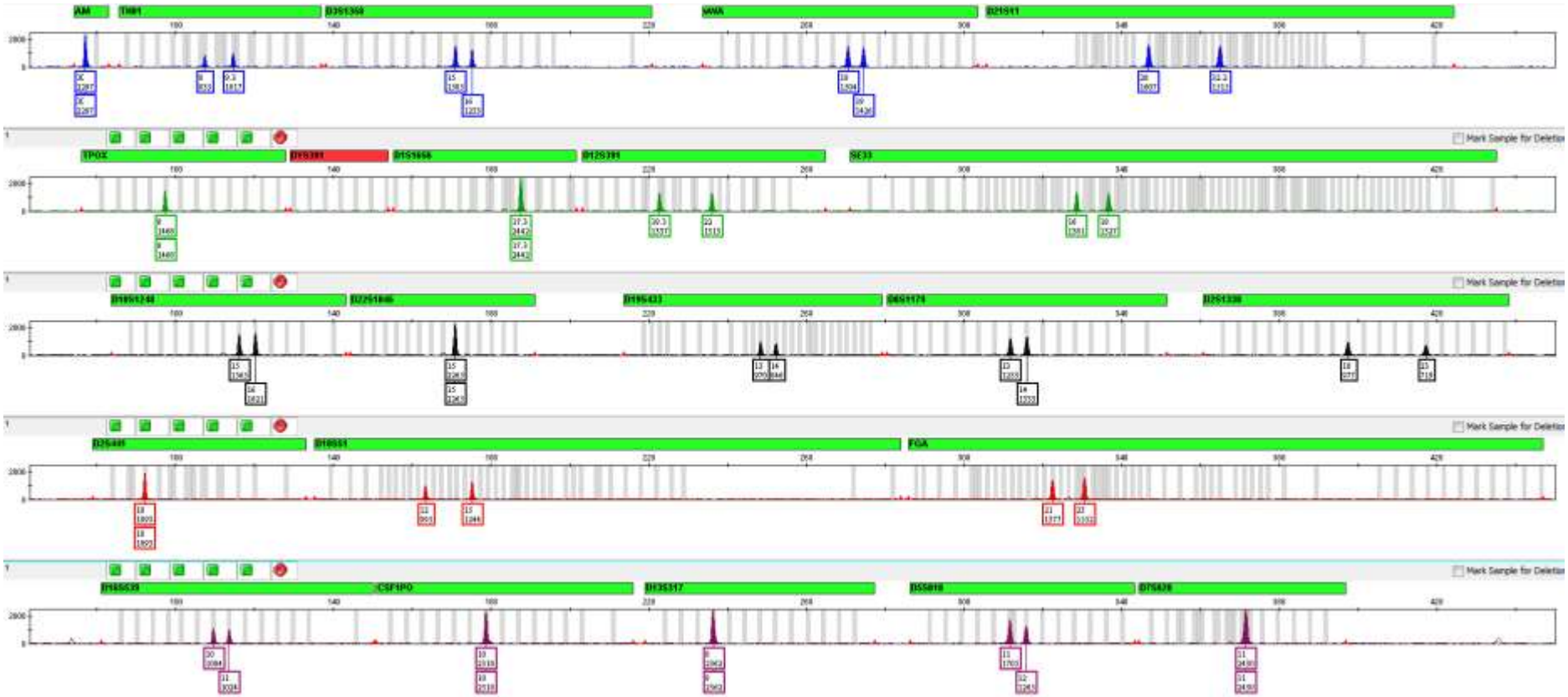
- 23 STR loci in 6 dyes (3500 use or 3130 upgrade required)
  - Includes SE33
- Includes Quality Sensors for detecting degraded and/or inhibited DNA
- 24plex GO!: direct amplification capabilities
  - Single source samples: 45 min amplification
- 24plex
  - Casework samples: 60 min amplification

Two separate kits

- Qiagen 24plex gives ~12 orders of magnitude improvement using the NIST 1036 data set

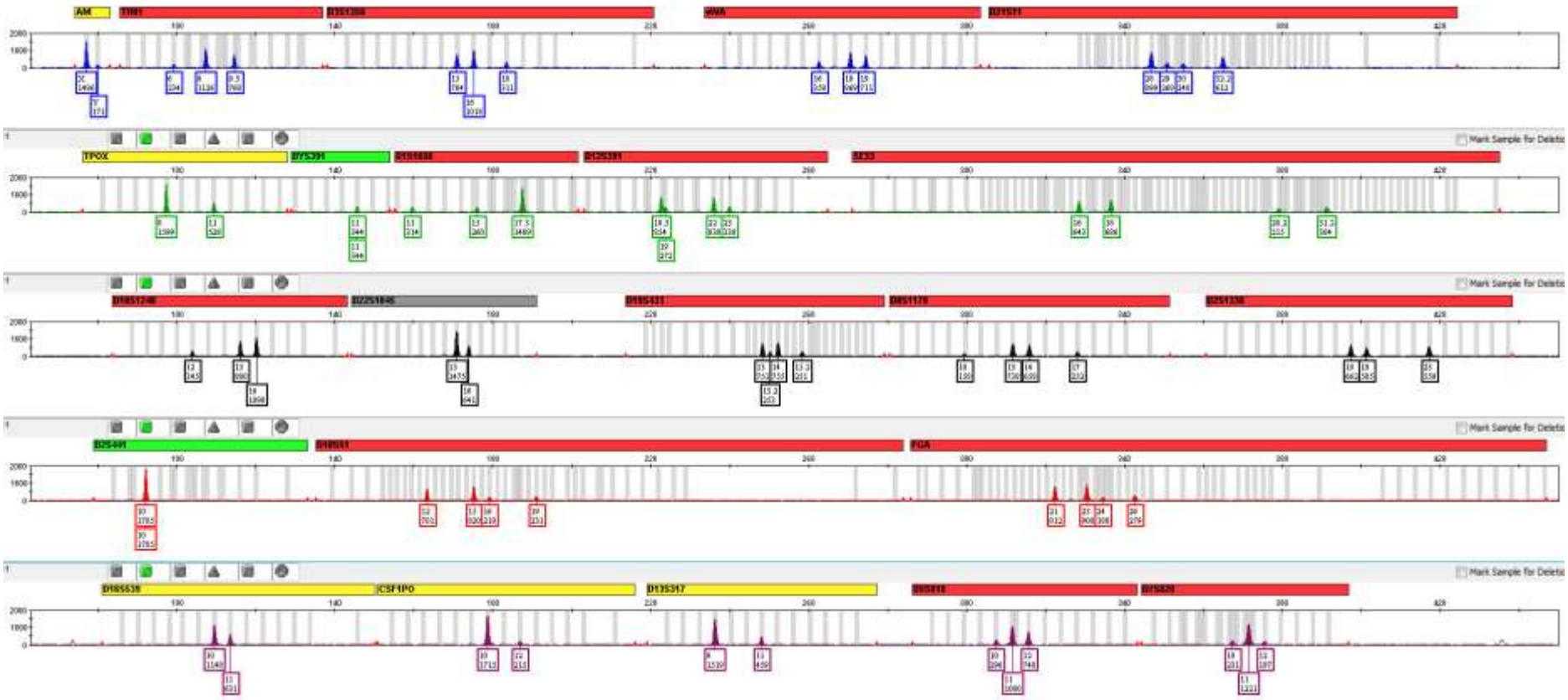
http://www.qiagen.com/resources/e-learning/webinars/human-identity-and-forensics/multiplex-pcr-global-str/

SRM 2391c is **fully concordant** at all loci for 24plex kit – **Component A Profile**



1 ng DNA, 30 cycles, 3500xl

SRM 2391c Mixture Component D



1 ng DNA, 30 cycles, 3500xl

NIST U.S. Population Samples

NIST U.S. Samples (>1450)

- **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)

Hill, C.R., et al. (2011) Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems. *Forensic Sci. Int. Genet.* 5(4): 269-275.

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* – 23 Y-STRs in PowerPlex Y23
25. Diegoli et al. (2014) *FSI Genetics* – 15 X-STRs
26. Purps et al. (2014) *FSI Genetics* – 23 Y-STRs in PowerPlex Y23 with global population comparison
27. Ballantyne et al. (2014) *Human Mutation* – 13 rapidly mutating (RM) Y-STRs with global comparison

NIST 1036 U.S. Population Samples

- 1032 males + 4 females
  - 361 Caucasians (2 female)
  - 342 African Americans (1 female)
  - 236 Hispanics
  - 97 Asians (1 female)

***Unrelated* samples**
All known or potential related individuals (based on autosomal & lineage marker testing) have been removed from the 1036 data set (e.g., only sons were used from father-son samples)

- Anonymous donors with self-identified ancestry
  - Interstate Blood Bank (Memphis, TN) – obtained in 2002
  - Millennium Biotech, Inc. (Ft. Lauderdale, FL) – obtained in 2001
  - DNA Diagnostics Center (Fairfield, OH) – obtained in 2007

- **Complete profiles with 29 autosomal STRs + PowerPlex Y23**
  - **Examined with multiple kits** and in-house primer sets enabling concordance

- Additional DNA results available on subsets of these samples
  - mtDNA control region/whole genome (AFDIL)
  - >100 SNPs (AIMs), 68 InDel markers, X-STRs (AFDIL)
  - NIST assays: miniSTRs, 26plex, >100 Y-STRs, 50 Y-SNPs

Data available on STRBase: **http://www.cstl.nist.gov/strbase/NISTpop.htm**

Benefits of NIST 1036 Data Set

- **Elimination of potential null alleles due to primer binding site mutations** through extensive concordance testing performed with different PCR primer sets from all available commercial STR kits

- **Ancestry testing performed** on DNA samples with autosomal SNPs, Y-SNPs, and mtDNA sequencing to verify self-declared ancestry categorization

- **Related individuals removed** based on Y-STR and mtDNA results

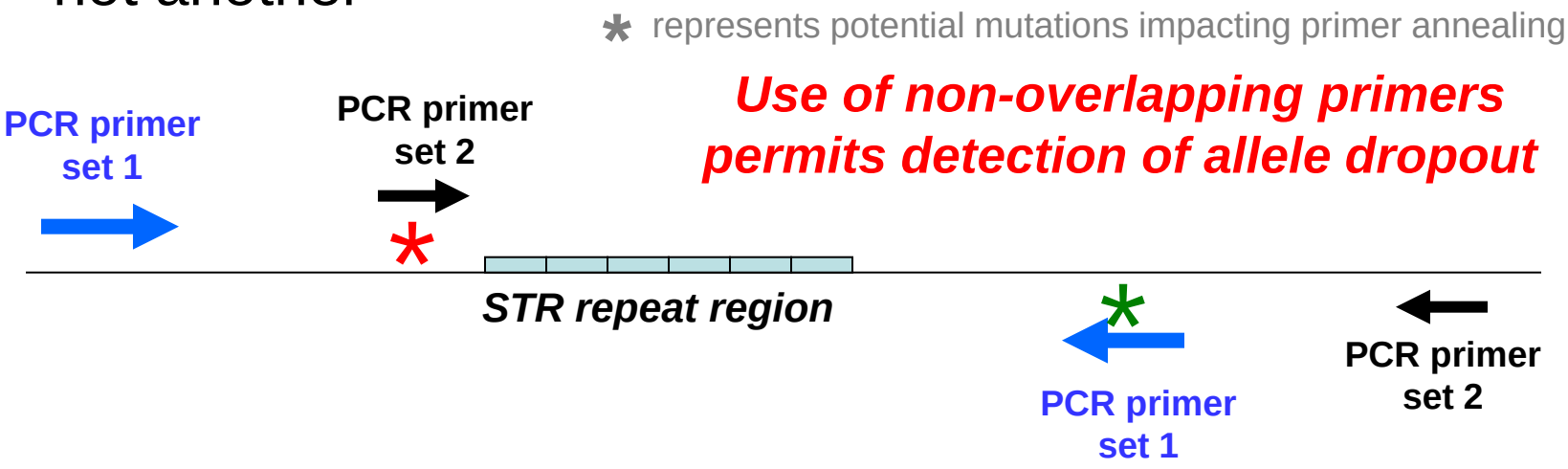
Concordance Testing at NIST

STR Kit Concordance Testing

- Many of these STR kits have different primer sequences for amplifying the same STR locus
- Need to analyze the same DNA samples with different STR typing kits looking for differences
- In some rare cases, allele dropout may occur due to mutations in primer binding regions

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

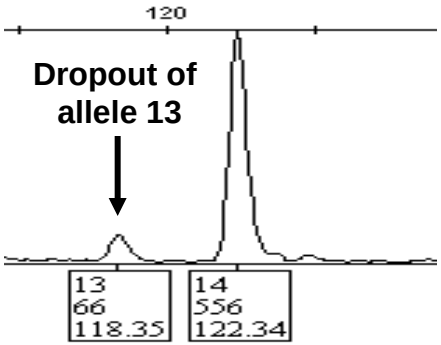


If no primer binding site mutations

If a primer binding site mutation exists

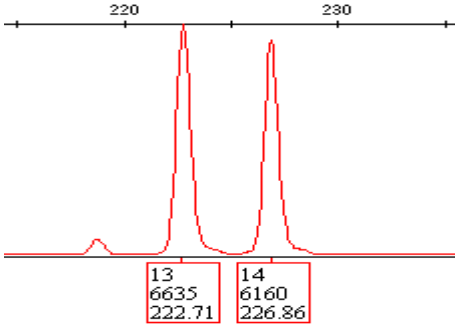
Example Primer Binding Site Mutation that Causes a Null Allele

Identifiler = 14,14



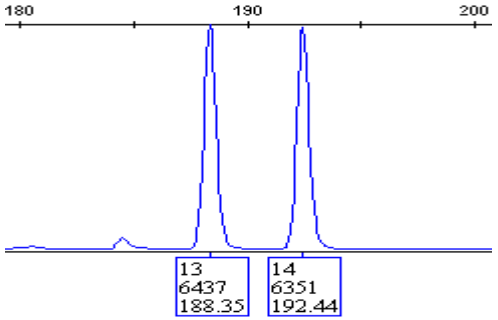
PHR = 11.9%

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

STR Kit Concordance Testing

Profiles in DNA Article Published April 2010

Article Type: Feature

Volume 13 No. 1, April 2010

Strategies for Concordance Testing

Carolyn R. Hill, Margaret C. Kline, David L. Duewer and John M. Butler
National Institute of Standards and Technology, Biochemical Science Division, Gaithersburg, Maryland, USA

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

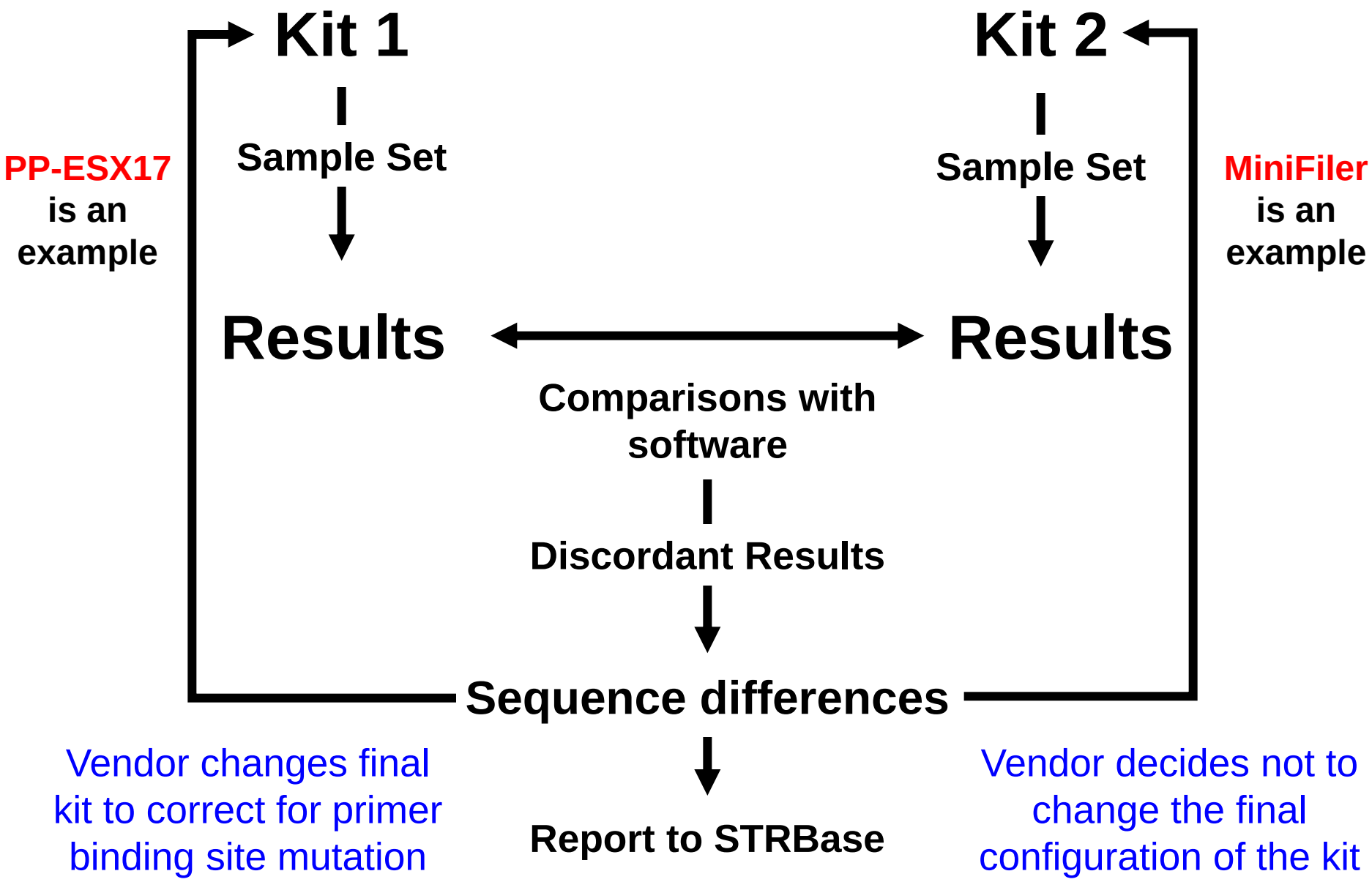
The 4 “S’s” of Concordance

- 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



Completed Concordance Studies

Applied Biosystems AmpFℓSTR Kits

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

GlobalFiler only examined with 50 bloodstains

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 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

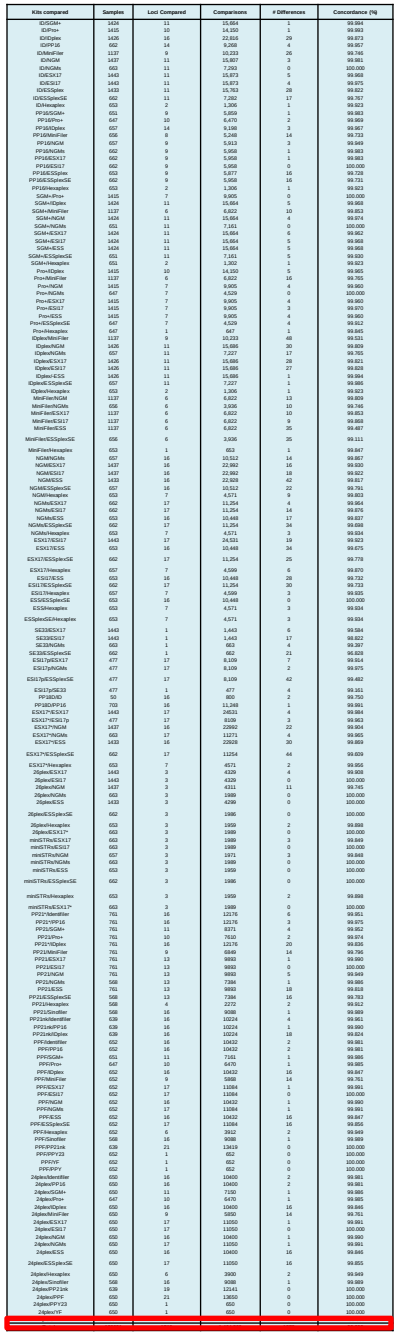
Carolyn R. Hill[a,*], David L. Duewer[a], Margaret C. Kline[a], Cynthia J. Sprecher[b], Robert S. McLaren[b], Dawn R. Rabbach[b], Benjamin E. Krenke[b], Martin G. Ensenberger[b], Patricia M. Fulmer[b], Douglas R. Storts[b], John M. Butler[a]

[a] National Institute of Standards and Technology, Chemical Science and Technology Laboratory, Gaithersburg, MD 20899-8312, USA
[b] Promega Corporation, Madison, WI 53711-5399, USA

Qiagen Investigator HID Kits

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

These STR kits are not currently for sale in the U.S.



Kits compared	Samples	Loci Compared	Comparisons	# Differences	Concordance (%)
IDSGM+	1439	11	15,664	1	99.994
IDPro+	1415	10	14,150	1	99.993
IDIDplex	1436	16	22,816	29	99.873
IDPP16	662	14	9,268	4	99.957
IDMiniFiler	1137	9	10,233	26	99.746
IDNGM	1437	11	15,807	3	99.981
IDNGMs	663	11	7,293	0	100.000
IDESX17	1443	11	15,873	5	99.968
IDESS17	1443	11	15,873	4	99.975
IDESSplex	1433	11	15,763	28	99.822
IDESSplexSE	662	11	7,282	17	99.767
IDHexplex	653	2	1,306	1	99.923
PP16SGM+	651	9	5,859	1	99.983
PP16Pro+	647	10	6,470	2	99.969
PP16Diplex	657	14	9,198	3	99.967
PP16MiniFiler	656	8	5,248	14	99.733
PP16NGM	657	9	5,913	3	99.949
PP16NGMs	662	9	5,958	1	99.983
PP16ESX17	662	9	5,958	1	99.983
PP16ESS17	662	9	5,958	0	100.000
PP16ESSplex	653	9	5,877	16	99.728
PP16ESSplexSE	662	9	5,958	16	99.731
PP16Hexaplex	653	2	1,306	1	99.923
SGM+Pro+	1415	7	9,905	0	100.000
SGM+Diplex	1434	11	15,664	5	99.968
SGM+MiniFiler	1137	6	6,822	10	99.853
SGM+NGM	1434	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+ESS17	1424	11	15,664	5	99.968
SGM+ESS	1434	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+Diplex	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+ESS17	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
DiplexMiniFiler	1137	9	10,233	48	99.531
DiplexNGM	1436	11	15,686	30	99.809
DiplexNGMs	657	11	7,227	17	99.765
DiplexESX17	1426	11	15,686	28	99.821
DiplexESS17	1436	11	15,686	27	99.828
DiplexESS	1426	11	15,686	1	99.994
DiplexESSplexSE	657	11	7,227	1	99.986
Diplexhexaplex	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
MiniFilerESS17	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
NGMESS17	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
NGMsESS17	662	17	11,254	14	99.876
NGMsESS	653	16	10,448	17	99.837
NGMsESSplexSE4	662	17	11,254	34	99.698
NGMsHexaplex	653	7	4,571	3	99.934
ESX17ESS17	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
ESS17ESS	653	16	10,448	28	99.732
ESS17ESSplexSE	662	17	11,254	30	99.733
ESS17Hexaplex	657	7	4,599	3	99.935
ESSESSplexSE	653	16	10,448	0	100.000
ESSHexaplex	653	7	4,571	3	99.934
ESSplexSEHexaplex	653	7	4,571	3	99.934
SE33ESX17	1443	1	1,443	6	99.584
SE33ESS17	1443	1	1,443	17	98.822
SE33NGMs	663	1	663	4	99.397
SE33ESSplexSE	662	1	662	21	96.828
ESS17plESX17	477	17	8,109	7	99.914
ESS17plNGMs	477	17	8,109	2	99.975
ESS17plESSplexSE	477	17	8,109	42	99.482
ESS17plSE33	477	1	477	4	99.161
PP16DID	50	16	800	2	99.750
PP16DPP16	703	16	11,248	1	99.991
ESX17IESX17	1443	17	24531	4	99.984
ESX17IESS17p	477	17	8109	3	99.963
ESX17YNGM	1437	16	22992	22	99.904
ESX17YNGMs	663	17	11271	4	99.965
ESX17YESS	1433	16	22928	30	99.869
ESX17YESSplexSE	662	17	11254	44	99.609
ESX17YHexaplex	653	7	4571	2	99.956
26plexESX17	1443	3	4339	4	99.908
26plexESS17	1443	3	4339	0	100.000
26plexNGM	1437	3	4311	11	99.745
26plexNGMs	663	3	1989	0	100.000
26plexESS	1433	3	4299	0	100.000
26plexESSplexSE	662	3	1986	0	100.000
26plexHexaplex	653	3	1959	2	99.898
26plexESX17*	663	3	1989	0	100.000
minSTRsESX17	663	3	1989	3	99.849
minSTRsESS17	663	3	1989	0	100.000
minSTRsNGM	657	3	1971	3	99.848
minSTRsNGMs	663	3	1989	0	100.000
minSTRsESS	653	3	1959	0	100.000
minSTRsESSplexSE	662	3	1986	0	100.000
minSTRsHexaplex	653	3	1959	2	99.898
minSTRsESX17*	663	3	1989	0	100.000
PP21YIdentifiler	761	16	12176	6	99.951
PP21YPP16	761	16	12176	3	99.975
PP21ySGM+	760	11	8371	4	99.952
PP21yPro+	761	10	7610	2	99.974
PP21YDiplex	761	16	12176	20	99.836
PP21MiniFiler	761	9	6849	14	99.796
PP21IESX17	761	13	9893	1	99.990
PP21IESS17	761	13	9893	0	100.000
PP21INGM	761	13	9893	5	99.949
PP21INGMs	568	13	7384	1	99.986
PP21IESS	761	13	9893	18	99.818
PP21IESSplexSE	568	13	7384	16	99.783
PP21IHexaplex	568	4	2272	2	99.912
PP21Smiffler	568	16	9088	1	99.989
PP21mIdentifiler	639	16	10224	4	99.961
PP21mPP16	639	16	10224	1	99.990
PP21mDiplex	639	16	10224	18	99.824
PPFIdentifiler	652	16	10432	2	99.981
PPIPP16	652	16	10432	2	99.981
PPISGM+	651	11	7161	1	99.986
PPIPro+	647	10	6470	1	99.985
PPIDiplex	652	16	10432	16	99.847
PPIMiniFiler	652	9	5868	14	99.761
PPIESX17	652	17	11084	1	99.991
PPIESS17	652	17	11084	0	100.000
PPINGM	652	16	10432	1	99.990
PPINGMs	652	17	11084	1	99.991
PPIESS	652	16	10432	16	99.847
PPIESSplexSE	652	17	11084	16	99.856
PPFHexaplex	652	6	3912	2	99.949
PPFSmiffler	568	16	9088	1	99.989
PPFPP21nk	639	21	13419	0	100.000
PPIPPY23	652	1	652	0	100.000
PPIYF	652	1	652	0	100.000
PPFPPY	652	1	652	0	100.000
24plexIdentifiler	650	16	10400	2	99.981
24plexPP16	650	16	10400	2	99.981
24plexSGM+	650	11	7150	1	99.986
24plexPro+	647	10	6470	1	99.985
24plexDiplex	650	16	10400	16	99.846
24plexMiniFiler	650	9	5850	14	99.761
24plexESX17	650	17	11050	1	99.991
24plexESS17	650	17	11050	0	100.000
24plexNGM	650	16	10400	1	99.990
24plexNGMs	650	17	11050	1	99.991
24plexESS	650	16	10400	16	99.846
24plexESSplexSE	650	17	11050	16	99.855
24plexHexaplex	650	6	3900	2	99.949
24plexSmiffler	568	16	9088	1	99.989
24plexPP21nk	639	19	12141	0	100.000
24plexPPY	650	21	13650	0	100.000
24plexPPY23	650	1	650	0	100.000
24plexYF	650	1	650	0	100.000

Completed Concordance Studies

	Samples Typed	Loci Compared	# Allele Comparisons Made	# Differences	Concordance (%)
Totals	**138,031**	**1712**	**1,404,800**	**1373**	**99.902**

1,404,031 allele comparisons

1,373 total differences

99.9% concordance

Kits (except Identifiler) were kindly provided by ***Promega, Qiagen and Applied Biosystems*** *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“

NIST SRM 2391b/2391c

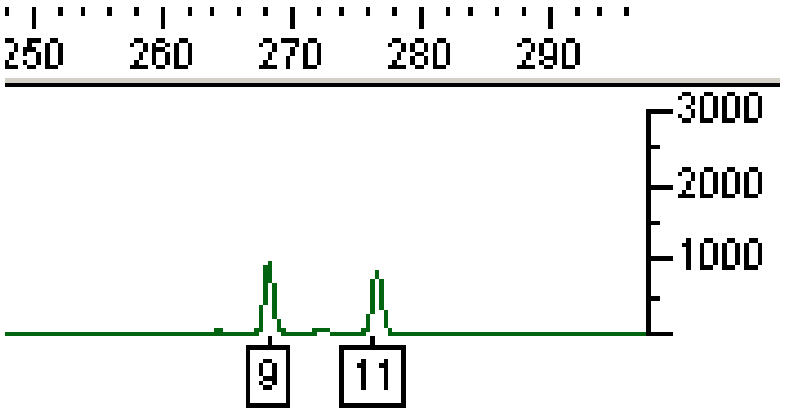
PCR-Based Profiling Standard

- The first set of samples run with new STR multiplex kits is SRM 2391b/SRM 2391c
- All new kits tested have been completely concordant with the certified values of all markers for each component for SRM 2391b and 2391c
- One exception for SRM 2391b: MiniFiler
  - Genomic 8 with D16S539

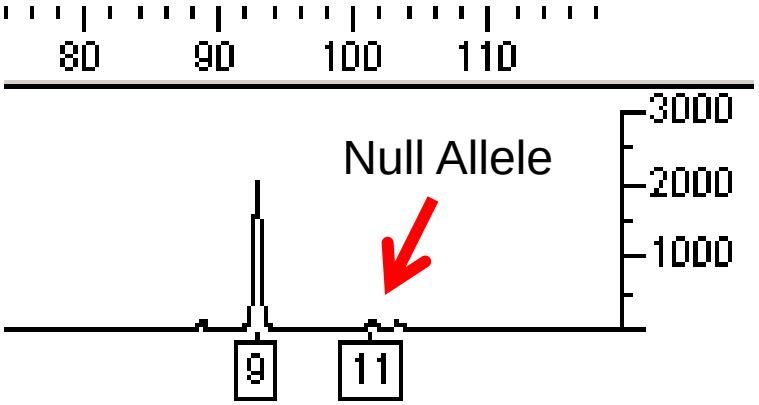
SRM 2391b Genomic 8 with D16S539

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

Identifiler

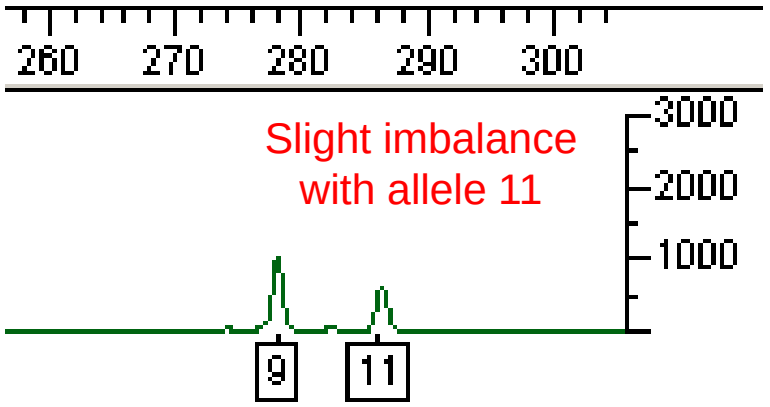


MiniFiler



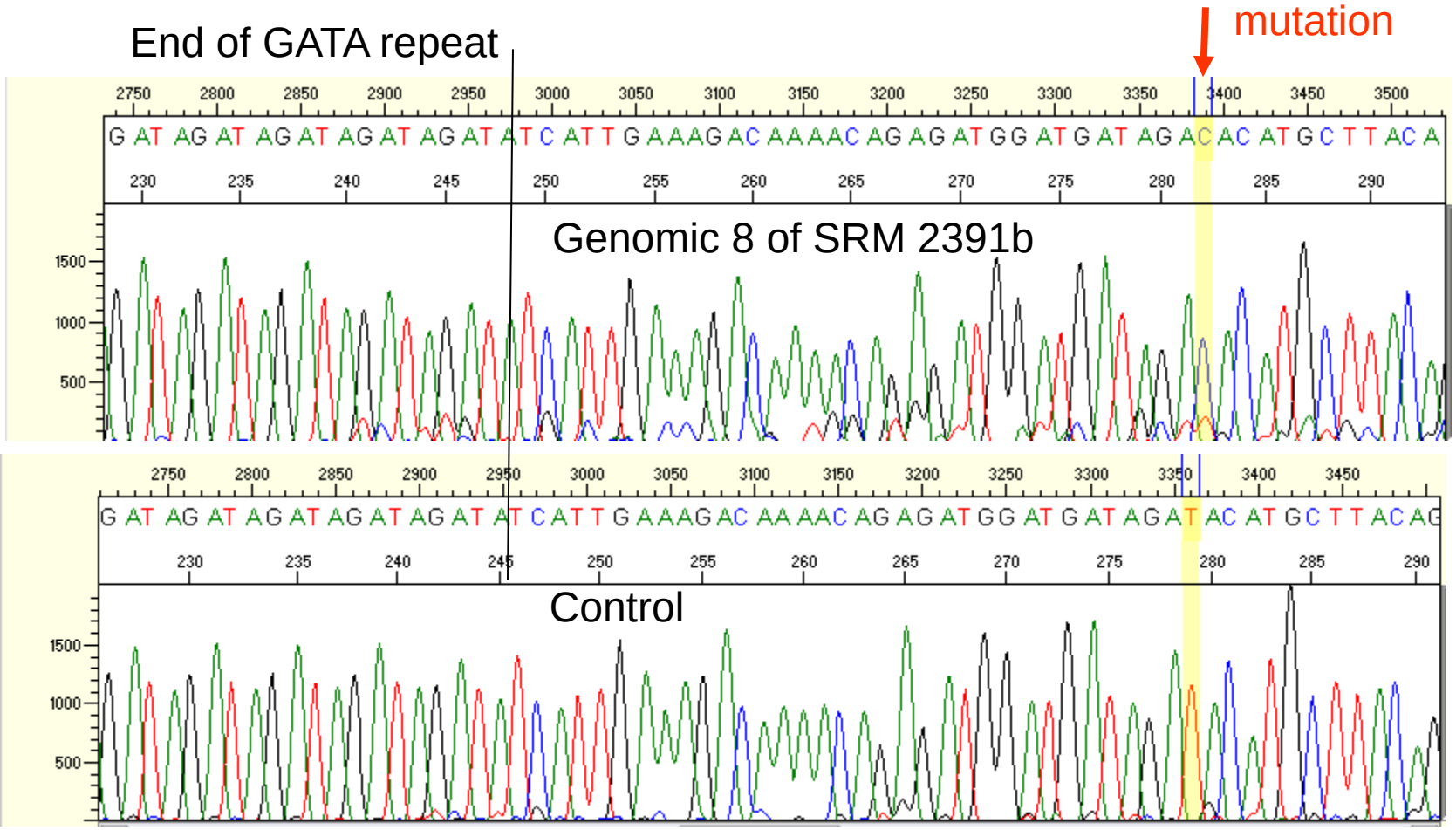
***Due to primer binding site mutation**

PowerPlex 16



D16S539 SRM 2391b Genomic 8

T→C mutation 34 bp downstream of the repeat

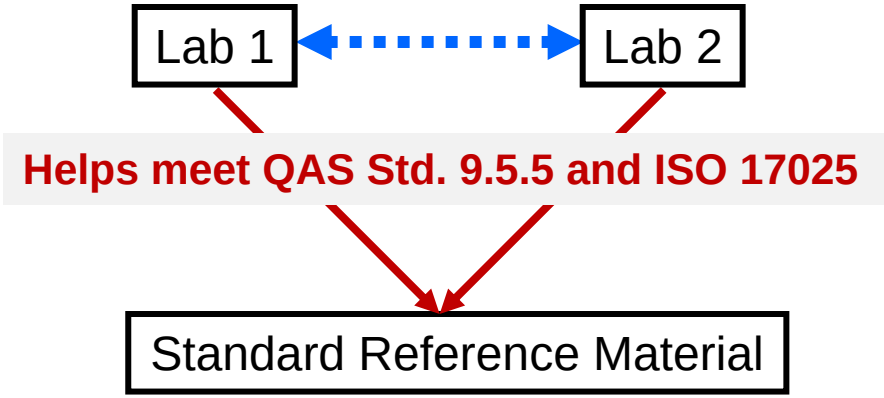


Position of the T→C probably affects the reverse primer of Minifiler and is the 3rd base found the 5'end of the Reverse PP16 primer. This could explain the imbalance of the allele seen when using PP16.

SRM 2391c:
PCR-Based DNA Profiling Standard

- Components A through D are DNA extracts in liquid form
- Components E and F are DNA spotted on 903 paper or FTA paper
- Certified values are for STR alleles based on length polymorphisms observed using capillary electrophoresis

Genomic DNAs characterized for the expanded CODIS core loci and Y-STRs



Calibration with SRMs enables confidence in comparisons of results between laboratories

Certified, Reference, & Information Values of SRM 2391c

- **Certified Values**: Value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account
  - **2 or more methods are used to compare values (i.e. Sanger sequencing, genotyping using multiple sets of primers)**

- **Reference Values**: High-confidence estimate of the true value but where all possible sources of bias have not been fully investigated by NIST
  - **Genotyping with only 2 sets of primers to compare**

- **Information Values**: Data that may be of interest and use to the SRM user, but insufficient information is available to access the confidence of the assignment
  - **Genotyping of only 1 kit is available**

Current Values for STR Loci with SRM 2391c

Certified Values		
Autosomal STR (24)	Y STR (17)	X STR (0)
D1S1656	DYS19	None
D2S1338	DYS385a	
D2S441	DYS385b	
D3S1358	DYS389I	
D5S818	DYS389II	
D7S820	DYS390	
D8S1179	DYS391	
D8S1115	DYS392	
D10S1248	DYS393	
D12S391	DYS437	
D13S317	DYS438	
D16S539	DYS439	
D18S51	DYS448	
D19S433	DYS456	
D21S11	DYS458	
D22S1045	DYS635	
CSF1PO	Y GATA H4	
FGA		
Penta D		
Penta E		
SE33		
TH01		
TPOX		
vWA		

Reference Values		
Autosomal STR (23)	Y STR (0)	X STR (0)
D1GATA113	None	None
D1S1627		
D1S1677		
D2S1776		
D3S3053		
D3S4529		
D4S2364		
D4S2408		
D5S2500		
D6S1017		
D6S474		
D9S1122		
D9S2157		
D10S1435		
D17S1301		
D17S974		
D18S853		
D20S1082		
D20S482		
F13A01		
F13B		
FESFPS		
LPL		

Information Values		
Autosomal STR (1)	Y STR (0)	X STR (0)
Penta C	None	None

STR Typing Kits and Primer Mixes

Original SRM 2391c certificate

Kit Provider			
Thermo Fisher (12)	Promega Corp. (8)	Qiagen Inc. (2)	Primer Mixes (2)
Identifiler	PowerPlex 16	ESSplex	26plex
Identifiler Plus	PowerPlex 16 HS	IDplex	miniSTRs
NGM	PowerPlex ESX 17		
NGM SElect	PowerPlex ESI 17		
COfiler	PowerPlex ES		
Profiler	PowerPlex S5		
Profiler Plus	PowerPlex Y		
Profiler Plus ID	FFFL		
SGM Plus			
SEfiler			
MiniFiler			
Yfiler			

100% Concordance with all kits

Updated SRM 2391c certificate

Kit Provider			
Thermo Fisher (14)	Promega Corp. (16)	Qiagen Inc. (9)	Primer Mixes (3)
Identifiler	PowerPlex 16	ESSplex	26plex
Identifiler Plus	PowerPlex 16 HS	IDplex	miniSTRs
NGM	PowerPlex ESX 17	ESSplex SE	RM Y STRs
NGM SElect	PowerPlex ESI 17	ESSplex SE Plus	
COfiler	PowerPlex ES	ESSplex SE GO!	
Profiler	PowerPlex S5	IDplex Plus	
Profiler Plus	PowerPlex Y	IDplex GO!	
Profiler Plus ID	FFFL	Argus X-12	
SGM Plus	PowerPlex ESI 17 Pro	DIPplex	
SEfiler	PowerPlex ESX 17 Fast		
MiniFiler	PowerPlex ESI 17 Fast		
Yfiler	PowerPlex 18D		
GlobalFIler	PowerPlex 21		
Yfiler Plus	PowerPlex CS7		
	PowerPlex Fusion		
	PowerPlex Y23		

Updated Values for STR Loci

Certified Values		
Autosomal STR (25)	Y STR (29)	X STR (0)
D1S1656	DYS19	None
D2S1338	DYS385a	
D2S441	DYS385b	
D3S1358	DYS389I	
D5S818	DYS389II	
D6S1043	DYS390	
D7S820	DYS391	
D8S1179	DYS392	
D8S1115	DYS393	
D10S1248	DYS437	
D12S391	DYS438	
D13S317	DYS439	
D16S539	DYS448	
D18S51	DYS456	
D19S433	DYS458	
D21S11	DYS635	
D22S1045	Y GATA H4	
CSF1PO	DYS449	
FGA	DYS460	
Penta D	DYS481	
Penta E	DYS518	
SE33	DYS533	
TH01	DYS549	
TPOX	DYS570	
vWA	DYS576	
	DYS627	
	DYS643	
	DYF387S1a	
	DYF387S1b	

Reference Values		
Autosomal STR (23)	Y STR (0)	X STR (0)
D1GATA113	None	None
D1S1627		
D1S1677		
D2S1776		
D3S3053		
D3S4529		
D4S2364		
D4S2408		
D5S2500		
D6S1017		
D6S474		
D9S1122		
D9S2157		
D10S1435		
D17S1301		
D17S974		
D18S853		
D20S1082		
D20S482		
F13A01		
F13B		
FESFPS		
LPL		

Information Values		
Autosomal STR (1)	Y STR (0)	X STR (12)
Penta C	None	DXS7132
		DXS7423
		DXS8378
		DXS10074
		DXS10079
		DXS10101
		DXS10103
		DXS10134
		DXS10135
		DXS10146
		DXS10148
		HPRTB

Argus X-12 kit

*Other Information Value Considerations:
-DIPplex kit
-PGM and MiSeq IISNPs

New Y-STR loci in commercial kits (Yfiler Plus & PPY23)

Update to be completed by Oct. 2014

Concordance Testing at NIST

- 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 2391b&c concordance
  - Several null alleles have been fixed before the final release of new STR multiplex kits

Characterization of STR Loci

Available in Commercial Kits

The 10 STR Loci Beyond the CODIS 13

STR Locus	Location	Repeat Motif	Allele Range*	# Alleles*
D2S1338	2q35	TGCC/TTCC	10 to 31	40
D19S433	19q12	AAGG/TAGG	5.2 to 20	36
Penta D	21q22.3	AAAGA	1.1 to 19	50
Penta E	15q26.2	AAAGA	5 to 32	53
D1S1656	1q42	TAGA	8 to 20.3	25
D12S391	12p13.2	AGAT/AGAC	13 to 27.2	52
D2S441	2p14	TCTA/TCAA	8 to 17	22
D10S1248	10q26.3	GGAA	7 to 19	13
D22S1045	22q12.3	ATT	7 to 20	14
SE33	6q14	AAAG‡	3 to 49	**178**

5 new European loci

**Allele range and number of observed alleles from Appendix 1, J.M. Butler (2012) Advanced Topics in Forensic DNA Typing: Methodology; ‡SE33 alleles have complex repeat structure*

25 Alleles Reported in the Literature for **D1S1656**

15 NIST observed alleles circled in red

Allele (Repeat #)	Promega ESX 17	Promega ESI 17	ABI NGM	Repeat Structure $[TAGA]_4[TGA]_{0-1}[TAGA]_nTAGG[TG]_5$	Reference
8	133 bp	222 bp	171 bp	$[TAGA]_8[TG]_5$	Phillips *et al.* (2010)
9	137 bp	226 bp	175 bp	$[TAGA]_9[TG]_5$	Phillips *et al.* (2010)
10 (a)	141 bp	230 bp	179 bp	$[TAGA]_{10}[TG]_5$	Lareu *et al.* (1998)
10 (b)	141 bp	230 bp	179 bp	$[TAGA]_{10}TAGG[TG]_5$	Phillips *et al.* (2010)
11	145 bp	234 bp	183 bp	$[TAGA]_{11}[TG]_5$	Lareu *et al.* (1998)
12 (a)	149 bp	238 bp	187 bp	$[TAGA]_{12}[TG]_5$	Lareu *et al.* (1998)
12 (b)	149 bp	238 bp	187 bp	$[TAGA]_{11}TAGG[TG]_5$	Lareu *et al.* (1998)
13 (a)	153 bp	242 bp	191 bp	$[TAGA]_{12}TAGG[TG]_5$	Lareu *et al.* (1998)
13 (b)	153 bp	242 bp	191 bp	$[TAGA]_{13}[TG]_5$	Phillips *et al.* (2010)
13.3	156 bp	245 bp	194 bp	$[TAGA]_1TGA[TAGA]_{11}TAGG[TG]_5$	Phillips *et al.* (2010)
14 (a)	157 bp	246 bp	195 bp	$[TAGA]_{13}TAGG[TG]_5$	Lareu *et al.* (1998)
14 (b)	157 bp	246 bp	195 bp	$[TAGA]_{14}[TG]_5$	Phillips *et al.* (2010)
14.3	160 bp	249 bp	198 bp	$[TAGA]_4TGA[TAGA]_9TAGG[TG]_5$	Phillips *et al.* (2010)
15	161 bp	250 bp	199 bp	$[TAGA]_{14}TAGG[TG]_5$	Lareu *et al.* (1998)
15.3	164 bp	253 bp	202 bp	$[TAGA]_4TGA[TAGA]_{10}TAGG[TG]_5$	Lareu *et al.* (1998)
16	165 bp	254 bp	203 bp	$[TAGA]_{15}TAGG[TG]_5$	Lareu *et al.* (1998)
16.3	168 bp	257 bp	206 bp	$[TAGA]_4TGA[TAGA]_{11}TAGG[TG]_5$	Lareu *et al.* (1998)
17	169 bp	258 bp	207 bp	$[TAGA]_{16}TAGG[TG]_5$	Lareu *et al.* (1998)
17.1	170 bp	259 bp	208 bp	Not published	Schröer *et al.* (2000)
17.3	172 bp	261 bp	210 bp	$[TAGA]_4TGA[TAGA]_{12}TAGG[TG]_5$	Lareu *et al.* (1998)
18	173 bp	262 bp	211 bp	$[TAGA]_{17}TAGG[TG]_5$	Phillips *et al.* (2010)
18.3	176 bp	265 bp	214 bp	$[TAGA]_4TGA[TAGA]_{13}TAGG[TG]_5$	Lareu *et al.* (1998)
19	177 bp	266 bp	215 bp	Not published	Asamura *et al.* (2008)
19.3	180 bp	269 bp	218 bp	$[TAGA]_4TGA[TAGA]_{14}TAGG[TG]_5$	Lareu *et al.* (1998)
20.3	184 bp	273 bp	222 bp	Not published	Gamero *et al.* (2000)

from Appendix 1, J.M. Butler (2012) Advanced Topics in Forensic DNA Typing: Methodology

NIST **U.S. Population Allele Frequencies**

D1S1656 (15 different alleles)

15 different alleles

Allele	African American (n=342)	Asian (n=97)	Caucasian (n=361)	Hispanic (n=236)
10	0.0146	0.0000	0.0028	0.0064
11	0.0453	0.0309	0.0776	0.0275
12	0.0643	0.0464	0.1163	0.0890
13	0.1009	0.1340	0.0665	0.1144
14	**0.2573**	0.0619	0.0789	0.1165
14.3	0.0073	0.0000	0.0028	0.0042
15	0.1579	**0.2784**	**0.1496**	0.1377
15.3	0.0292	0.0000	0.0582	0.0508
16	0.1096	0.2010	0.1357	**0.1758**
16.3	0.1023	0.0155	0.0609	0.0508
17	0.0278	0.0722	0.0471	0.0424
17.3	0.0497	0.0876	0.1330	0.1483
18	0.0029	0.0155	0.0055	0.0064
18.3	0.0234	0.0515	0.0499	0.0254
19.3	0.0073	0.0052	0.0152	0.0042

N=1036

(only unrelated samples used; fathers removed from this sample set)

D1S1656 Characteristics

- **15 alleles** observed
- **93 genotypes** observed
- **>89% heterozygotes** (heterozygosity = 0.8890)
- **0.0224 Probability of Identity** (P_I)

$$P_I = \sum (genotype\ \ frequencies)^2$$

These values have been calculated for all 29 STR loci across the U.S. population samples examined

Loci sorted on Probability of Identity (P_I) values

Locus	Alleles Observed	Genotypes Observed	Het (obs)	P_I Value n=1036
SE33	52	304	0.9353	0.0066
Penta E	23	138	0.8996	0.0147
D2S1338	13	68	0.8793	0.0220
D1S1656	15	93	0.8890	0.0224
D18S51	22	93	0.8687	0.0258
D12S391	24	113	0.8813	0.0271
FGA	27	96	0.8745	0.0308
D6S1043	27	109	0.8494	0.0321
Penta D	16	74	0.8552	0.0382
D21S11	27	86	0.8330	0.0403
D8S1179	11	46	0.7992	0.0558
D19S433	16	78	0.8118	0.0559
vWA	11	39	0.8060	0.0611
F13A01	16	56	0.7809	0.0678
D7S820	11	32	0.7944	0.0726
D16S539	9	28	0.7761	0.0749
D13S317	8	29	0.7674	0.0765
TH01	8	24	0.7471	0.0766
Penta C	12	49	0.7732	0.0769
D2S441	15	43	0.7828	0.0841
D10S1248	12	39	0.7819	0.0845
D3S1358	11	30	0.7519	0.0915
D22S1045	11	44	0.7606	0.0921
F13B	7	20	0.6911	0.0973
CSF1PO	9	31	0.7558	0.1054
D5S818	9	34	0.7297	0.1104
FESFPS	12	36	0.7230	0.1128
LPL	9	27	0.7027	0.1336
TPOX	9	28	0.6902	0.1358

Better for mixtures (more alleles seen)

29 STR Loci present in STR kits

rank ordered by their variability

N=1036

(only unrelated samples used)

There are several loci more polymorphic than the **current CODIS 13 STRs**

361 Caucasians
342 African Americans
236 Hispanics
97 Asians

Better for kinship (low mutation rate)

Probability of Identity Combinations
(assuming unrelated individuals)

STR Kit or Core Set of Loci	Total N=1036	Caucasians (n=361)	African Am. (n=342)	Hispanics (n=236)	Asians (n=97)
CODIS 13	5.02E-16	2.97E-15	1.14E-15	1.36E-15	1.71E-14
Identifiler	6.18E-19	6.87E-18	1.04E-18	2.73E-18	5.31E-17
PowerPlex 16	2.82E-19	4.24E-18	6.09E-19	1.26E-18	2.55E-17
PowerPlex 18D	3.47E-22	9.82E-21	5.60E-22	2.54E-21	7.92E-20
ESS 12	3.04E-16	9.66E-16	9.25E-16	2.60E-15	3.42E-14
ESI 16 / ESX 16 / NGM	2.80E-20	2.20E-19	6.23E-20	4.03E-19	9.83E-18
ESI 17 / ESX 17 / NGM SElect	1.85E-22	1.74E-21	6.71E-22	3.97E-21	1.87E-19
CODIS 20	9.35E-24	7.32E-23	6.12E-23	8.43E-23	4.22E-21
GlobalFiler	7.73E-28	1.30E-26	3.20E-27	2.27E-26	1.81E-24
PowerPlex Fusion	6.58E-29	2.35E-27	1.59E-28	2.12E-27	1.42E-25
All 29 autosomal STRs	2.24E-37	7.36E-35	3.16E-37	2.93E-35	4.02E-32
29 autoSTRs + DYS391	1.07E-37	3.26E-35	1.77E-37	1.29E-35	2.81E-32

~8-13 orders of magnitude improvement for total P_I (n=1036)

NIST U.S. Population Data

- The data from our 1036 U.S. population samples is currently available on STRBase:

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

- A summary of the NIST 1036 data set has been published in Profiles in DNA for autosomal and YSTR loci

- Population Data announcements have been published in *FSI: Genetics* for
  - 29 autosomal STR loci (*Hill et al*)
  - 23 Y-STR loci (*Coble et al*)


Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

Letter to the Editor

U.S. population data for 29 autosomal STR loci

run and population statistics were confirmed using the PowerMarker v3.25 statistics program [10].

- Added to CODIS PopStats software in Sept 2013

Summary

- Additional STR loci are important as DNA databases grow larger each year: the power of discrimination increases as new loci are added
  - Adding seven new loci (CODIS 13 vs CODIS 20) adds approximately 8 orders of magnitude improvement

- Commercial companies are continuing to release larger STR multiplex kits to meet the needs of the forensic community

- NIST has a set of 1036 unrelated U.S. population samples that have been used to fully characterize 29 autosomal STR loci available in commercial STR multiplex kits

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Final version of this presentation available at:
http://www.cstl.nist.gov/strbase/NISTpub.htm