



# Characterization of Additional STR Loci Beyond the 13 CODIS Loci

Becky Hill and John Butler

National Institute of Standards and Technology

Bode Technology 1st Annual Advanced DNA Technology  
Mid-Atlantic Workshop

Charlottesville, VA

September 20, 2012

# Outline of Topics to Discuss

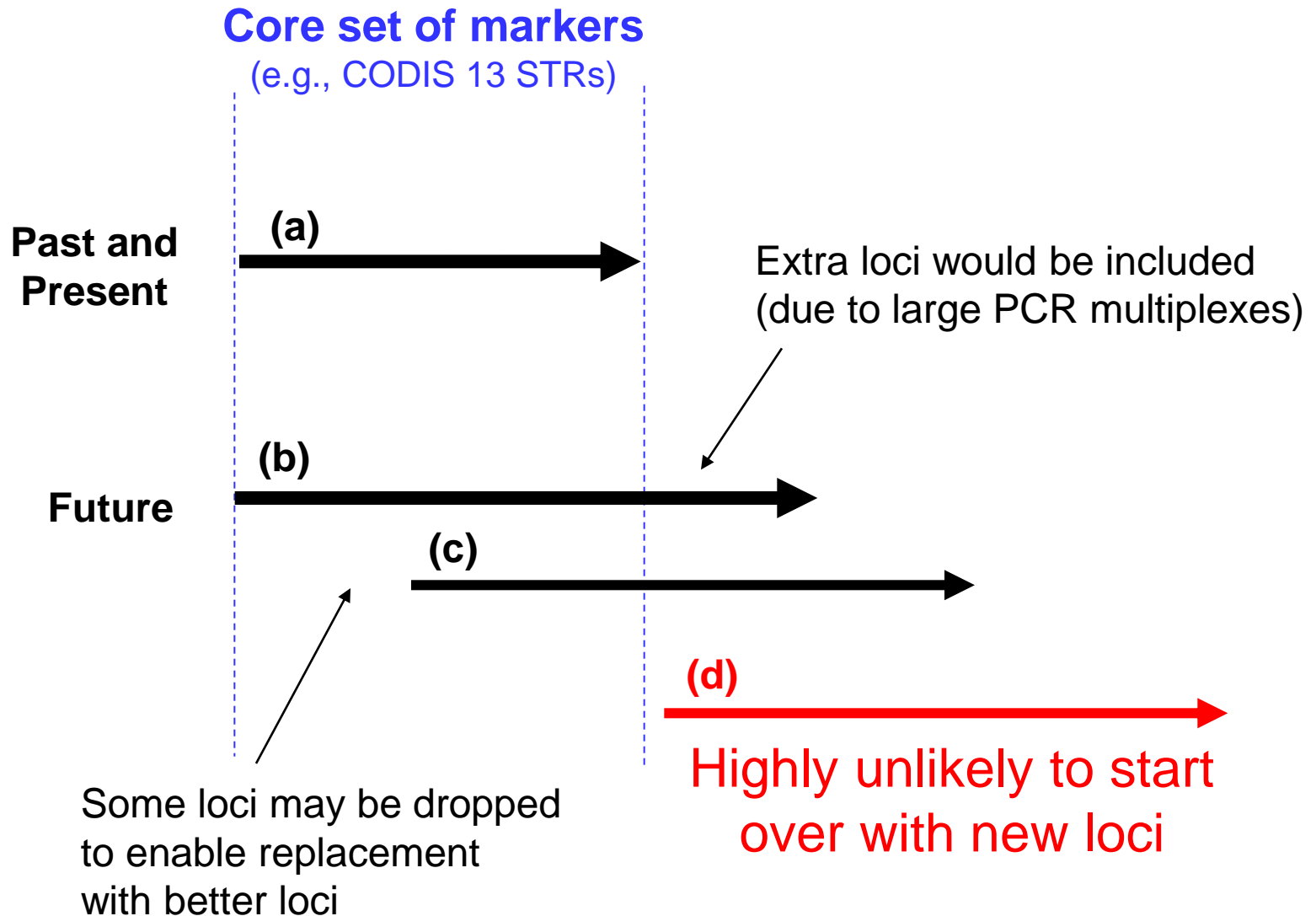
- Need for additional loci
  - Growth in U.S. and other national DNA databases
- Locus characteristics
- Population data and statistical analysis
- New STR kits available with additional loci
  - GlobalFiler (Life Technologies)
  - PowerPlex Fusion (Promega)

**Need for Additional Loci**

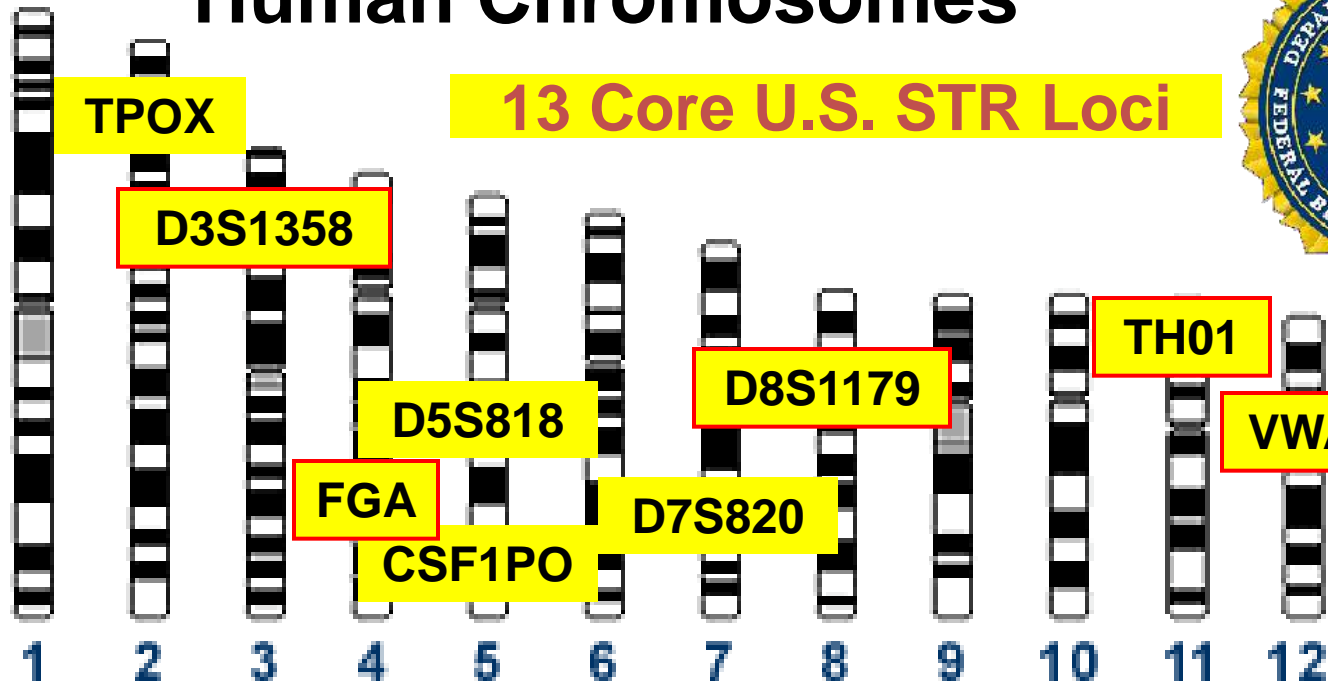
# Additional STR Loci in the Future?

- More loci will be needed for more complex kinship analyses and extended applications
  - Example: Y-STRs needed for familial searching
- Immigration testing needs more than 13 STRs
- Larger DNA databases will require more loci
  - CODIS database currently has 11.5 million profiles and it continues to quickly grow

# Possible scenarios for extending sets of genetic markers to be used in national DNA databases

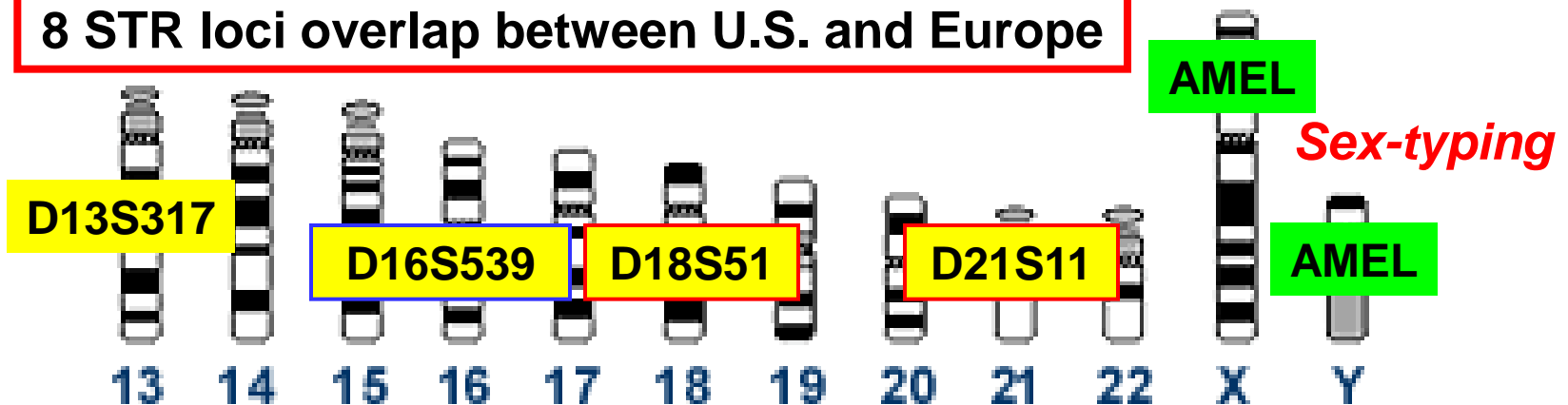


# Position of Forensic STR Markers on Human Chromosomes



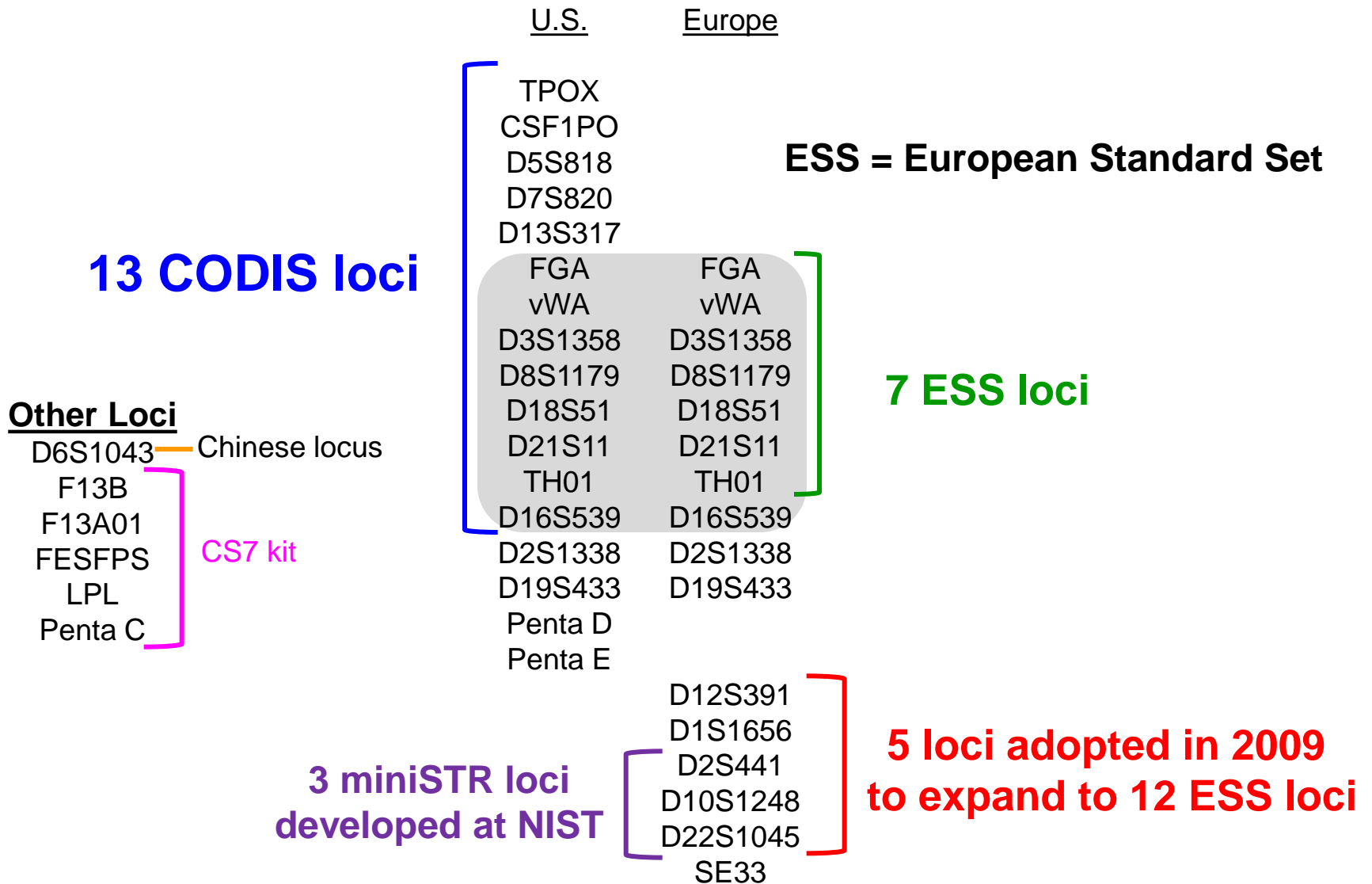
1997

**8 STR loci overlap between U.S. and Europe**



# 29 Autosomal STR Markers

## Present in Commercial STR Multiplex Kits



# Expanded U.S. Core Loci

Forensic Science International: Genetics 6 (2012) e52–e54



Letter to the Editor

## Expanding the CODIS core loci in the United States

Dear Editor:

After over a decade of operation, the National DNA Index System (NDIS) continues to grow in importance and size [1]. While the STR DNA technology has remained relatively consistent, other key aspects of the NDIS program have been reevaluated and revisions implemented. For example, based upon recommendations of the Scientific Working Group on DNA Analysis Methods, the Director of the Federal Bureau of Investigation (FBI) issued revised Quality Assurance Standards (QAS) for Forensic DNA

major reasons for expanding the CODIS core loci in the United States:

- (1) To reduce the likelihood of adventitious matches [7] as the number of profiles stored at NDIS continues to increase each year (expected to total over 10 million profiles by the time of this publication). There are no signs that this trend will slow down as States expand the coverage of their DNA database programs and increase laboratory efficiency and capacity.
- (2) To increase international compatibility to assist law enforcement data sharing efforts.
- (3) To increase discrimination power to aid missing persons cases.

Hares, D.R. (2012a) Expanding the CODIS core loci in the United States. *Forensic Sci. Int. Genet.* 6(1), e52-4.

Forensic Science International: Genetics 6 (2012) e135



Letter to the Editor

## Addendum to expanding the CODIS core loci in the United States

Dear Editor,

An important objective in proposing new CODIS core loci is to ensure that all loci would be available for all potential manufacturers. During the evaluation process, appropriate steps were taken to document access to all proposed core loci. Since

publication of the proposed list of core loci, additional information has come to our attention indicating that there may be outstanding issues with respect to some of the proposed loci. Consequently, to ensure the availability for all interested manufacturers in accordance with our stated objective, we are withdrawing Penta D and Penta E as proposed CODIS core loci and recommending the revised listing of core loci in Table 1. Manufacturers are still encouraged to attempt loci in Section B, in ranked order of preference, for inclusion in potential kits provided the impact on the kit's sensitivity and overall performance is negligible. Please

Hares, D.R. (2012b) Addendum to expanding the CODIS core loci in the United States. *Forensic Sci. Int. Genet.* 6(5), e135.



# Required and Recommended CODIS Core Loci

**Table 1**

Revised ranked list of CODIS core loci.

Locus
<b>Section A (required) Required Loci</b>
Amelogenin
D18S51
FGA
D21S11
D8S1179
vWA
D13S317
D16S539
D7S820
TH01
D3S1358
D5S818
CSF1PO
D2S1338
D19S433
D1S1656
D12S391
D2S441
D10S1248
DYS391
<b>Section B (in order of preference) Recommended Loci</b>
TPOX
D22S1045
SE33

Penta D and Penta E were removed from this list in the addendum

Y-STR to confirm Amelogenin null alleles

No longer required

# Commercially Available STR Kits

## Applied Biosystems (18)

- ~~AmpFISTR Blue (1996)~~
- ~~AmpFISTR Green I (1997)~~
- Profiler (1997)
- Profiler Plus (1997)
- COfiler (1998)
- SGM Plus (1999)
- **Identifiler** (2001)
- Profiler Plus ID (2001)
- ~~SEfiler (2002)~~
- **Yfiler** (2004)
- MiniFiler (2007)
- SEfiler Plus (2007)
- Sinofiler (2008) – China only
- **Identifiler Direct** (2009)
- NGM (2009)
- **Identifiler Plus** (2010)
- NGM SElect (2010)
- **GlobalFiler** (2012)

## Promega Corporation (17)

- PowerPlex 1.1 (1997)
- PowerPlex 1.2 (1998)
- PowerPlex 2.1 (1999)
- **PowerPlex 16** (2000)
- PowerPlex ES (2002)
- **PowerPlex Y (2003)**
- PowerPlex S5 (2007)
- **PowerPlex 16 HS** (2009)
- PowerPlex ESX 16 (2009)
- PowerPlex ESX 17 (2009)
- PowerPlex ESI 16 (2009)
- PowerPlex ESI 17 (2009)
- PowerPlex CS7 (2009)
- PowerPlex 18D (2011)
- **PowerPlex Y23 (2012)**
- PowerPlex 21 (2012)
- **PowerPlex Fusion** (2012)

## Qiagen (10) kits in 2010

*Primarily selling kits in Europe  
Due to patent restrictions  
cannot sell in U.S.*

### Investigator kits

- ESSplex
- ESSplex SE
- Decaplex SE
- IDplex
- Nonaplex ESS
- Hexaplex ESS
- HDplex
- Triplex AFS QS
- Triplex DSF
- Argus X-12

**>1/3 of all STR kits were released in the last four years**

# Most Commonly Used STR Kits in the U.S.

## 29 STR Loci

Locus	CODIS 13	CODIS 20	ESS 12	PP 16	PP 18D	PP ES/ESX 16	PP ES/ESX 17	PP 21	PP CS7	PP Fusion	Profiler Plus	COfiler	SGM Plus	SEfiler Plus	SinoFiler	MiniFiler	Identifiler	NGM	NGM Select	GlobalFiler	
	Required loci			Promega STR kits						Life Technologies (ABI) STR kits											
D1S1656																					
F13B																					
TPOX																					
D2S441																					
D2S1338																					
D3S1358																					
FGA																					
CSF1PO																					
D5S818																					
F13A01																					
D6S1043																					
SE33																					
D7S820																					
LPL																					
D8S1179																					
Penta C																					
D10S1248																					
TH01																					
D12S391																					
vWA																					
D13S317																					
FESFPS																					
Penta E																					
D16S539																					
D18S51																					
D19S433																					
D21S11																					
Penta D																					
D22S1045																					
Amelogenin																					
DYS391																					

# NIST U.S. Population Samples

# 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/biotech/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

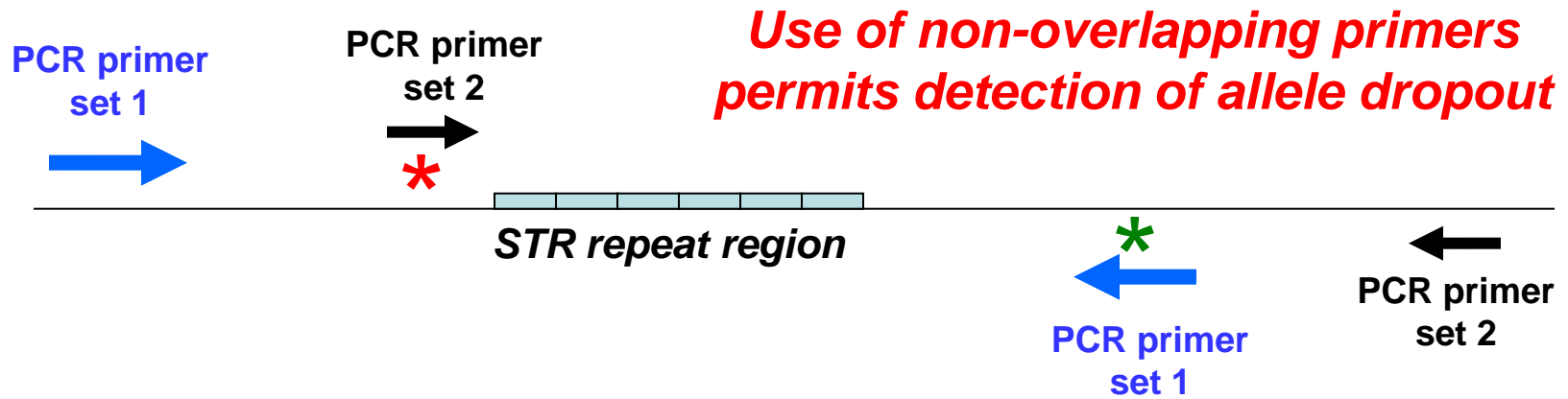
- Many of these STR kits have different primer sequences for amplifying the same STR locus
- Need to analyze the same DNA samples with different STR typing kits looking for differences
- In some rare cases, allele dropout may occur due to mutations in primer binding regions



# 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

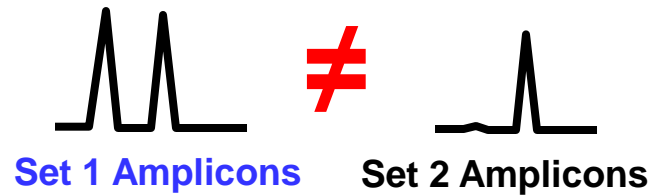
\* represents potential mutations impacting primer annealing



**If no primer binding site mutations**



**If a primer binding site mutation exists**





# Completed Concordance Studies

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	23	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/ES17	1443	11	15,873	5	99.968
ID/ES17	1443	11	15,873	4	99.975
ID/ES17	1433	11	15,763	28	99.822
ID/ES17	662	11	7,292	17	99.767
ID/Hexaplex	663	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	666	8	5,248	14	99.731
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/ES17	662	9	5,958	0	100.000
PP16/ESS	653	9	5,877	16	99.726
PP16/ESSpleGE	662	9	5,958	16	99.731
PP16/Hexaplex	663	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	661	11	7,161	0	100.000
SGM+/ESX17	1424	11	15,664	6	99.862
SGM+/ES17	1424	11	15,664	5	99.968
SGM+/ESS	1424	11	15,664	5	99.968
SGM+/ESSpleGE	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+/ES17	1415	7	9,905	3	99.970
Pro+/ESS	1415	7	9,905	4	99.960
Pro+/ESSpleGE	647	7	4,529	4	99.912
DPlex/Hexaplex	647	1	848	1	99.846
DPlex/MiniFiler	1137	9	10,233	48	99.531
DPlex/SGM+	1426	11	15,689	20	99.873
DPlex/NGMs	657	11	7,227	17	99.765
DPlex/ESX17	1426	11	15,686	28	99.821
DPlex/ES17	1426	11	15,686	27	99.828
DPlex/ESS	1426	11	15,686	1	99.994
DPlex/ESSpleGE	657	11	7,227	1	99.986
DPlex/Hexaplex	653	2	1,306	1	99.923
MiniFiler/NGM	1137	6	6,822	13	99.809
MiniFiler/NGMs	656	6	3,336	10	99.746
MiniFiler/ESX17	1137	6	6,822	10	99.853
MiniFiler/ES17	1137	6	6,822	9	99.869
MiniFiler/ESS	1137	6	6,822	35	99.487
MiniFiler/ESSpleGE	656	6	3,336	35	99.111
MiniFiler/Hexaplex	653	1	653	1	99.847
NGM/NGMs	657	16	10,512	14	99.867
NGM/ESX17	1437	16	22,952	16	99.930
NGM/ES17	1437	16	22,952	18	99.822
NGM/ESS	1433	16	22,928	42	99.817
NGM/ESSpleGE	657	16	10,512	22	99.793
NGM/Hexaplex	653	7	4,571	3	99.833
NGMs/ESX17	662	17	11,254	4	99.964
NGMs/ES17	662	17	11,254	14	99.876
NGMs/ESS	663	16	10,448	17	99.837
NGMs/ESSpleGE	662	17	11,254	34	99.698
NGMs/Hexaplex	663	7	4,571	3	99.834
ESX17/ES17	1443	17	24,531	19	99.923
ESX17/ESS	663	16	10,448	34	99.675
ESX17/ESSpleGE	662	17	11,254	25	99.778
ESX17/Hexaplex	657	7	4,569	6	99.870
ES17/ESS	653	16	10,448	28	99.732
ES17/ESSpleGE	662	17	11,254	30	99.733
ES17/Hexaplex	657	7	4,569	3	99.825
ES17/Hexaplex	653	16	10,448	0	100.000
ES17/Hexaplex	663	7	4,571	3	99.834
ES17/Hexaplex	653	7	4,571	3	99.834
ES17/Hexaplex	1443	1	1,443	6	99.584
SE33/ES17	1443	1	1,443	17	98.822
SE33/NGMs	663	1	663	4	99.397
SE33/ESSpleGE	662	1	662	21	96.828
ES17/ESX17	477	17	8,109	7	99.814
ES17/NGMs	477	17	8,109	2	99.975
ES17/ESSpleGE	477	17	8,109	42	99.482
ES17/ESS	477	1	477	4	99.161
PP18/ID	50	16	800	2	99.750
PP18/PP16	703	16	11,248	1	99.991
ESX17/ESX17	1443	17	24,031	4	99.884
ESX17/ES17	477	17	8,109	3	99.963
ESX17/NGM	1437	16	22,962	22	99.804
ESX17/NGMs	663	17	11,271	4	99.965
ESX17/ESS	1433	16	22,928	30	99.869
ESX17/ESSpleGE	662	17	11,254	44	99.609
ESX17/Hexaplex	653	7	4,571	2	99.956
26plex/ESX17	1443	3	4,329	4	99.908
26plex/ES17	1443	3	4,329	0	100.000
26plex/NGM	1437	3	4,311	11	99.745
26plex/NGMs	663	3	1,989	0	100.000
26plex/ESS	1433	3	4,299	0	100.000
26plex/ESSpleGE	662	3	1,986	0	100.000
26plex/Hexaplex	653	3	1,959	2	99.898
26plex/ESX17	663	3	1,989	0	100.000
minSTR/ESX17	663	3	1,989	3	99.849
minSTR/ES17	663	3	1,989	0	100.000
minSTR/NGM	657	3	1,971	3	99.848
minSTR/NGMs	663	3	1,989	0	100.000
minSTR/ESS	653	3	1,959	0	100.000
minSTR/ESSpleGE	662	3	1,986	0	100.000
minSTR/Hexaplex	653	3	1,959	2	99.898
minSTR/ESX17	663	3	1,989	0	100.000
PP21/MiniFiler	761	16	12,176	6	99.501
PP21/PP16	761	16	12,176	3	99.975
PP21/SGM+	761	11	8,371	4	99.824
PP21/Pro+	761	10	7,610	2	99.974
PP21/IDPlex	761	16	12,176	20	99.836
PP21/MiniFiler	761	9	6,849	14	99.736
PP21/ESX17	761	13	8,993	1	99.990
PP21/ES17	761	13	8,993	0	100.000
PP21/NGM	761	13	8,993	5	99.949
PP21/NGMs	568	13	7,384	1	99.986
PP21/ESS	761	13	8,993	18	99.818
PP21/ESSpleGE	568	13	7,384	16	99.783
PP21/Hexaplex	568	4	2,272	1	99.956
PP21/Identifiler	568	16	3,068	1	99.989
PP21a/Identifiler	639	16	10,224	4	99.961
PP21a/PP16	639	16	10,224	1	99.990
<b>Totals</b>	<b>114144</b>	<b>1245</b>	<b>1,104,031</b>	<b>1224</b>	<b>99.889</b>

Kits compared	Samples	Loci Compared	Comparisons	# Differences	Concordance (%)
<b>128</b>	<b>114144</b>	<b>1245</b>	<b>1,104,031</b>	<b>1224</b>	<b>99.889</b>

**1,104,031** allele comparisons  
**1,224** total differences  
**99.89%** concordance

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



# Characterization of STR Loci

## Available in Commercial Kits

# The 10 STR Loci Beyond the CODIS 13

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

5 new European loci

\*Allele range and number of observed alleles from Appendix 1, J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*; <sup>‡</sup>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] <sub>4</sub> [TGA] <sub>0-1</sub> [TAGA] <sub>n</sub> TAGG[TG] <sub>5</sub>	Reference
8	133 bp	222 bp	171 bp	[TAGA] <sub>8</sub> [TG] <sub>5</sub>	Phillips <i>et al.</i> (2010)
9	137 bp	226 bp	175 bp	[TAGA] <sub>9</sub> [TG] <sub>5</sub>	Phillips <i>et al.</i> (2010)
10 (a)	141 bp	230 bp	179 bp	[TAGA] <sub>10</sub> [TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
10 (b)	141 bp	230 bp	179 bp	[TAGA] <sub>10</sub> TAGG[TG] <sub>5</sub>	Phillips <i>et al.</i> (2010)
11	145 bp	234 bp	183 bp	[TAGA] <sub>11</sub> [TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
12 (a)	149 bp	238 bp	187 bp	[TAGA] <sub>12</sub> [TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
12 (b)	149 bp	238 bp	187 bp	[TAGA] <sub>11</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
13 (a)	153 bp	242 bp	191 bp	[TAGA] <sub>12</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
13 (b)	153 bp	242 bp	191 bp	[TAGA] <sub>13</sub> [TG] <sub>5</sub>	Phillips <i>et al.</i> (2010)
13.3	156 bp	245 bp	194 bp	[TAGA] <sub>1</sub> TGA[TAGA] <sub>11</sub> TAGG[TG] <sub>5</sub>	Phillips <i>et al.</i> (2010)
14 (a)	157 bp	246 bp	195 bp	[TAGA] <sub>13</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
14 (b)	157 bp	246 bp	195 bp	[TAGA] <sub>14</sub> [TG] <sub>5</sub>	Phillips <i>et al.</i> (2010)
14.3	160 bp	249 bp	198 bp	[TAGA] <sub>4</sub> TGA[TAGA] <sub>9</sub> TAGG[TG] <sub>5</sub>	Phillips <i>et al.</i> (2010)
15	161 bp	250 bp	199 bp	[TAGA] <sub>14</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
15.3	164 bp	253 bp	202 bp	[TAGA] <sub>4</sub> TGA[TAGA] <sub>10</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
16	165 bp	254 bp	203 bp	[TAGA] <sub>15</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
16.3	168 bp	257 bp	206 bp	[TAGA] <sub>4</sub> TGA[TAGA] <sub>11</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
17	169 bp	258 bp	207 bp	[TAGA] <sub>16</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
17.1	170 bp	259 bp	208 bp	Not published	Schröer <i>et al.</i> (2000)
17.3	172 bp	261 bp	210 bp	[TAGA] <sub>4</sub> TGA[TAGA] <sub>12</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
18	173 bp	262 bp	211 bp	[TAGA] <sub>17</sub> TAGG[TG] <sub>5</sub>	Phillips <i>et al.</i> (2010)
18.3	176 bp	265 bp	214 bp	[TAGA] <sub>4</sub> TGA[TAGA] <sub>13</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
19	177 bp	266 bp	215 bp	Not published	Asamura <i>et al.</i> (2008)
19.3	180 bp	269 bp	218 bp	[TAGA] <sub>4</sub> TGA[TAGA] <sub>14</sub> TAGG[TG] <sub>5</sub>	Lareu <i>et al.</i> (1998)
20.3	184 bp	273 bp	222 bp	Not published	Gamero <i>et al.</i> (2000)

# 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	<b>0.2573</b>	0.0619	0.0789	0.1165
14.3	0.0073	0.0000	0.0028	0.0042
15	0.1579	<b>0.2784</b>	<b>0.1496</b>	0.1377
15.3	0.0292	0.0000	0.0582	0.0508
16	0.1096	0.2010	0.1357	<b>0.1758</b>
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 ( $P_I$ )**

$$P_I = \sum (\textit{genotype frequencies})^2$$

**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

29 STR Loci  
present in STR kits  
rank ordered by their  
variability

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

Better for mixtures (more alleles seen)

**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)
<b>CODIS 13</b>	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
<b>ESS 12</b>	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
<b>CODIS 20</b>	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
<b>All 29 autosomal STRs</b>	2.24E-37	7.36E-35	3.16E-37	2.93E-35	4.02E-32
<b>29 autoSTRs + DYS391</b>	1.07E-37	3.26E-35	1.77E-37	1.29E-35	2.81E-32

18 orders of magnitude  
improvement



# NIST U.S. Population Data

- We plan on making the data from our 1036 U.S. population samples available on STRBase:

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

- A summary of the NIST 1036 data set was recently submitted to *Profiles in DNA* for autosomal and YSTR loci
- Population Data announcements will be submitted to FSI: Genetics (soon!) for
  - 29 autosomal STR loci (*Hill et al*)
  - 23 Y-STR loci (*Coble et al*)

# New STR Multiplex Kits

## Recently Launched

# GlobalFiler STR Kit

Launched Friday, September 14, 2012

## Human Identification

GlobalFiler™ Kit

Go Faster

Go Further

Go Global

Powered by 6-Dye™

Human Identification Home



## Introducing the world's most powerful STR kit

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

Go Faster ▶

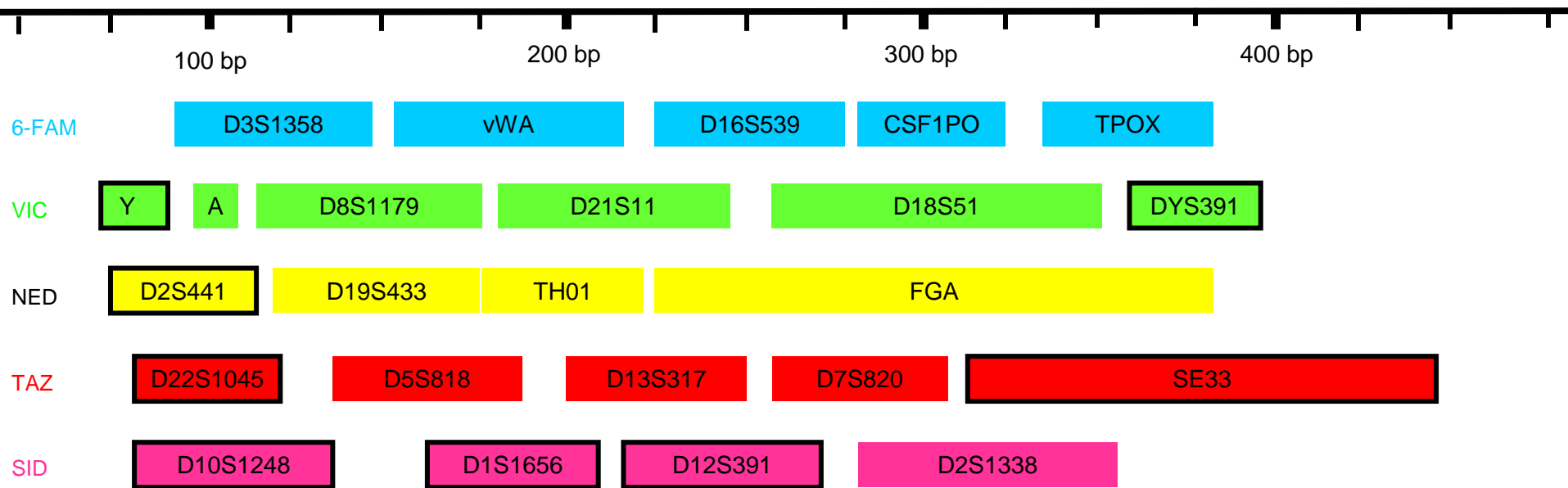
Go Further ▶

Go Global ▶

Powered  
by 6-Dye™ ▶

# Applied Biosystems GlobalFiler

24plex



- 24 STR loci in 6 dyes (3500 instrument use only)
- Direct amplification capabilities
  - Single Source Samples: 40 min amplification
  - Casework Samples: 80 min amplification
- Largest products <460 bp, 10 markers <220 bp (miniSTRs)
- Allelic ladder includes 343 total alleles



# PowerPlex Fusion

PowerPlex® Fusion System

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.

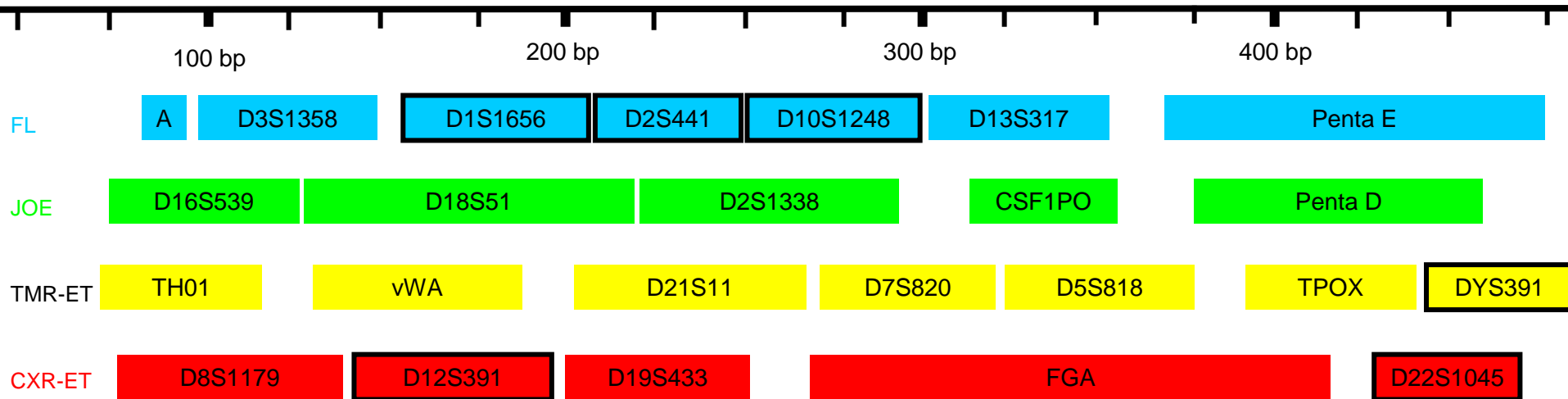
The PowerPlex® Fusion System provides all of the materials needed for co-amplification and five-color fluorescent detection of 24 loci (23 STR loci and Amelogenin), including the CODIS core loci and the European Standard Set (ESS) loci. With 24 loci, the system offers the most STR loci and highest discrimination 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.





# Promega PowerPlex FUSION

24plex

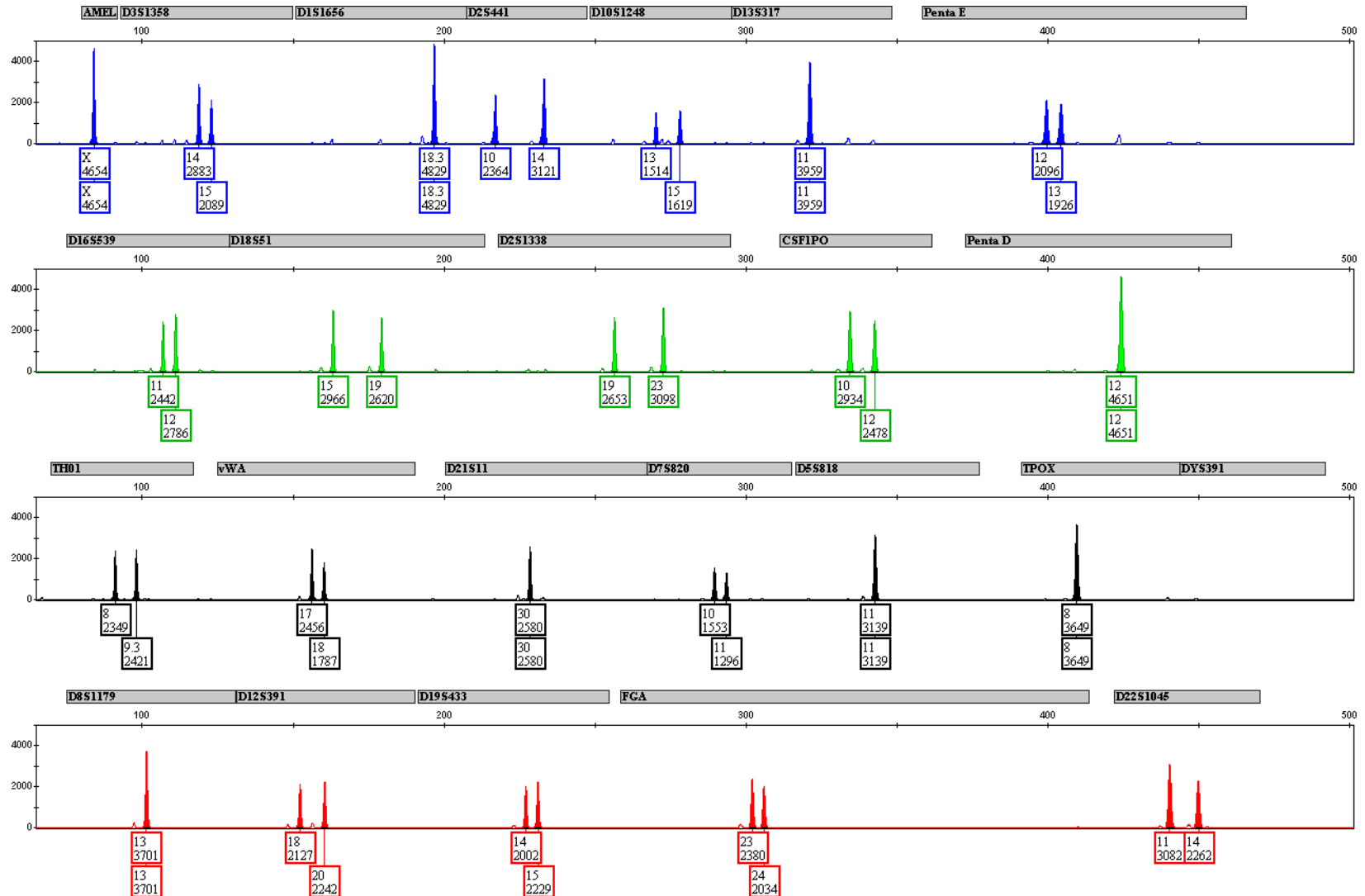


- 24 STR loci in 5 dyes (3130 and 3500 instrument use)
- Direct amplification capabilities: 85 min amp
- Largest products <500 bp
- Full profiles using 100 pg at 30 cycles

# NIST Concordance Testing with PowerPlex Fusion

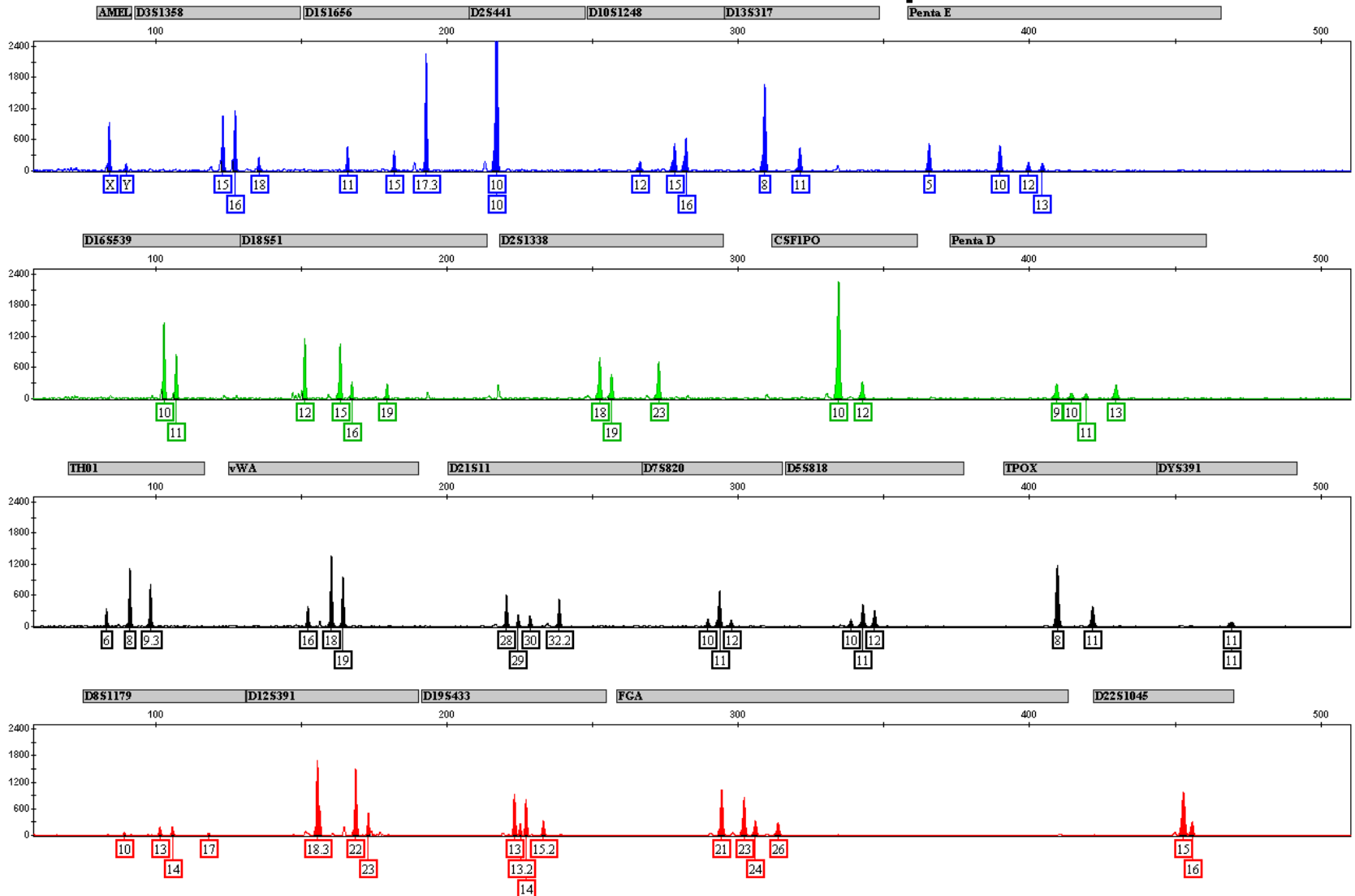
- PP Fusion results compared to all other kits tested including:  
Sinofiler/NGM/Identifiler/Yfiler/IDplex/ESSplex/PP16/PP21 kits with **652 unrelated individuals** (NIST U.S. population set )
- PP Fusion is **fully concordant with NIST SRMs 2391b&c** certified values
- No PP Fusion **null alleles**
- No PP Fusion **discordance** with other PowerPlex kits, discordance with ABI or Qiagen kits is on their end and are previously documented

# SRM 2391b&c were **fully concordant** at all loci for PP Fusion kit – **9947A Profile**



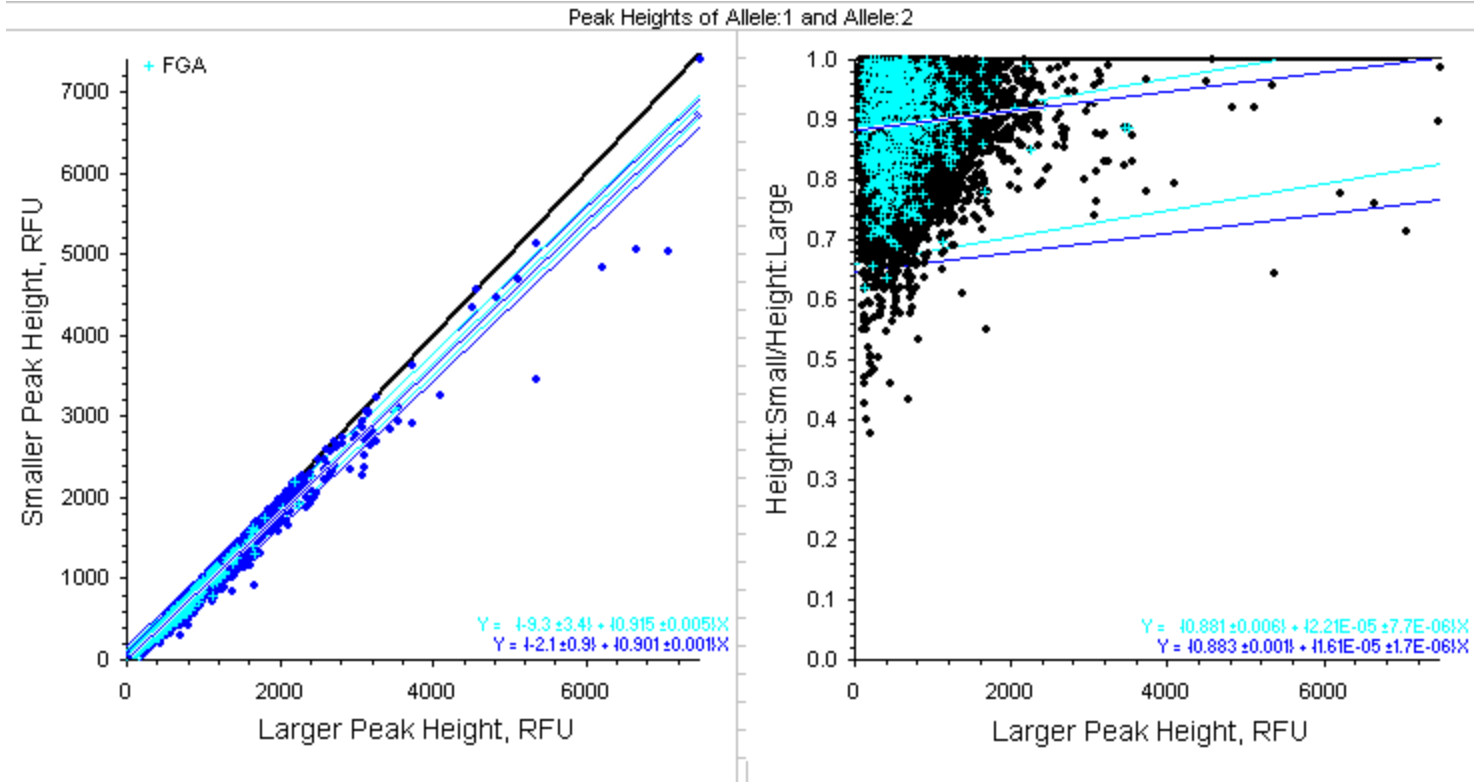
1 ng DNA, 30 cycles

# SRM 2391c Mixture Component D

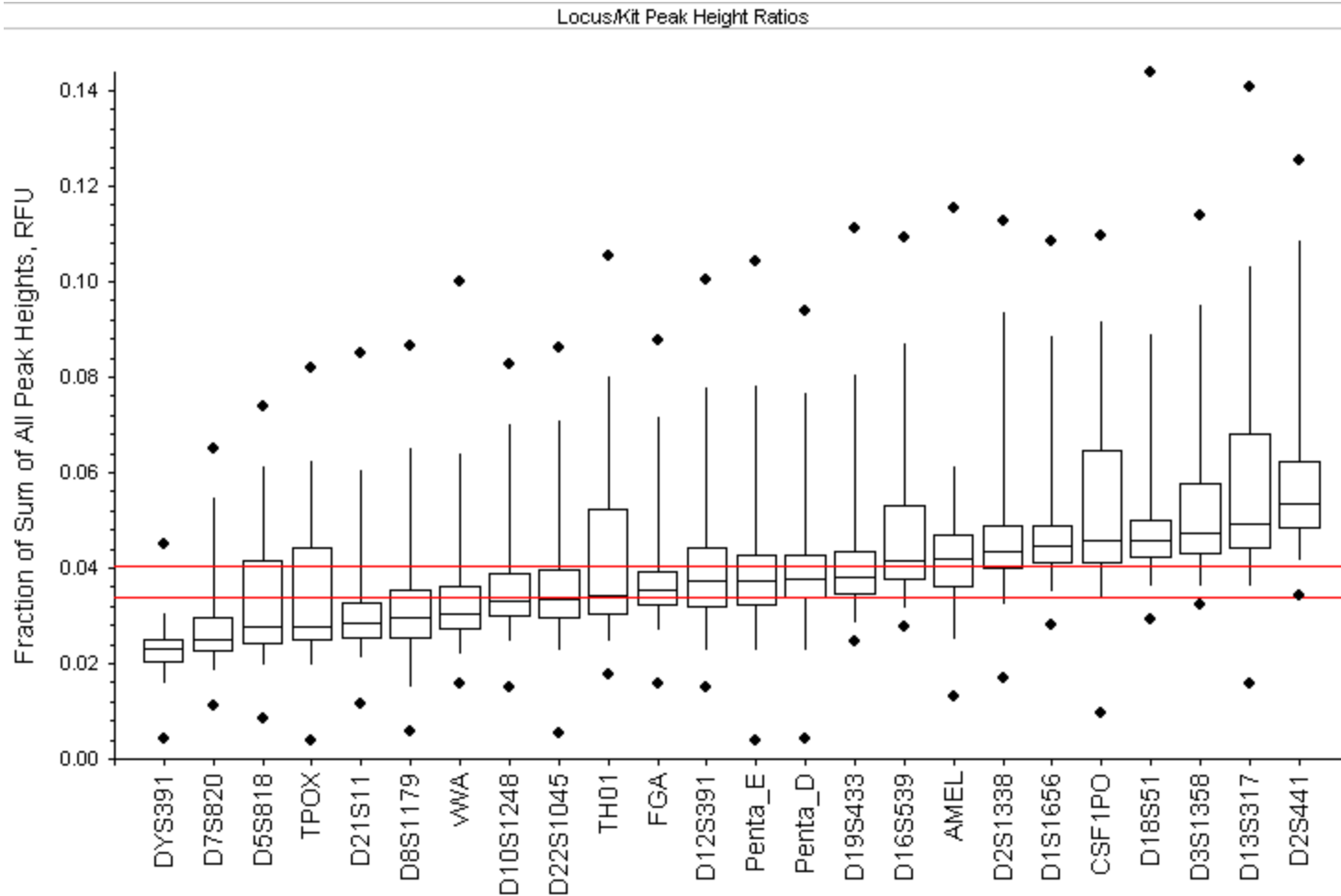


\*This sample was low-injected because the default had too much bleed through

# Peak Height Ratios



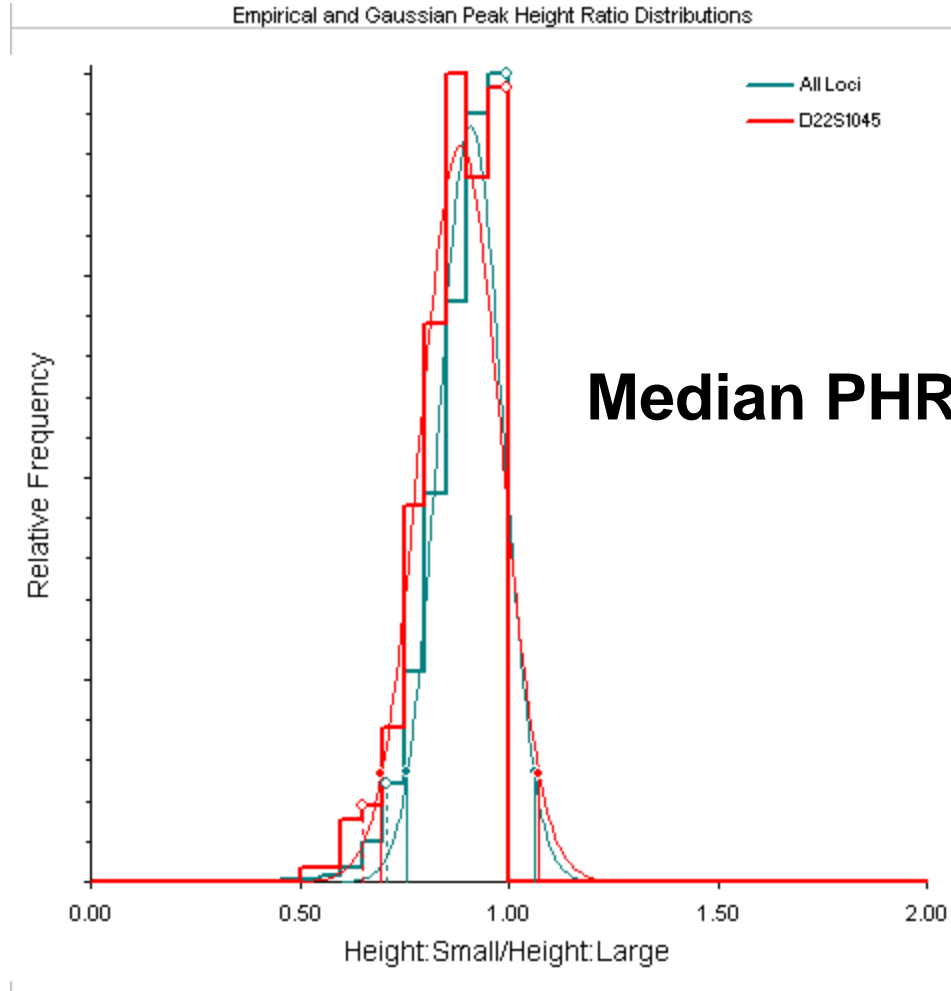
# PHR Balance Across All Loci



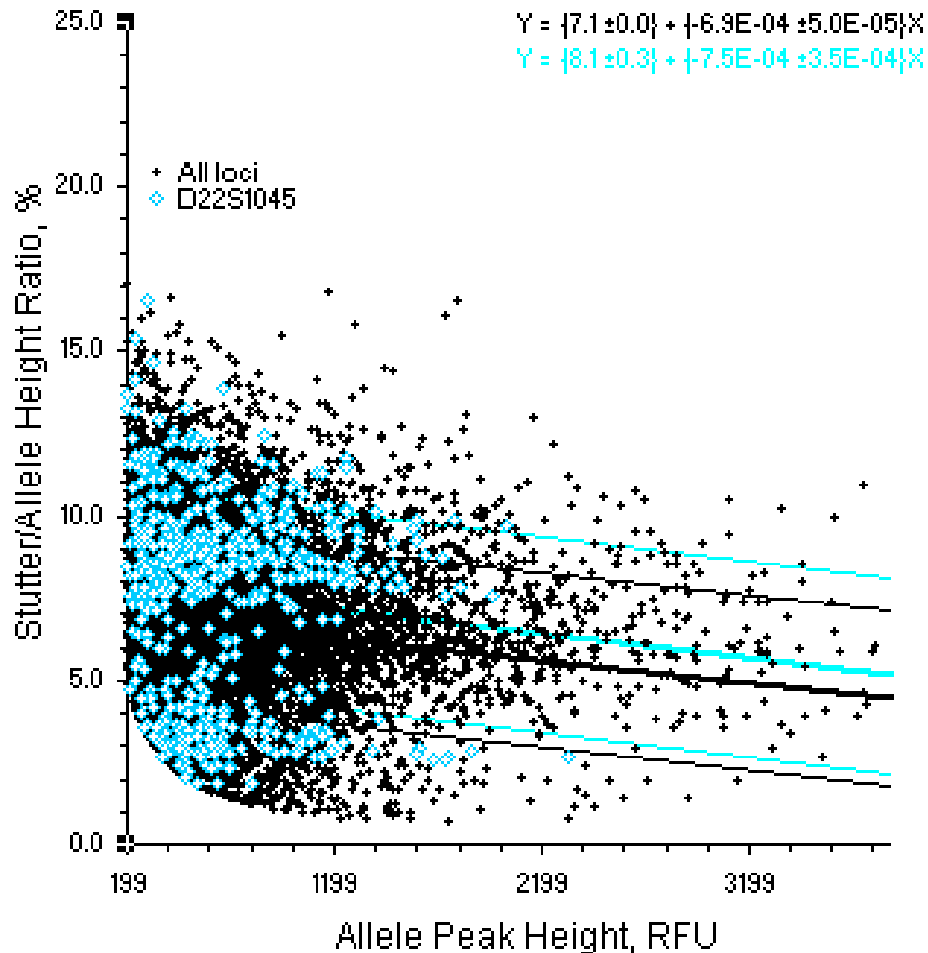
"Boxes" enclose 50% of the Locus/Sample Ratios, centerlines denote the median, "whiskers" span central 95%, dots denote the min and max.

Horizontal red lines denote the Kfactor-defined confidence interval on the mean of the medians.

# Heterozygote Peak Balance



# Minus Stutter Observed (Big Picture View)



\*D22S1045  
stutter is in blue  
(trinucleotide locus)



# Locus-Specific Stutter Values

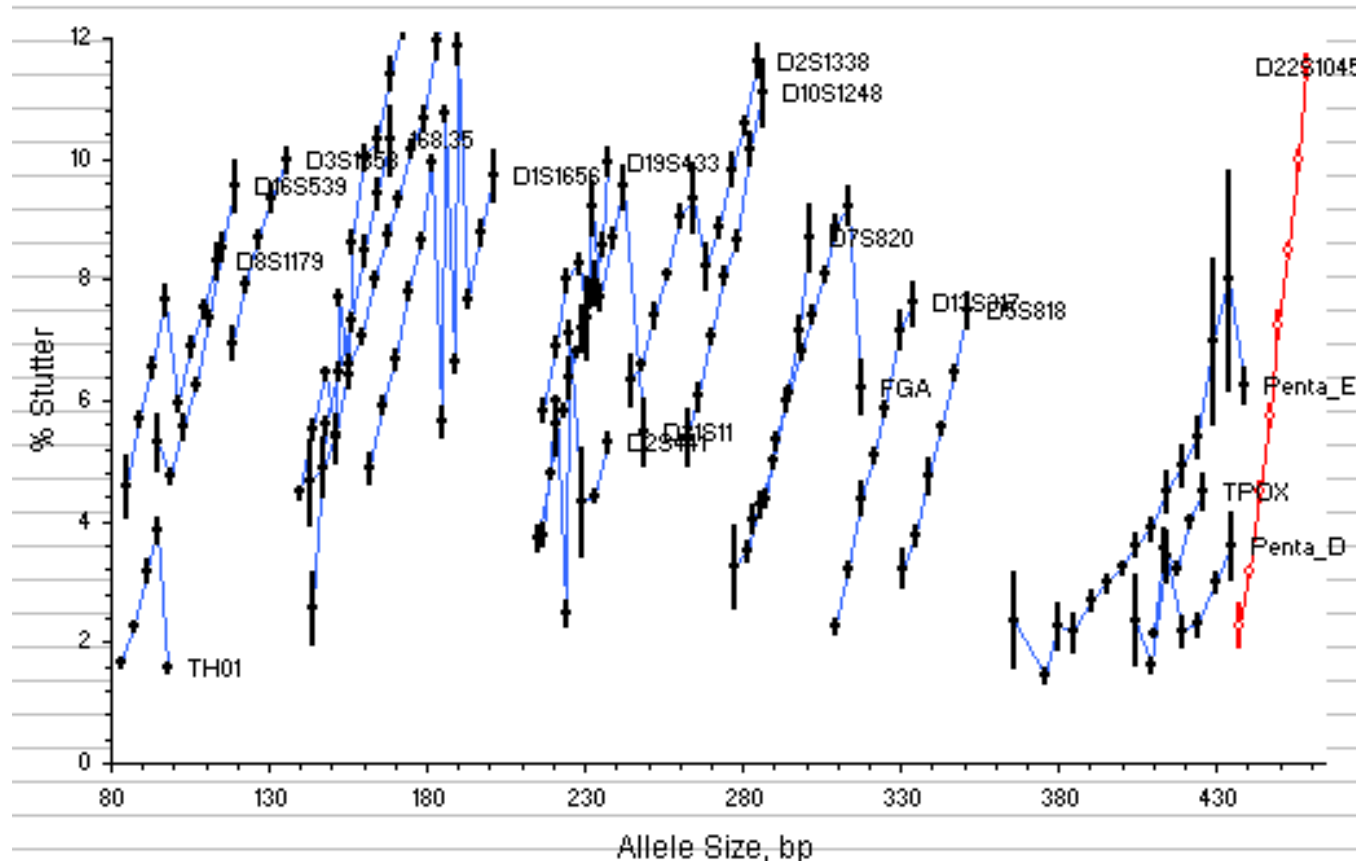
Created on: 23-May-12 16:37:36		Percentage Stutter																	
From Version: 29-Feb-12		Locus	#Ratio	Mean	SD	Median	MADe	Min	1%	2.5%	5%	10%	25%	75%	90%	95%	97.5%	99%	Max
Filter	Setting	TH01	432	2.5	1.1	2.2	1.0	0.9	1.1	1.2	1.3	1.4	1.7	3.2	4.0	4.5	5.2	5.8	10.0
Fetch	Stored profiles	Penta_D	427	2.9	1.6	2.5	1.2	0.7	0.8	1.0	1.1	1.3	1.8	3.5	4.9	5.9	6.4	7.4	12.7
Min PkHt	200	TPOX	452	3.3	1.3	3.2	1.5	0.9	1.5	1.6	1.7	1.8	2.2	4.1	4.9	5.8	6.3	6.9	9.6
Max PkHt	4000	Penta_E	685	4.0	2.4	3.5	1.6	0.7	0.9	1.1	1.3	1.7	2.6	4.7	6.8	8.8	11.3	12.8	15.3
Max %Stutter	30	D7S820	428	5.2	1.7	4.9	1.6	1.8	2.5	2.7	2.8	3.1	4.0	6.2	7.5	8.7	9.1	10.3	11.2
Allow Forwards	No	D2S441	711	5.1	1.6	5.1	1.5	1.0	1.9	2.3	2.7	3.2	4.0	6.1	6.9	7.7	8.7	9.7	14.7
Min #Ratio	7	D13S317	526	5.1	1.9	5.2	1.7	1.2	1.7	1.8	2.0	2.5	4.0	6.2	7.4	8.1	8.8	10.0	17.1
Bp/win	0.50	D5S818	388	5.8	1.6	5.9	1.3	1.8	2.6	3.0	3.4	3.8	4.9	6.7	7.6	8.2	9.1	9.8	13.7
		CSF1PO	452	6.1	1.6	6.0	1.4	1.6	2.4	2.8	3.4	4.2	5.2	7.0	8.3	9.0	9.8	10.6	12.4
		D16S539	496	6.5	1.7	6.3	1.7	2.3	3.6	3.8	4.0	4.4	5.3	7.6	8.9	9.5	10.3	10.8	14.3
		D19S433	588	6.6	1.6	6.5	1.4	1.8	3.3	3.6	4.3	4.7	5.6	7.5	8.6	9.4	10.2	10.7	12.9
		D8S1179	522	6.8	1.5	6.7	1.4	2.5	3.3	3.8	4.3	5.1	5.8	7.8	8.6	9.4	10.2	10.9	11.8
		vWA	461	6.9	2.3	6.8	1.9	0.9	1.2	1.5	2.3	4.3	5.6	8.2	9.6	10.4	11.4	12.3	20.6
		FGA	644	6.9	1.7	6.8	1.7	3.4	3.7	3.9	4.2	4.7	5.7	8.1	9.2	9.8	10.3	11.0	12.6
		D10S1248	453	8.0	1.8	7.7	1.6	3.7	4.7	5.0	5.5	6.0	6.8	8.9	10.5	11.4	12.0	13.2	15.3
		D21S11	633	8.1	1.6	8.0	1.4	4.1	4.9	5.3	5.7	6.1	7.1	9.0	10.2	10.8	12.0	12.9	15.3
		D1S1656	813	8.2	2.0	8.1	2.2	2.3	4.2	4.9	5.3	5.8	6.7	9.7	10.9	11.5	12.2	12.8	15.6
		D22S1045	552	7.6	3.0	8.3	2.7	1.8	2.2	2.4	2.8	3.0	4.5	9.6	11.2	11.7	12.4	13.5	16.6
		D12S391	696	8.6	2.7	8.3	3.0	3.7	4.0	4.3	4.6	5.0	6.5	10.5	12.4	13.2	14.3	15.3	16.8
		D18S51	745	8.4	2.2	8.4	2.3	3.6	4.3	4.8	5.1	5.4	6.8	9.8	11.4	12.4	13.1	13.6	16.6
		D2S1338	834	8.7	1.9	8.5	2.0	4.0	5.4	5.6	5.9	6.3	7.2	9.9	11.2	11.8	12.7	13.4	14.9
		D3S1358	484	8.6	1.6	8.5	1.5	3.1	5.4	5.9	6.2	6.7	7.5	9.5	10.6	11.2	11.8	12.6	16.1

Filter	Setting
Fetch	Stored profiles
Min PkHt	200
Max PkHt	4000
Max %Stutter	30
Allow Forwards	No
Min #Ratio	7
Bp/win	0.50

**Settings Used for Analysis**

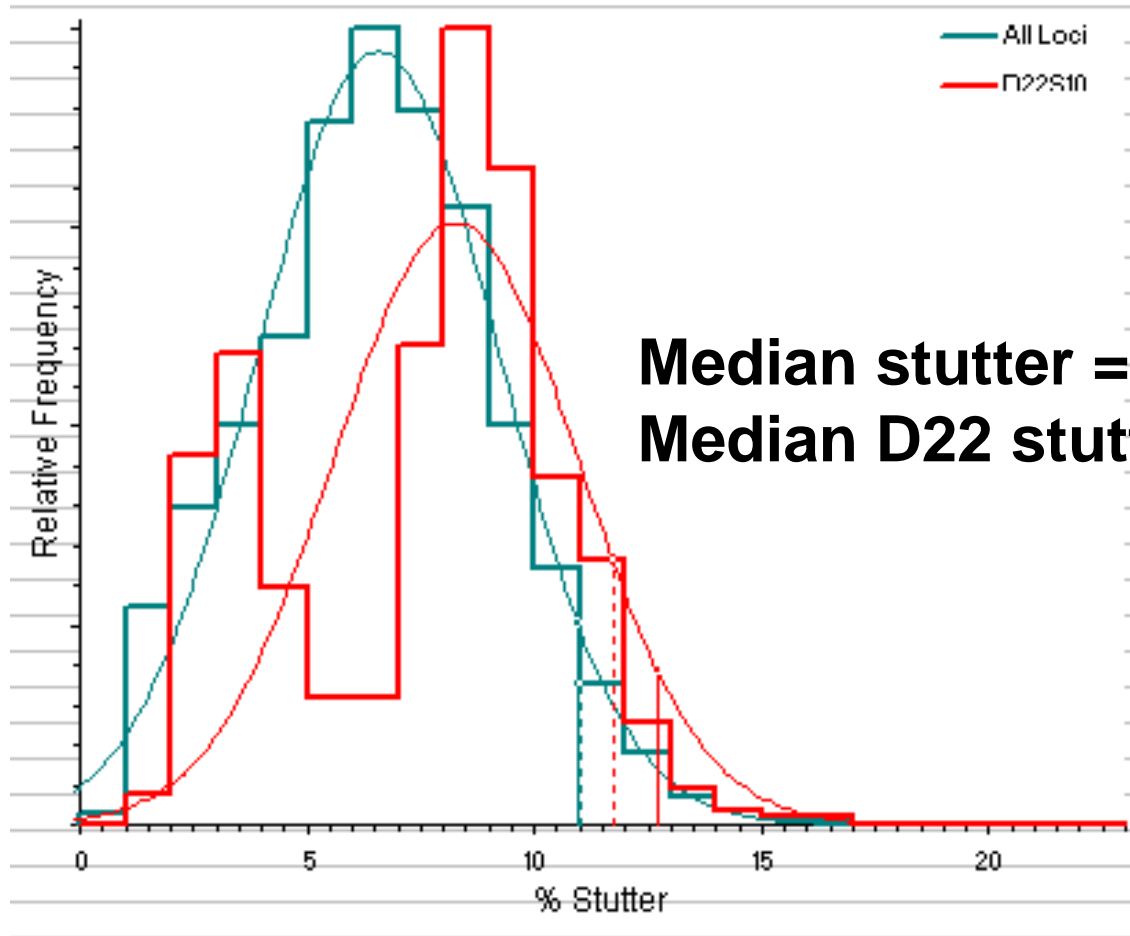
\*Verified Max stutter amounts

# Stutter Trends (Size Scale)



\*D22S1045 is in red

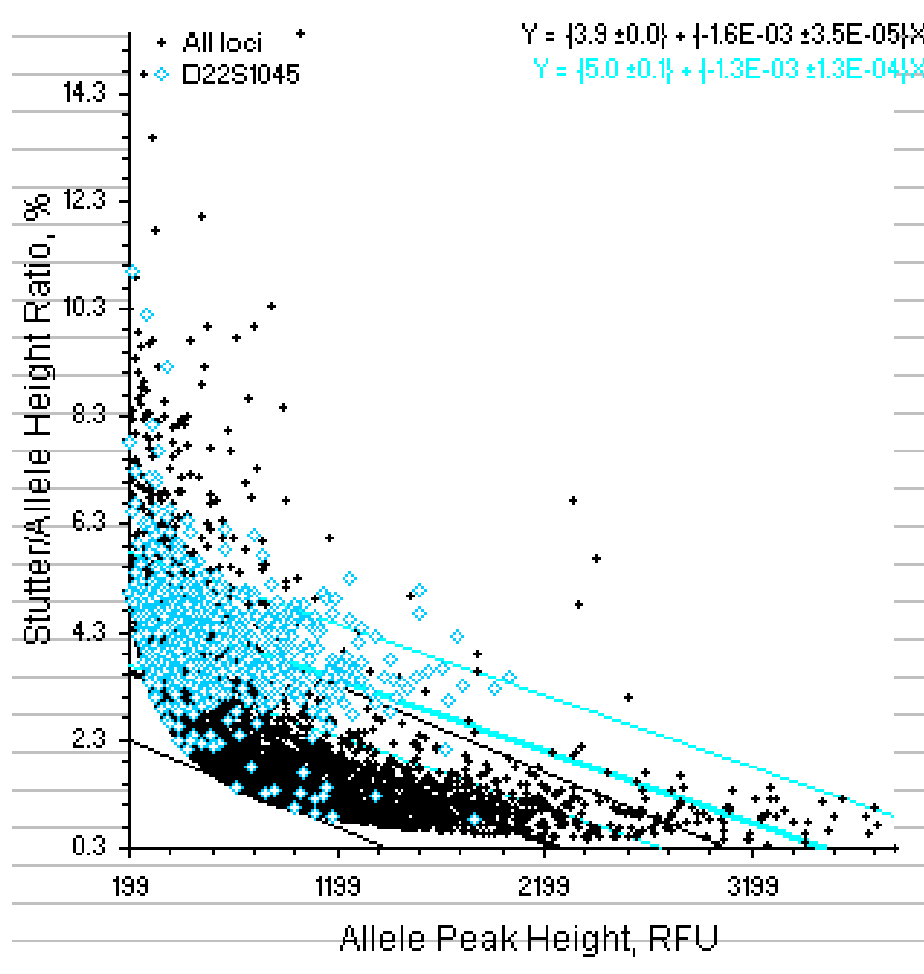
# Stutter Density Plot



**Median stutter = 6.57%**

**Median D22 stutter (in red) = 8.26%**

# Forward Stutter Observed (Big Picture View)



\*D22S1045  
stutter is in blue  
(trinucleotide locus)

# Locus-Specific Stutter Values

Created on: 23-May-12 17:00:59		Percentage Stutter																	
From Version: 29-Feb-12		Locus	#Ratio	Mean	SD	Median	MADe	Min	1%	2.5%	5%	10%	25%	75%	90%	95%	97.5%	99%	Max
Filter	Setting	FGA	92	1.7	1.0	1.3	0.8	0.4	0.4	0.5	0.5	0.6	1.0	2.1	3.1	3.5	4.1	5.4	6.0
Fetch	Stored profiles	D3S1358	316	1.9	1.2	1.5	0.7	0.4	0.5	0.6	0.7	0.8	1.1	2.2	3.4	4.5	5.4	6.0	7.0
Min PkHt	200	D19S433	26	1.9	1.5	1.5	0.6	0.3	0.4	0.4	0.6	0.9	1.1	2.0	3.2	3.7	5.5	7.2	8.3
Max PkHt	4000	D13S317	348	1.8	1.2	1.5	0.6	0.4	0.6	0.7	0.8	0.9	1.2	2.1	3.0	3.9	4.4	8.4	10.2
Max %Stutter	30	D2S441	417	1.9	1.2	1.6	0.8	0.4	0.6	0.6	0.7	0.8	1.1	2.2	3.2	4.0	4.7	5.5	9.9
Allow Forwards	No	D5S818	169	1.9	1.1	1.6	0.6	0.6	0.6	0.7	0.8	1.0	1.2	2.1	3.5	4.2	4.8	5.4	6.3
Min #Ratio	7	D12S391	133	1.9	1.2	1.6	0.7	0.3	0.5	0.6	0.7	0.8	1.2	2.1	3.2	4.5	4.9	6.2	7.3
BpWin	0.50	D1S1656	514	2.0	1.0	1.7	0.7	0.3	0.7	0.8	0.9	1.1	1.3	2.3	3.3	4.1	4.9	5.5	8.2
		TH01	56	2.1	1.2	1.8	0.8	0.3	0.4	0.7	0.9	1.0	1.4	2.5	3.5	4.1	5.2	6.2	6.8
		D16S539	329	2.4	1.8	1.8	1.0	0.5	0.7	0.7	0.8	1.0	1.3	3.0	4.5	6.2	8.0	8.3	11.7
		D8S1179	67	2.1	1.6	1.8	0.7	0.7	0.9	1.0	1.0	1.0	1.2	2.3	3.1	4.2	4.4	7.5	13.4
		D18S51	441	2.2	1.3	1.9	0.9	0.6	0.6	0.8	0.9	1.0	1.3	2.7	3.7	4.7	5.7	6.9	8.8
		D2S1338	176	2.4	1.5	2.0	1.1	0.5	0.5	0.7	0.8	1.0	1.3	2.9	4.0	5.6	6.9	7.7	8.5
		CSF1PO	422	2.6	1.5	2.3	1.1	0.6	0.7	0.8	1.0	1.2	1.5	3.1	4.5	5.2	7.1	7.9	11.1
		D21S11	289	2.6	1.4	2.4	1.2	0.8	0.8	0.9	1.0	1.2	1.6	3.4	4.7	5.2	6.1	6.6	9.8
		vWA	113	2.7	1.7	2.4	1.1	0.7	0.8	0.9	0.9	1.2	1.7	3.1	4.0	5.2	6.0	6.5	15.4
		D10S1248	159	2.7	1.6	2.4	1.4	0.5	0.5	0.7	0.8	1.0	1.6	3.5	4.9	5.5	6.1	7.8	10.9
		TPOX	35	2.8	1.6	2.4	1.3	0.6	0.6	0.6	0.7	1.0	1.7	3.5	5.2	5.6	6.1	6.5	6.8
		Penta_D	188	3.0	1.9	2.6	1.5	0.6	0.7	0.8	0.9	1.2	1.7	3.9	5.3	6.4	7.6	9.4	14.6
		Penta_E	177	3.6	2.4	2.8	1.8	0.5	0.7	1.0	1.1	1.3	1.9	4.6	7.3	8.4	9.2	10.0	12.0
		D7S820	124	3.2	1.6	3.2	1.7	0.6	0.8	0.9	1.0	1.2	1.9	4.2	5.4	5.9	6.5	7.3	7.8
		D22S1045	540	4.1	1.1	4.1	0.9	0.6	1.1	1.5	2.4	2.9	3.5	4.7	5.2	5.9	6.4	7.5	11.0

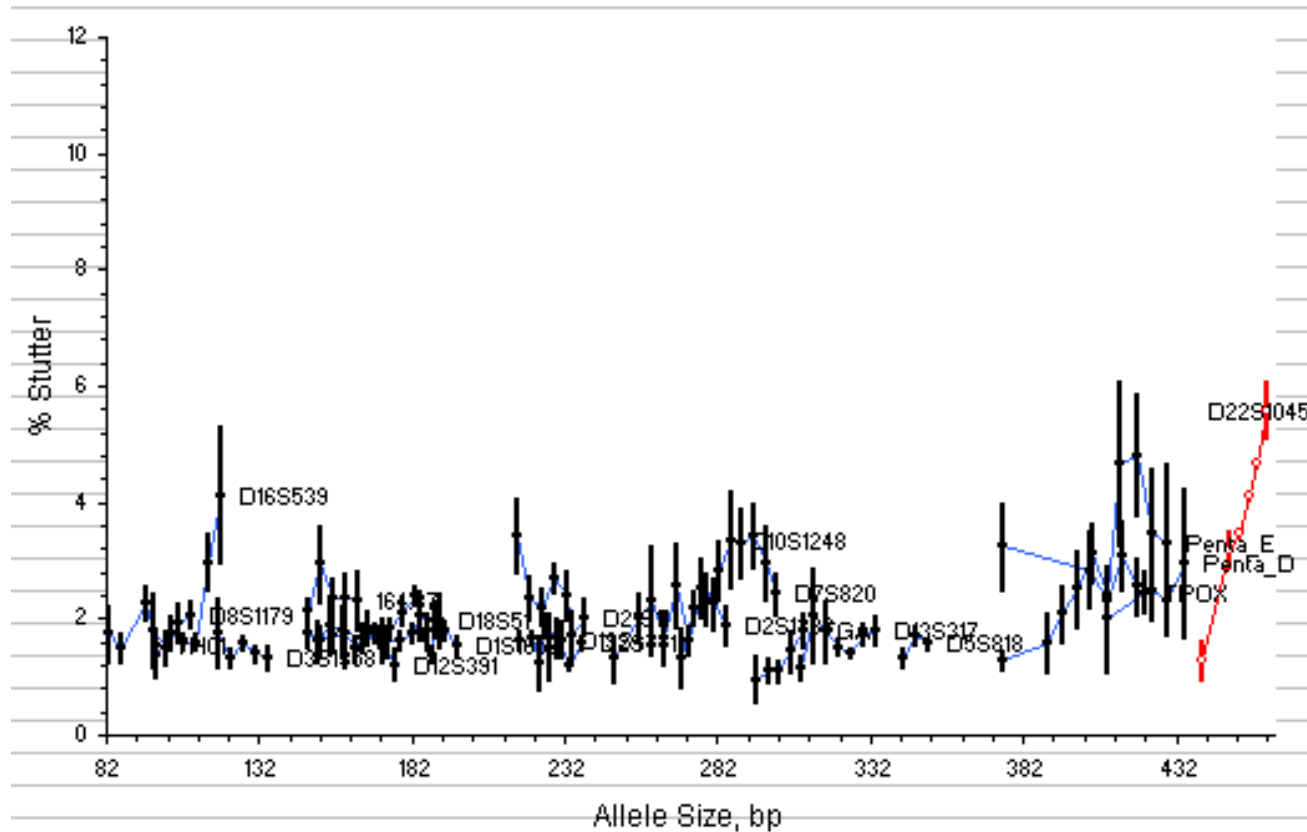
Filter	Setting
Fetch	Stored profiles
Min PkHt	200
Max PkHt	4000
Max %Stutter	30
Allow Forwards	No
Min #Ratio	7
BpWin	0.50



**Settings Used for Analysis**

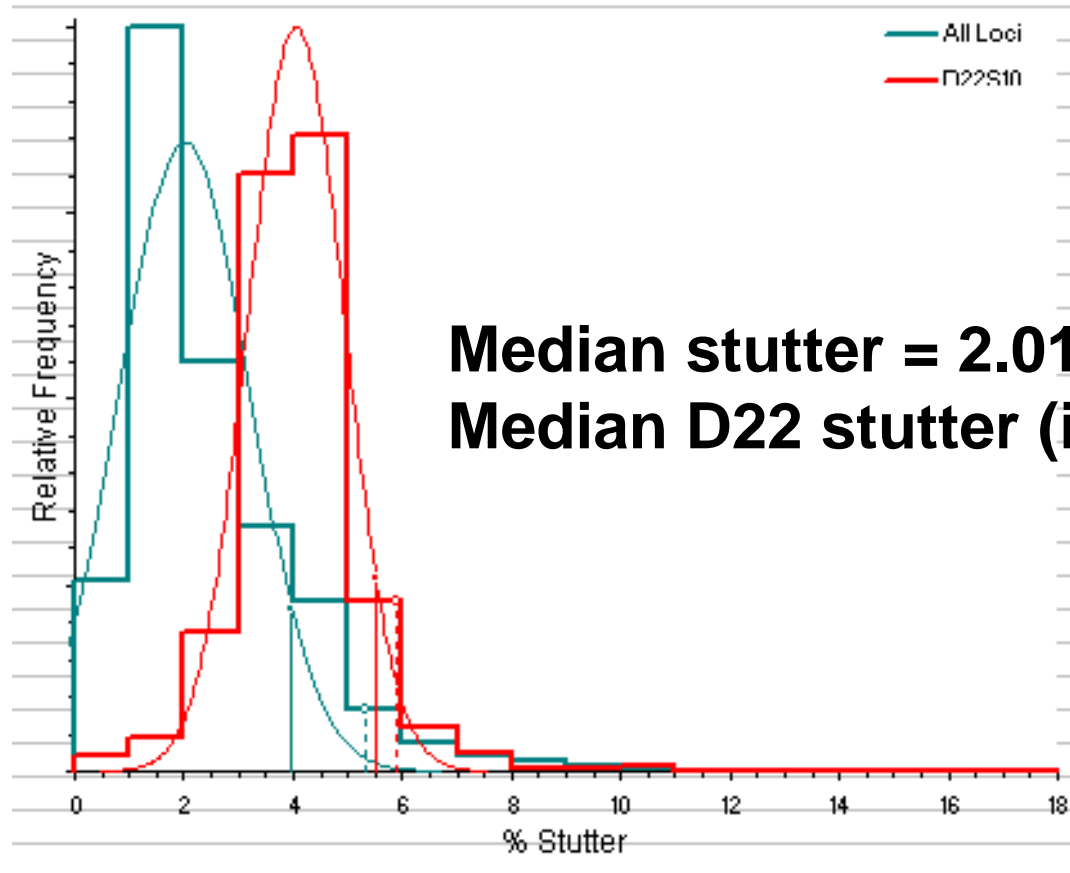
\*Verified Max stutter amounts

# Stutter Trends (Size Scale)



\*D22S1045 is in red

# Stutter Density Plot



# Summary

- Additional STR loci are important as DNA databases grow larger each year
- NIST has a set of 1036 U.S. Population Samples that have been used to fully characterize 29 autosomal STR loci
- Commercial companies are continuing to release larger STR multiplexes to meet the needs of the forensic community



# SAVE THE DATE

## Forensics@NIST

Three day symposium on cutting edge  
forensic science research at NIST

# 2012

**Date:** November 28-30<sup>th</sup>, 2012

**Time:** 9:00 am to 5:00 pm

**Location:** NIST (Gaithersburg, Maryland)

**For more information:**

[www.nist.gov/oles/forensics-2012.cfm](http://www.nist.gov/oles/forensics-2012.cfm)

**Note: registration is required (free)**



# Acknowledgments

**NIST Funding:** Interagency Agreement 2008-DN-R-121 between the **National Institute of Justice** and NIST Office of Law Enforcement Standards

**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 endorsement by the National Institute of Standards and Technology nor does 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 or policies of the US Department of Justice or the National Institute of Standards and Technology.

## **NIST Team for This Work**



**John Butler**



**Dave Duewer**



**Margaret Kline**



**Mike Coble**

**A special thanks to Applied Biosystems, Promega, and Qiagen for providing the kits used in this study**

**Contact Info:** [becky.hill@nist.gov](mailto:becky.hill@nist.gov), **301-975-4275**