ISHI Workshop on New Loci and Kits
October 10, 2013 (Atlanta, GA)
New Autosomal and Y-STR Loci and Kits: Making Data Driven Decisions

Introductory Remarks

John M. Butler

Special Assistant to the Director for Forensic Science

National Institute of Standards and Technology (NIST)

NIST FORENSIC SCIENCES

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Workshop Planned Schedule

1:00 – 1:20 pm Welcome and Introductory Remarks

1:20 – 2:00 pm NIST Studies: Kit Concordance and U.S. Population Data

2:00 – 2:30 pm Experience with PowerPlex Fusion

2:30 – 2:45 pm BREAK

2:45 – 3:15 pm Experience with GlobalFiler

3:15 – 3:40 pm NIST Studies with New Y-STR Loci & Kits

3:40 – 4:00 pm STRBase Resources and Additional Information

Additional U.S. Core CODIS Loci Are Coming…

D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6: e52-e54

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

What	Why	Who/How	When
Form a Working Group (WG) to discuss initial selection	Establishes target goals	CODIS Core Loci Working Group with FBI Chair and 5 members; Web meetings	May 2010 - present
Announce proposed additional CODIS core loci	Sets desired target goals and informs manufacturers	WG Chair; Publish proposed listing of CODIS core loci	April 2011 online (published Jan 2012)
Ongoing Progress Reports	Provides updates for DNA community	WG Chair; Present updates on status of CODIS Core Loci project at meetings	2010-2012
Implementation Considerations & Strategy	Identify issues for implementation and timeline	WG	June 2011 - present
Manufacturers develop prototype kits	Creates tools to meet target goals	Manufacturers; Provide status reports to WG for timeline	2011-2012
Test and validate prototype kits	Examines if target goals can be met	Validation Laboratories; Follow QAS compliant validation plan	Beginning in 2012
Review and evaluate data from validation	Evaluates if desired performance is obtained	NIST, SWGDAM and FBI; Provide feedback, if any, to Manufacturers	In conjunction with and at the conclusion of validation
Selection of new CODIS core loci	Allows protocols to be established	FBI; seek input from DNA community and stakeholders; Notify Congress	After evaluation of validation data and kit production factors
Implementation of new CODIS core loci at the National DNA Index System	Enables target goals to be met	All NDIS-participating labs	~ 24 months after selection of new CODIS core loci

http://www.fbi.gov/about-us/lab/biometric-analysis/codis/planned-process-and-timeline-for-implementation-of-additional-codis-core-loci

We will not discuss FBI Project data

- **This workshop will NOT discuss Consortium Validation Project data being used by the FBI CODIS Unit in the U.S. core loci expansion**

- We will discuss STR loci and what we know about the latest autosomal and Y-STR kits

Product Disclaimer

- **We will mention commercial STR kit names and information, but we are 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 the speakers** and do not necessarily represent the official position of the National Institute of Standards and Technology or the U.S. Department of Justice. **The NIST Applied Genetics Group receives or has received funding from the FBI Laboratory and the National Institute of Justice.**

Expanding the U.S. CODIS Core Loci

D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6(1): e52-e54
Addendum to expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. (2012) 6(5): e135

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Letter to the Editor

Expanding the CODIS core loci in the United States

CODIS Core Loci Working Group
Formed in May 2010 to make recommendations to FBI CODIS Unit

Douglas Hares (Chair) – FBI
John Butler – NIST
Cecelia Crouse – FL PBSO
Brad Jenkins – VA DFS
Ken Konzak – CA DOJ
Taylor Scott – IL SP

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.

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

D.R. Hares (2012) *Forensic Sci. Int. Genet.* 6(1):e52-e54

- **To reduce the likelihood of adventitious matches** as the number of profiles stored at NDIS continues to increase each year

- **To increase international compatibility** to assist law enforcement data sharing efforts

- **To increase discrimination power to aid missing persons cases**

International Comparability

Currently there are ***29 autosomal STR markers*** *present in commercial kits*

13 CODIS loci

U.S.	Europe
TPOX	
CSF1PO	
D5S818	
D7S820	
D13S317	
FGA	FGA
vWA	vWA
D3S1358	D3S1358
D8S1179	D8S1179
D18S51	D18S51
D21S11	D21S11
TH01	TH01
D16S539	D16S539
D2S1338	D2S1338
D19S433	D19S433
Penta D	
Penta E	

ESS = European Standard Set

7 ESS loci

+5 additional loci
In PowerPlex CS7
F13B
FES/FPS
F13A01
LPL
Penta C

D12S391
D1S1656
D2S441
D10S1248
D22S1045

3 miniSTR loci developed at NIST

5 loci adopted in 2009 to expand to 12 ESS loci

SE33 → Core locus for Germany

Locus used in China ← **D6S1043**

Amelogenin for Sex-Typing

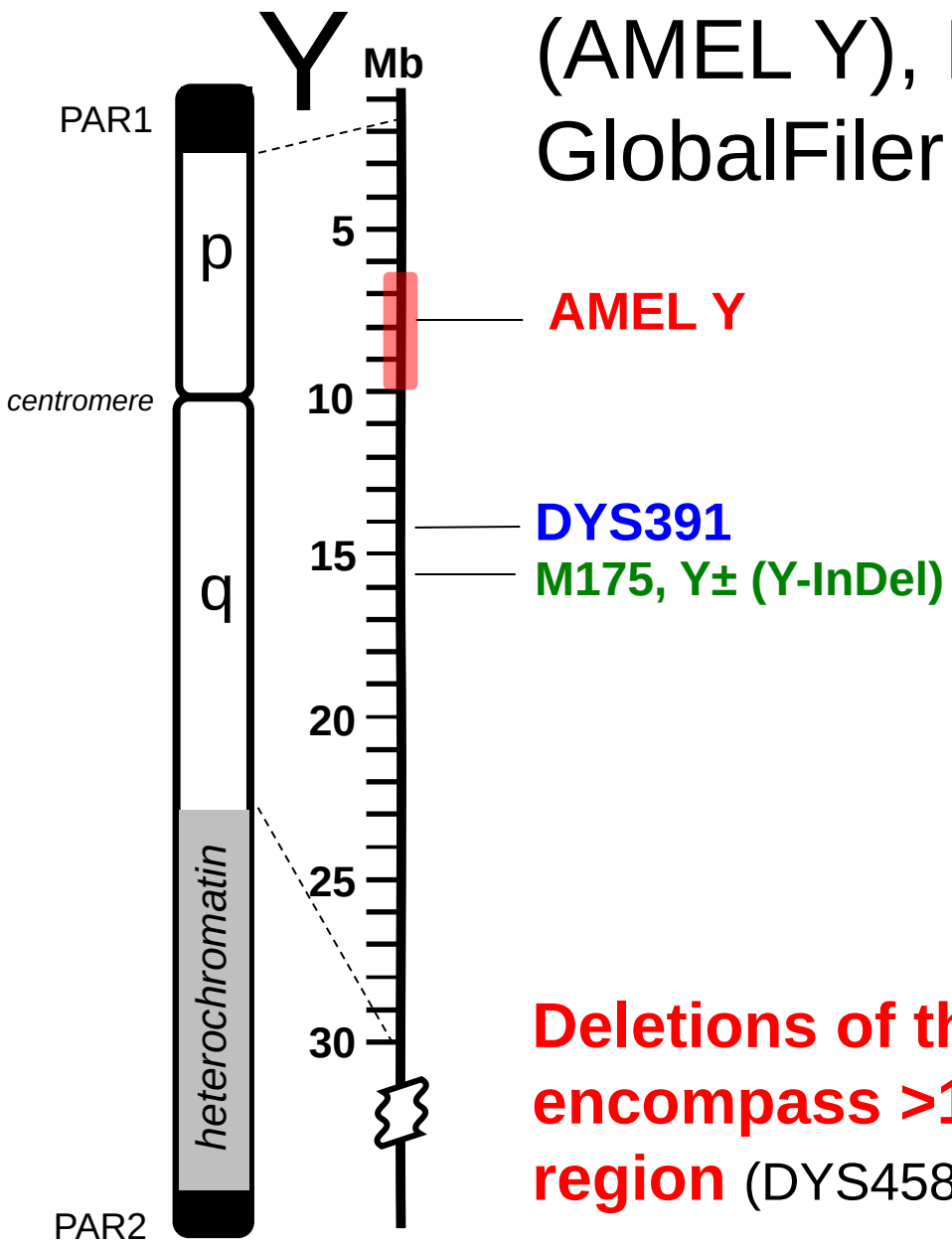
- Deletions and primer site polymorphisms can lead to incorrect sex-typing results
- Amelogenin is located at 6.74 Mb on ChrY (short arm) and 11.31 Mb on ChrX
- Using another marker on the Y-chromosome can help verify male DNA samples (e.g., DYS391)

Why Consider DYS391?

- **DYS391 is located on the long arm of the Y-chromosome over 7 Mb away from amelogenin**. Thus, it is likely to be detected in the event of an amelogenin Y deletion that could make a male sample falsely appear as a female (X,-).
- DYS391 is not very polymorphic. From a data set of 97,575 haplotypes available on the Y-Chromosome Haplotype Reference Database, over half of them possess allele 10. However, only two null alleles have been reported and 0.01% duplication events (11 total) have been seen in over 700 different population groups from around the world. Thus**, it is a stable locus with a relatively narrow allele range**.
- DYS391 has a mutation rate of 0.26%, which is comparable to most autosomal STRs commonly in use. There have been 38 mutations observed so far in the 14,621 meioses reported in the literature and compiled on YHRD.

DYS391 data as of October 2011

Relative positions of amelogenin (AMEL Y), DYS391, and Y-indel in GlobalFiler



AMEL Y deletion is most commonly seen in males of Indian subcontinent origin

Chang *et al.* (2007) *Forensic Sci. Int.* 166: 115-120

12/649 Malaysian males showed no AMEL Y

Cadenas *et al.* (2007) *Forensic Sci. Int.* 166: 155-163

5/77 Nepal males showed no AMEL Y

Deletions of the Y-chromosome can encompass >1 Mb around the AMEL Y region (DYS458 is often lost in these situations)

Novel Y-indel in GlobalFiler Kit

- Can be either “1” (deletion) or “2” (insertion)
- Small size (81 or 86 nt) enabling successful results with degraded DNA samples
- Likely an insertion/deletion (InDel) known as M175 (175th marker discovered by Peter Underhill from Stanford University using DHPLC)
  - Exhibits deletion of “TTCTC” with Y-SNP Haplogroup O individuals (East or SE Asians)
  - See van Oven et al. (2012) *J Human Genet* 57: 65-69
- Most samples will be “2” (the ancestral “insertion” form) unless they are Asian in origin

Reference List Compiled for Workshop

205 Articles and Websites

Autosomal STR Topics	#
European & US Core Loci	9
STR kits & new assays	31
NIST U.S. population data	6
On-line population databases	6
Population data on new STR loci	18
Information on STR loci	17
Concordance studies	12
Amelogenin & anomalies	28
D12S391 & vWA LD studies	5
	132

Y-STR Topics	#
Y-STR haplotype databases	8
Y-STR kits	3
PowerPlex Y23 population data	3
Rapidly mutating (RM) Y-STRs	2
Early Y-STR work at NIST	11
Impact of additional Y-STR loci	14
Y-STR mutations	26
Y-STR profile anomalies	6
	73

Autosomal STR Loci

Autosomal STR Kits

Chr	STR Locus	Repeat	Allele Range (Butler et al. 2012)	CODIS 13 (US 1997-present)	CODIS 20 (US future)	ESS 12 (EU 2009-present)
				required		
1q31	F13B	AAAT	6 to 11			
1q42	D1S1656	TAGA	10 to 19.3		■	■
2p25.3	**TPOX**	AATG	5 to 13	■		
2p14	D2S441	TCWA	8 to 17		■	■
2q35	D2S1338	TKCC	15 to 27		■	
3p21.31	**D3S1358**	TCTR	11 to 20	■	■	■
4q31.3	**FGA**	YTYY	16.2 to 43.2	■	■	■
5q23.2	**D5S818**	AGAT	7 to 15	■	■	
5q33.1	**CSF1PO**	AGAT	7 to 15	■	■	
6p24	F13A01	AAAG	3.2 to 17			
6q14	SE33	AAAG	6.3 to 36			
6q15	D6S1043	AGAY	8 to 26			
7q21.11	**D7S820**	GATA	6 to 14	■	■	
8p22	LPL	AAAT	7 to 15			
8q24.13	**D8S1179**	TCTR	8 to 18	■	■	■
9p13	Penta C	AAAAC	5 to 16			
10q26.3	D10S1248	GGAA	8 to 19		■	■
11p15.5	**TH01**	TCAT	5 to 11	■	■	■
12p13.31	**vWA**	TCTR	11 to 21	■	■	■
12p13.2	D12S391	AGAY	14 to 27		■	■
13q31.1	**D13S317**	TATC	8 to 15	■	■	
15q25	FESFPS	ATTT	5 to 14			
15q26.2	Penta E	AAAGA	5 to 25			
16q24.1	**D16S539**	GATA	5 to 15	■	■	
18q21.33	**D18S51**	AGAA	9 to 28	■	■	■
19q12	D19S433	WAGG	9 to 18.2		■	
21q21.1	**D21S11**	TCTR	24.2 to 39	■	■	■
21q22.3	Penta D	AAAGA	2.2 to 17			
22q12.3	D22S1045	ATT	8 to 19			■
Xp, Yp	Amelogenin	--	--		■	
Yq11.21	DYS391	TCTA	7 to 13		■	

Promega STR kits						
PowerPlex 16 (HS)	PowerPlex 18D	PowerPlex ESI/ESX 16	PowerPlex ESI/ESX 17	PowerPlex 21	PowerPlex CS7	**PowerPlex Fusion**
					■	
		■	■	■		■
■	■			■		■
		■	■			■
	■	■	■	■		■
■	■	■	■	■		■
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					■	
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■	■			■	■	
					■	
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					■	
■	■	■	■	■		■

Life Technologies (ABI) STR kits										
Profiler Plus	COfiler	SGM Plus	SEfiler Plus	SinoFiler	MiniFiler	**Identifiler (Plus)**	VeriFiler	NGM	NGM SElect	**GlobalFiler**
							■	■	■	■
	■					■				■
							■	■	■	■
		■	■	■	■	■	■	■	■	■
■		■	■	■		■		■	■	■
■		■	■	■	■	■		■	■	■
	■			■	■	■				■
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■	■			■	■	■				■
■		■	■	■		■		■	■	■
							■	■	■	■
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■		■	■	■		■		■	■	■
				■			■	■	■	■
					■	■				■
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■		■	■	■	■	■		■	■	■
		■	■	■	■	■	■	■	■	■
							■	■	■	■
										■

Qiagen STR kits					
ESSplex	ESSplex SE	Hexaplex ESS	Nonaplex ESS	Decaplex SE	IDplex
■	■	■	■		
					■
■	■	■	■		
■	■			■	■
■	■		■	■	■
■	■		■	■	■
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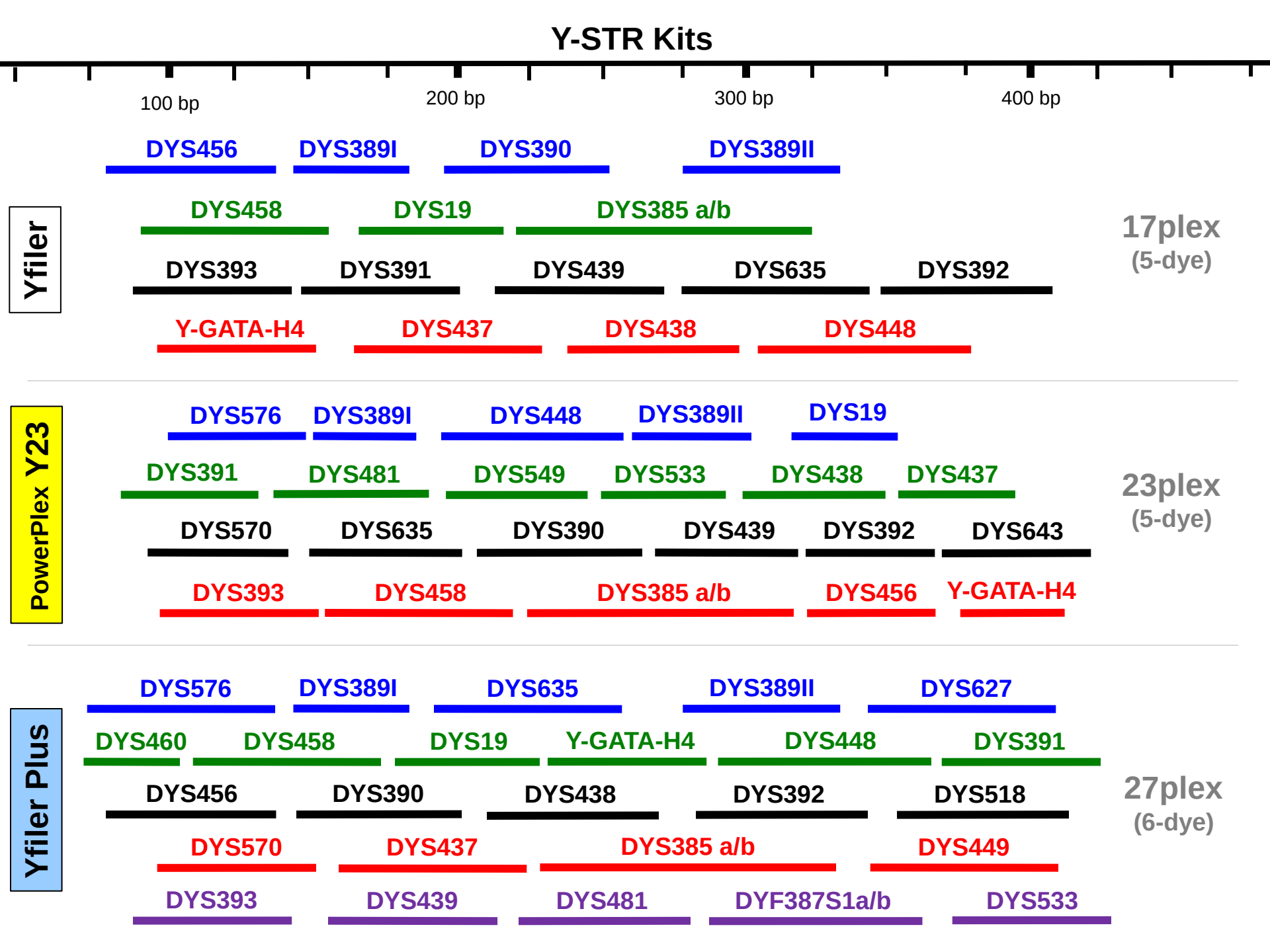
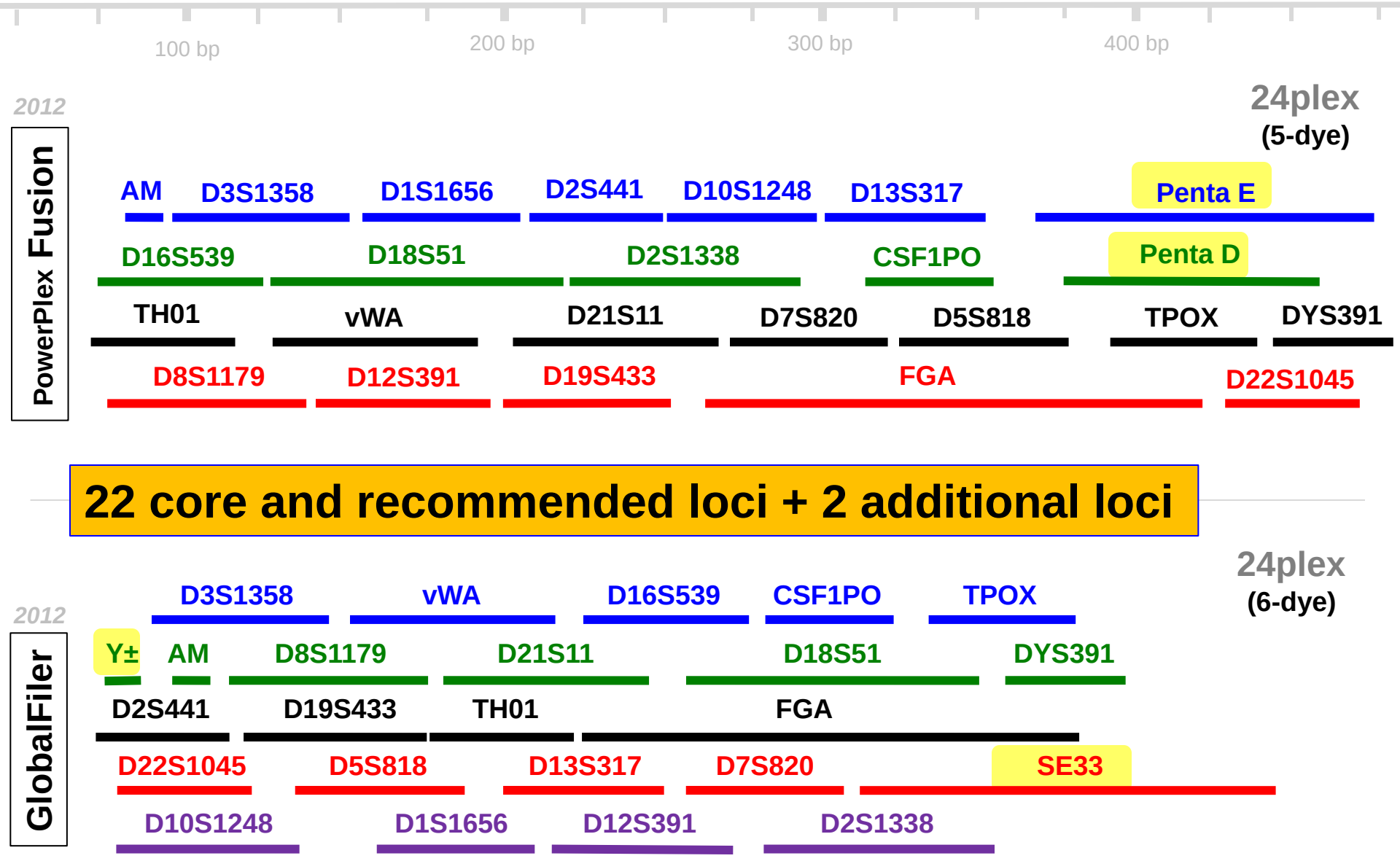
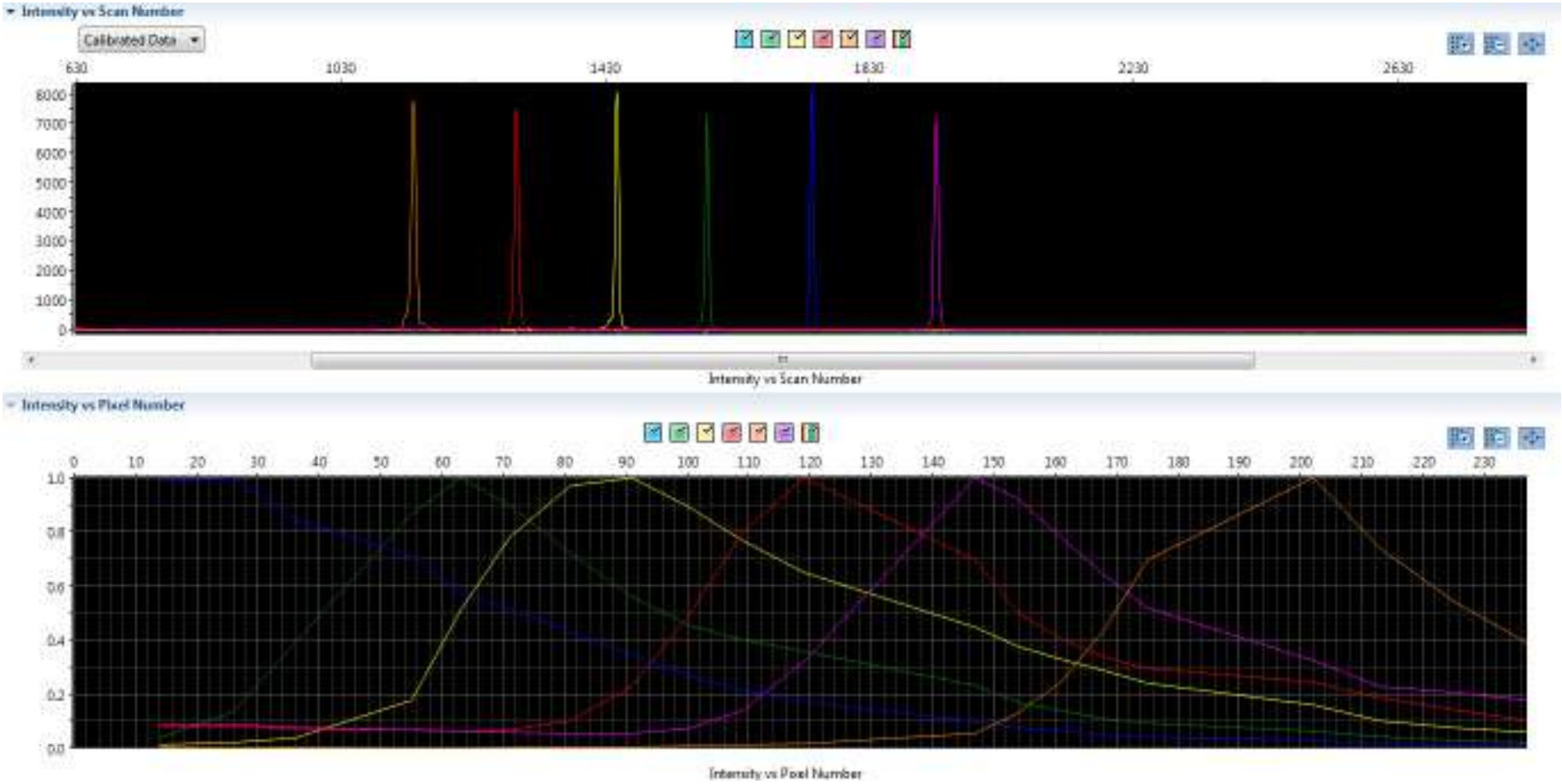
					■


Y-STR Kits
100 bp
200 bp
300 bp
400 bp
Yfiler
DYS456
DYS389I
DYS390
DYS389II
DYS458
DYS19
DYS385 a/b
DYS393
DYS391
DYS439
DYS635
DYS392
Y-GATA-H4
DYS437
DYS438
DYS448
17plex
(5-dye)
PowerPlex Y23
DYS576
DYS389I
DYS448
DYS389II
DYS19
DYS391
DYS481
DYS549
DYS533
DYS438
DYS437
DYS570
DYS635
DYS390
DYS439
DYS392
DYS643
DYS393
DYS458
DYS385 a/b
DYS456
Y-GATA-H4
23plex
(5-dye)
Yfiler Plus
DYS576
DYS389I
DYS635
DYS389II
DYS627
DYS460
DYS458
DYS19
Y-GATA-H4
DYS448
DYS391
DYS456
DYS390
DYS438
DYS392
DYS518
DYS570
DYS437
DYS385 a/b
DYS449
DYS393
DYS439
DYS481
DYF387S1a/b
DYS533
27plex
(6-dye)

STR Marker Layouts for New U.S. Kits

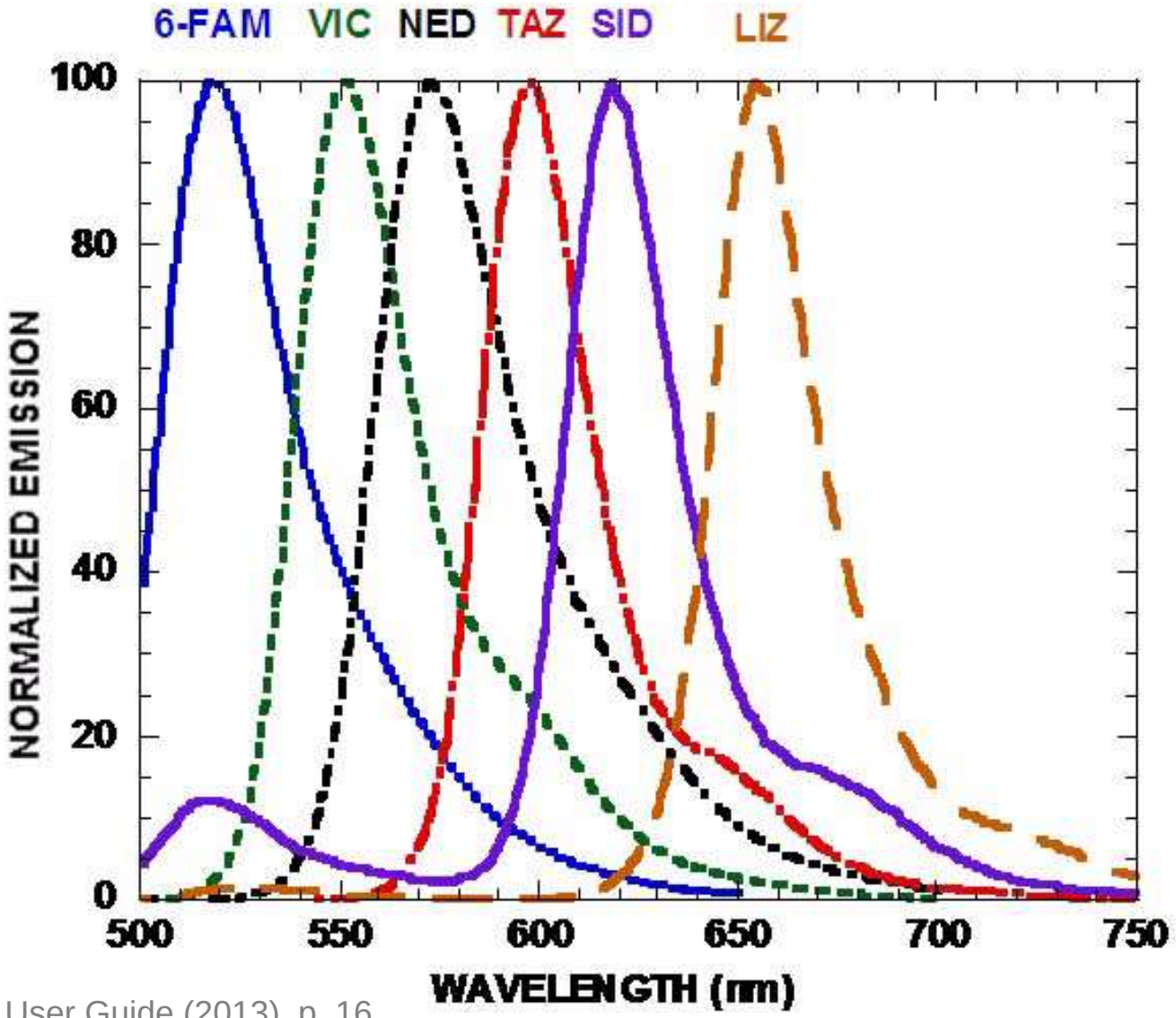


New Life Technologies STR Kits Require 6-Dye Detection



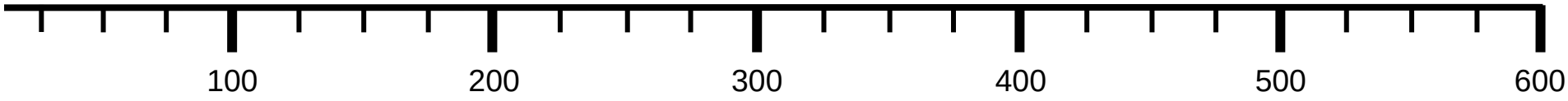
6-dye spectral (from GlobalFiler manual) on ABI 3500

Fluorescence Emission Spectra of 6 Dyes Present in the GlobalFiler STR Kit



From GlobalFiler User Guide (2013), p. 16

Different Internal Size Standards



Local Southern sizing requires two size standard peaks on either size of measured allele
(a hole in the sizing ability exists in this range)

Life Technologies/ABI

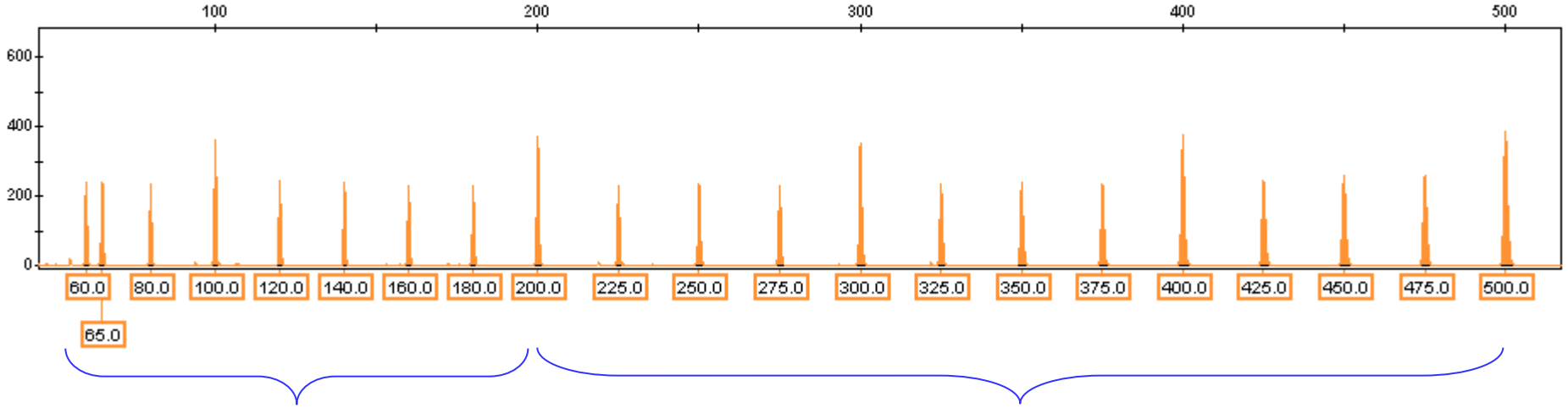
GS500-ROX and GS500-LIZ

GS600-LIZ

Promega

ILS-500

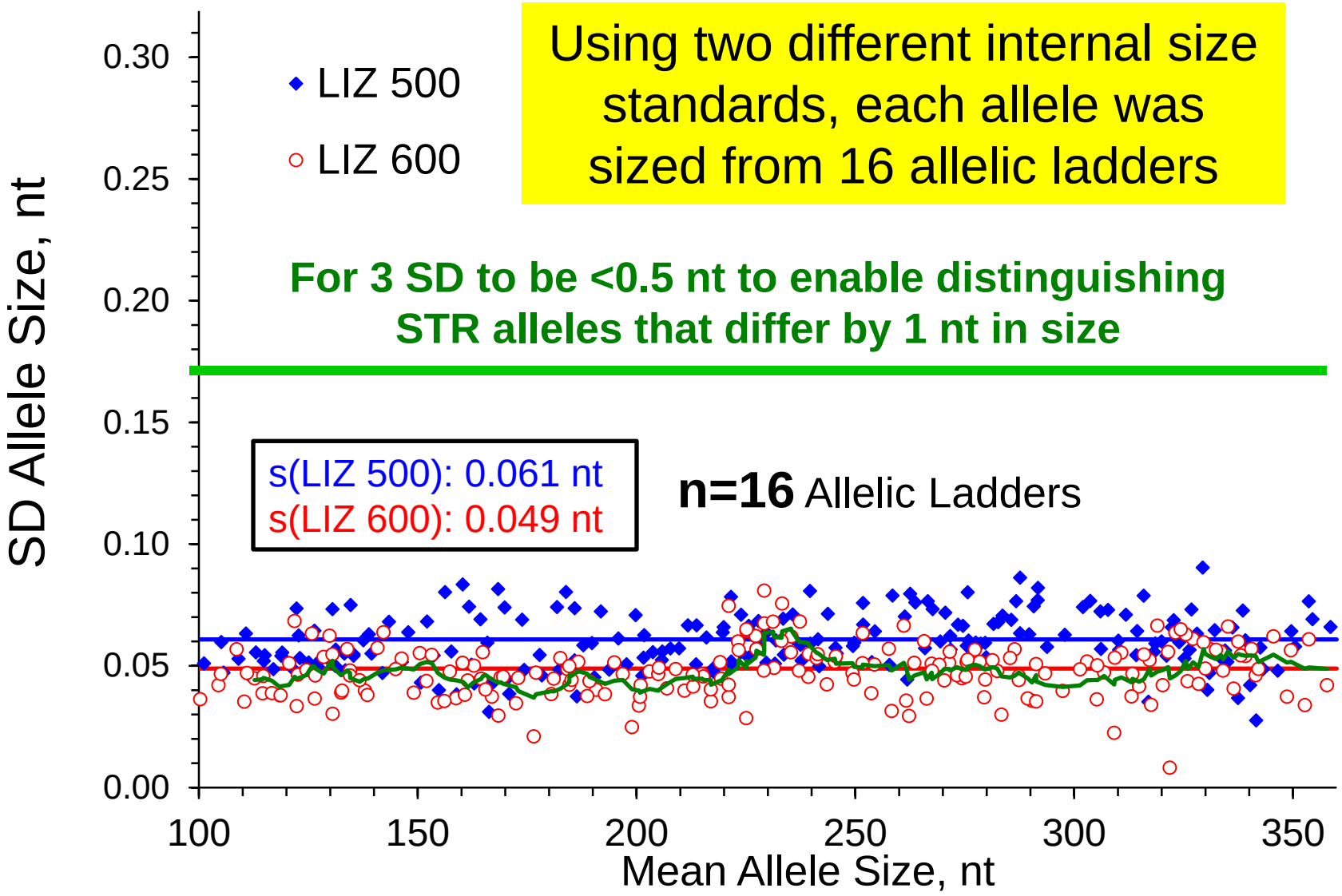
New Promega Size Standard



Labeled with 5-dye (orange)
Contains 21 fragments with even spacing
Low end: 60 & 65 bp
High end: 475 & 500 bp

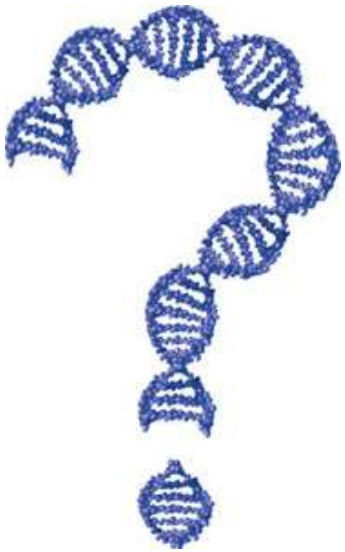
Local Southern sizing possible from 66 bp to 474 bp

Precision Data from ABI 3500



Validation data from Erica Butts (NIST)

Questions for Workshop Participants



- **STR kit(s) in your lab?**
  - Currently in use: Identifiler, PP16, Pro/CO
  - Considering: Fusion, GlobalFiler, other
- **Y-STR kit(s):** PPY, PPY23, Yfiler, Yfiler Plus
- **CE instrument(s)?**
  - Currently: ABI 310, ABI 3130xl, ABI 3500
  - Considering: 3500, 3130xl (6-dye conversion)
- **Analysis software?**
  - GeneMapperID, GMID-X, GeneMarkerHID, OSIRIS

NIST SRM 2391c

Component D is a mixture

- **Contains certified values** for **29 autosomal STR loci** and **17 Y-STR loci** available in commercial kits (plus some additional reference values for miniSTRs)
- In 2013, **we plan to add certified values for the six additional Y-STR loci in PowerPlex Y23** and new loci included in Life Technologies' Yfiler Plus kit

Full STR allele sequence coverage is planned to aid future next-generation sequencing efforts

Acknowledgments

$ National Institute of Justice and NIST OLES

Becky Hill and Mike Coble (NIST Applied Genetics Group)

Contact info:

john.butler@nist.gov

+1-301-975-4049

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