National Institute of Standards and Technology
U.S. Department of Commerce

NIST FORENSIC SCIENCES

NIST SRM 2391d: PCR-Based DNA Profiling Standard

Where Are We Now?

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National Institute of Standards and Technology

Potomac Regional Symposium on Forensic DNA Analysis

Richmond, VA

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Topics for Discussion

- Why are we working on a new SRM if we are already selling one?
- Brief historical perspective of NIST SRM production
- What all is involved with developing an SRM?
- Value assignment for SRM 2391d
- What markers, kits, and instruments will be included?
- SRM 2391d data and interesting examples
- How do I make my own materials traceable to the SRM?
- When will SRM 2391d be available to purchase?

Development of the Next PCR-Based DNA Profiling Standard

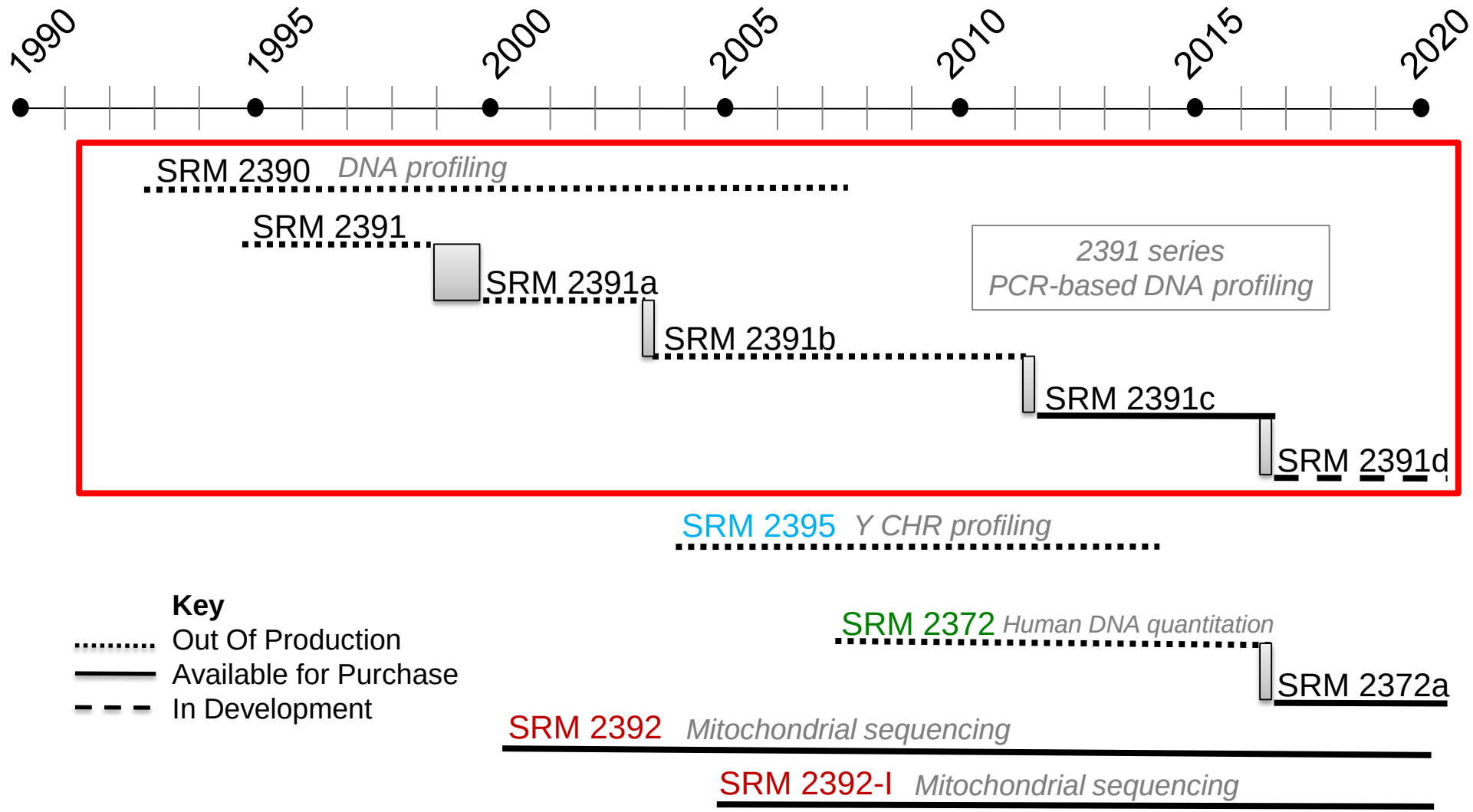
- As a successor to SRM 2391c
  - Inventory may be depleted by fall 2019
  - Develop SRM 2391d now to ensure availability when needed

Note: SRM 2391c can still be used until the expiration date (February 3, 2020) if stored properly.

- **Next Generation Sequencing** is used for certification in addition to **Capillary Electrophoresis** testing
  - Length- and sequence-based genotypes are provided
  - Information values are included for all commercially available forensic markers **beyond STR markers**

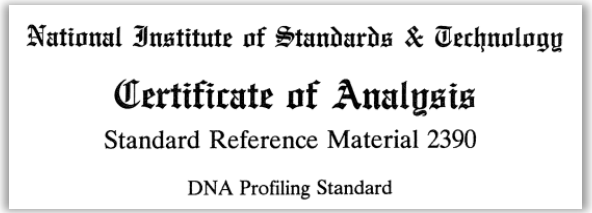
Goal: SRM 2391d will be the most comprehensive NIST forensic SRM to date

NIST Forensic SRM Timeline

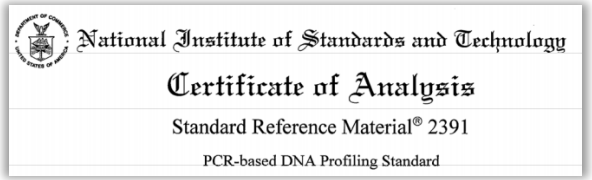


NIST Forensic DNA SRMs
Historical Perspective: Past, Present, Future

Past


National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material 2390
DNA Profiling Standard

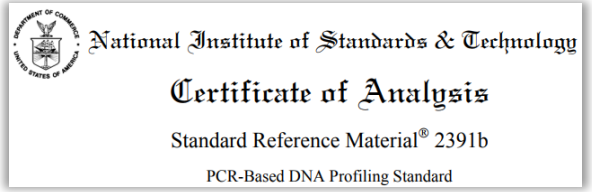
RFLP Testing & DNA Probes (1990)


National Institute of Standards and Technology
Certificate of Analysis
Standard Reference Material® 2391
PCR-based DNA Profiling Standard

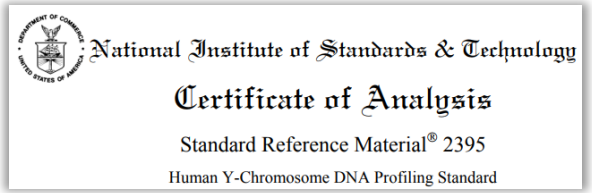
PCR-Based Testing (1995)
- VNTR, Dot Blot
- STR typing (updated 1998)


National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2391a
PCR-based DNA Profiling Standard

PCR-Based Testing (2000)
- Focus on STR typing
- VNTR, Dot Blot

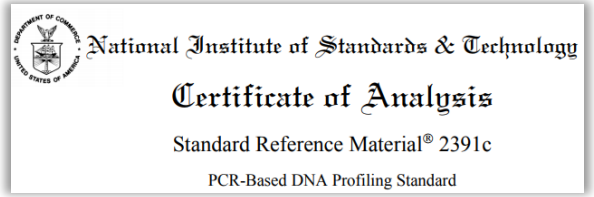

National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2391b
PCR-Based DNA Profiling Standard

PCR-Based Testing (2003)
- Autosomal STR loci
- More STR loci added (updated 2008)


National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2395
Human Y-Chromosome DNA Profiling Standard

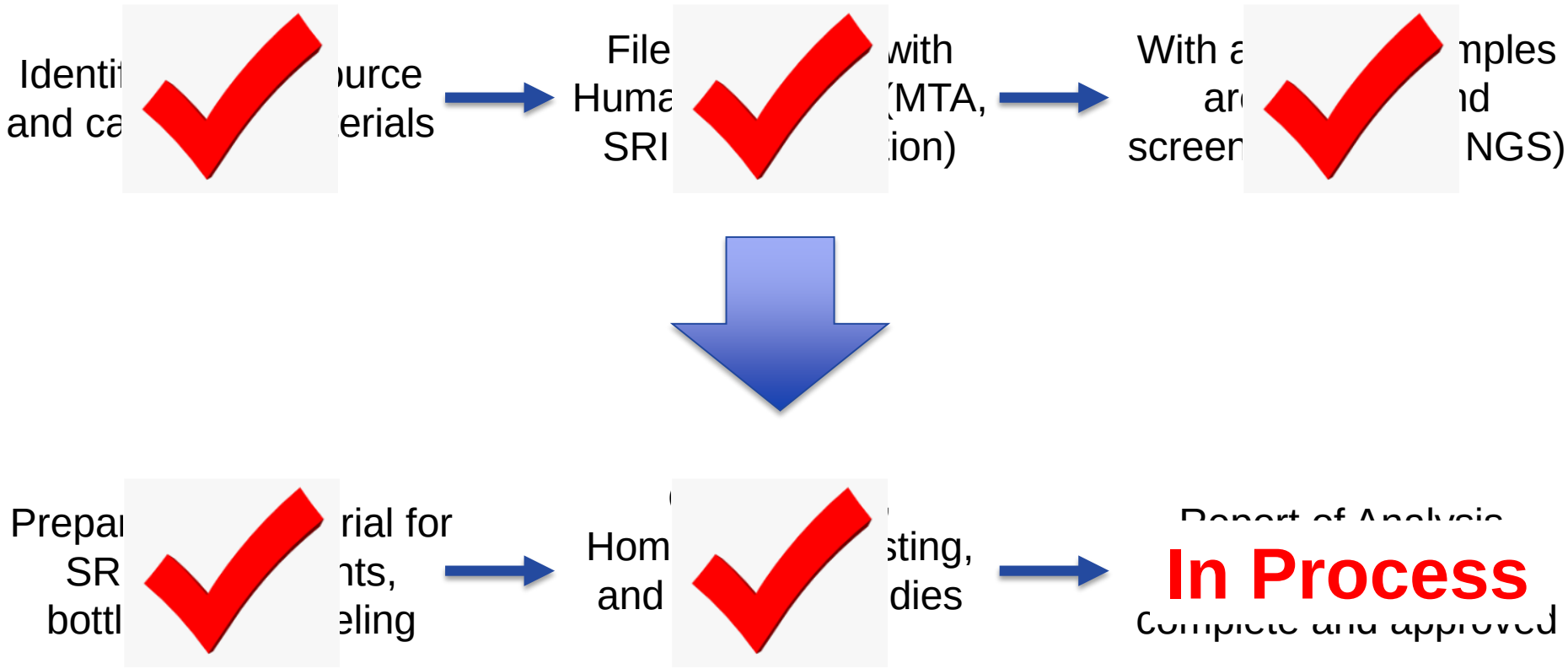
PCR-Based Y-STR Testing (2003)
- Y-STR loci
- More Y-STR loci added (updated 2008)

Present


National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2391c
PCR-Based DNA Profiling Standard

PCR-Based STR Testing (2011)
- Autosomal and Y-STR loci
- More autosomal and Y-STR loci, X-STR loci, and Indels added (updated 2015)
- Identity and Ancestry SNPs, and Y-Indel added (updated 2017)

Steps to Develop SRM 2391d



Goal: SRM 2391d available for purchase by Summer 2019

Challenges Encountered (so far…)

- Keeping up with new markers
  - CODIS 13 → now the CODIS 20
  - New SNP markers for ancestry and eye/hair color predictions
- Keeping up with new technologies
  - Next generation sequencing (full sequence strings)
  - New CE instruments and STR kits

Challenges Encountered (so far…)

- Procuring samples

  - Commercial sources are requiring fees for secondary distribution
  - and/or forbidding secondary distribution altogether
  - *Biorepositories have general concerns about cell line profiles being searched in DNA databases*
  - *Blood banks with proper consent are the way moving forward…*
    - *Recently received NIST HSPO approval*

Before SRM 2391d Development…

There was Human Subject Protections paperwork

Excluded Human Data/Specimens Form

Complete this form when your research study fits into one of the categories below **and** this is the primary use of the specimens and/or data. The form should be routed through your OU for approval and submitted to the HSPO for acknowledgement and tracking before beginning the work on this study.

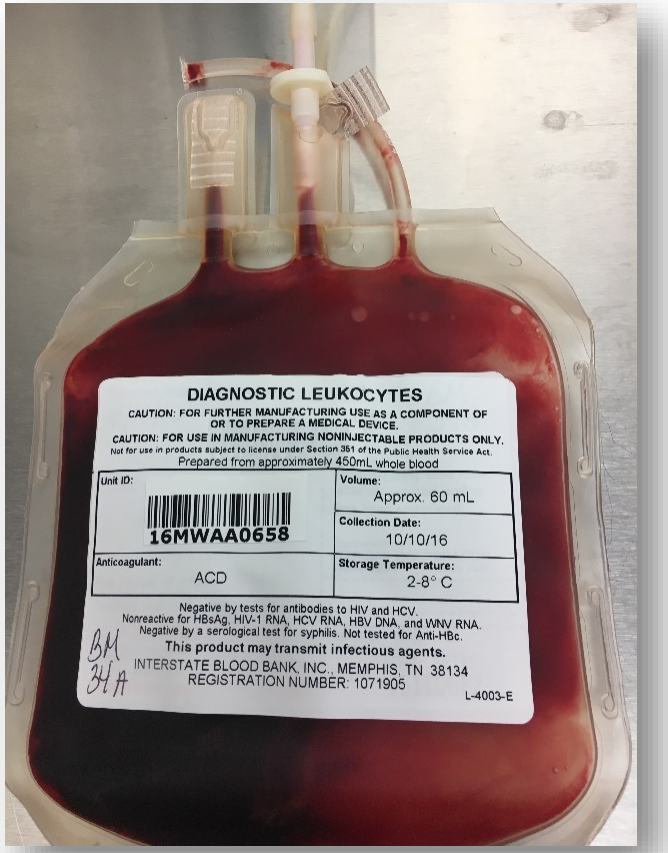
MML-16-0045-EXCL

And approval

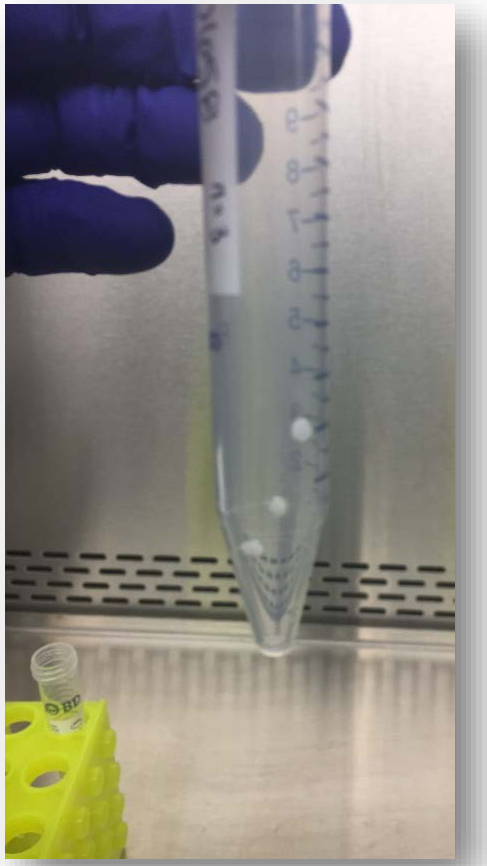
The HSPO has received your proposed project using only excluded specimens and/or data that meet the criteria for *not human subjects research* as defined in Department of Commerce Regulations, 15 CFR 27, also known as the Common Rule (45 CFR 46, Subpart A), for the Protection of Human Subjects. As indicated in your documentation, these specimens and/or data are from 1) deceased individual(s), 2) established cell lines, 3) human embryonic stem cells from the NIH hESC registry, and/or 4) derivatives of material originally obtained from humans and do not contain information identifying the subjects providing the specimens associated with the data. This determination is valid only for this project. You are responsible for conducting this project as outlined in the above documents. This project may proceed with no further requirement for review by the HSPO, but may require other agreements (MOU, MTA, DUA etc.), grant, contract, RACO (IAA) and/or OU approvals before your project may begin. In the event that there is a change to the above-described project that may affect this determination status, send a description of the change to the HSPO. The HSPO will re-evaluate the project, if necessary.

SRM 2391d:
Production, Bottling, and Labeling

The Beginning Stages

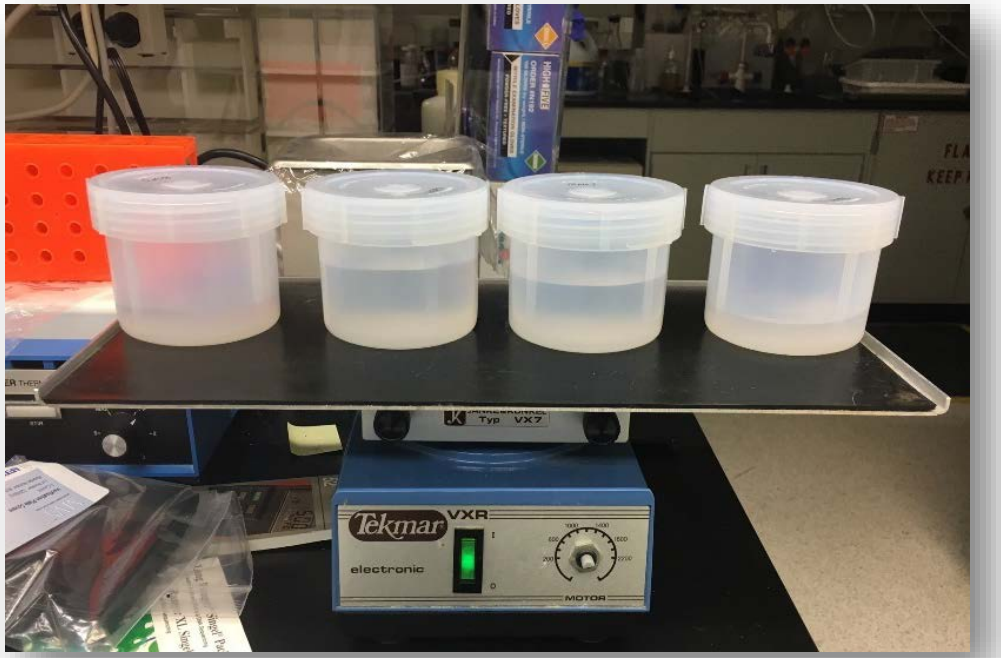


15 buffy coat samples were purchased from Interstate Blood Bank

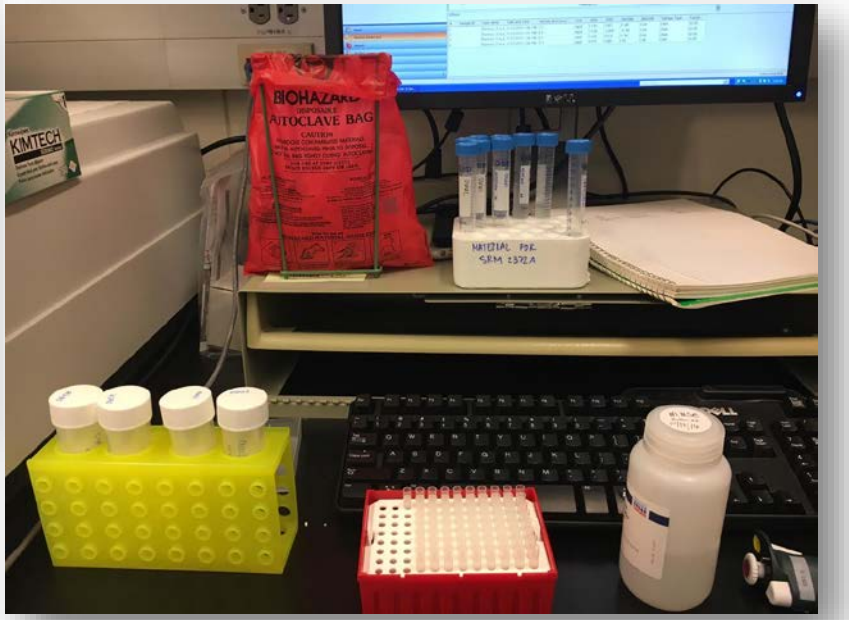


DNA was extracted using a manual method for high quality and high yield

The Beginning Stages



DNA was allowed to solubilize and reconstitute in Teflon pots in TE^{-4}



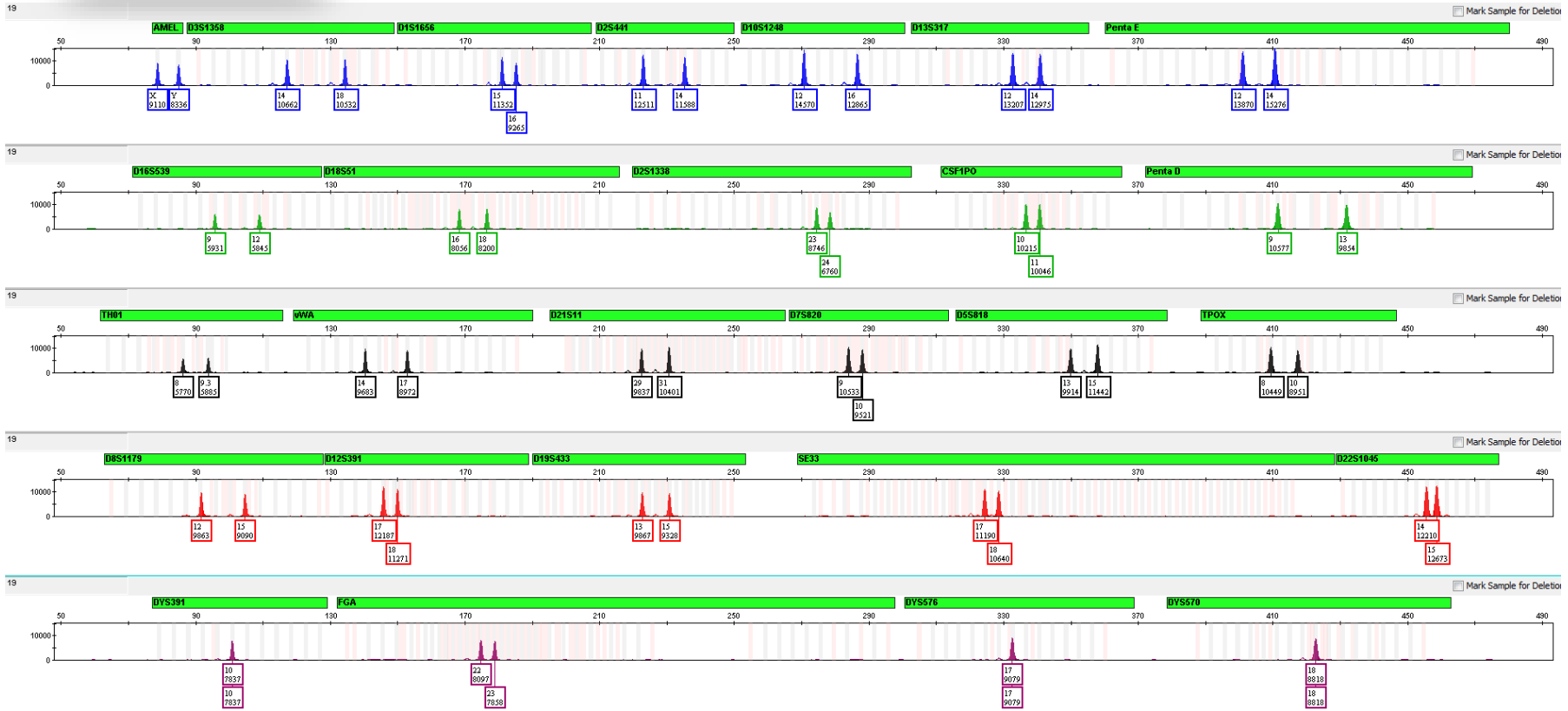
DNA was initially quality checked for purity and concentration on a NanoDrop

Preliminary Screening – CE & STR kits

Promega

ThermoFisher SCIENTIFIC

QIAGEN



Screening 15 human blood samples as potential candidate materials for SRM 2391d

Final Sample Selection and Certification

Final Set of Components Identified

Homogeneity

Value Assignment: Certified, Reference, and Information Values

Stability

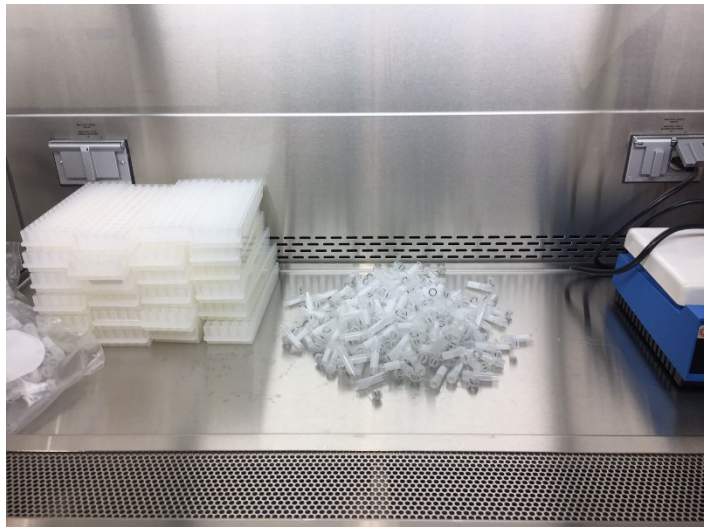
Bottling and Labeling Process

Teflon tubes had to be individually cleaned and dried before production

Teflon tubes were used for Components A-D (extracted DNA) so we had to clean ~10,000 tubes

Sarstedt tubes were used for Component E and came sterile (2,500 tubes)

Bottling and Labeling Process



Individual tubes placed into racks prior to filling

Band-Aids are applied to prevent blisters for those screwing caps onto each tube

Bottling and Labeling Process

Ready for bottling

Labelers check the caps, add a SRM label, and verify volume of product

*We have moved the labeling process to the lab

Completed Units for Testing

Homogeneity, stability and certification

~2200 units of each component

SRM 2391d:
Value Assignment

How are SRM 2391d values assigned?

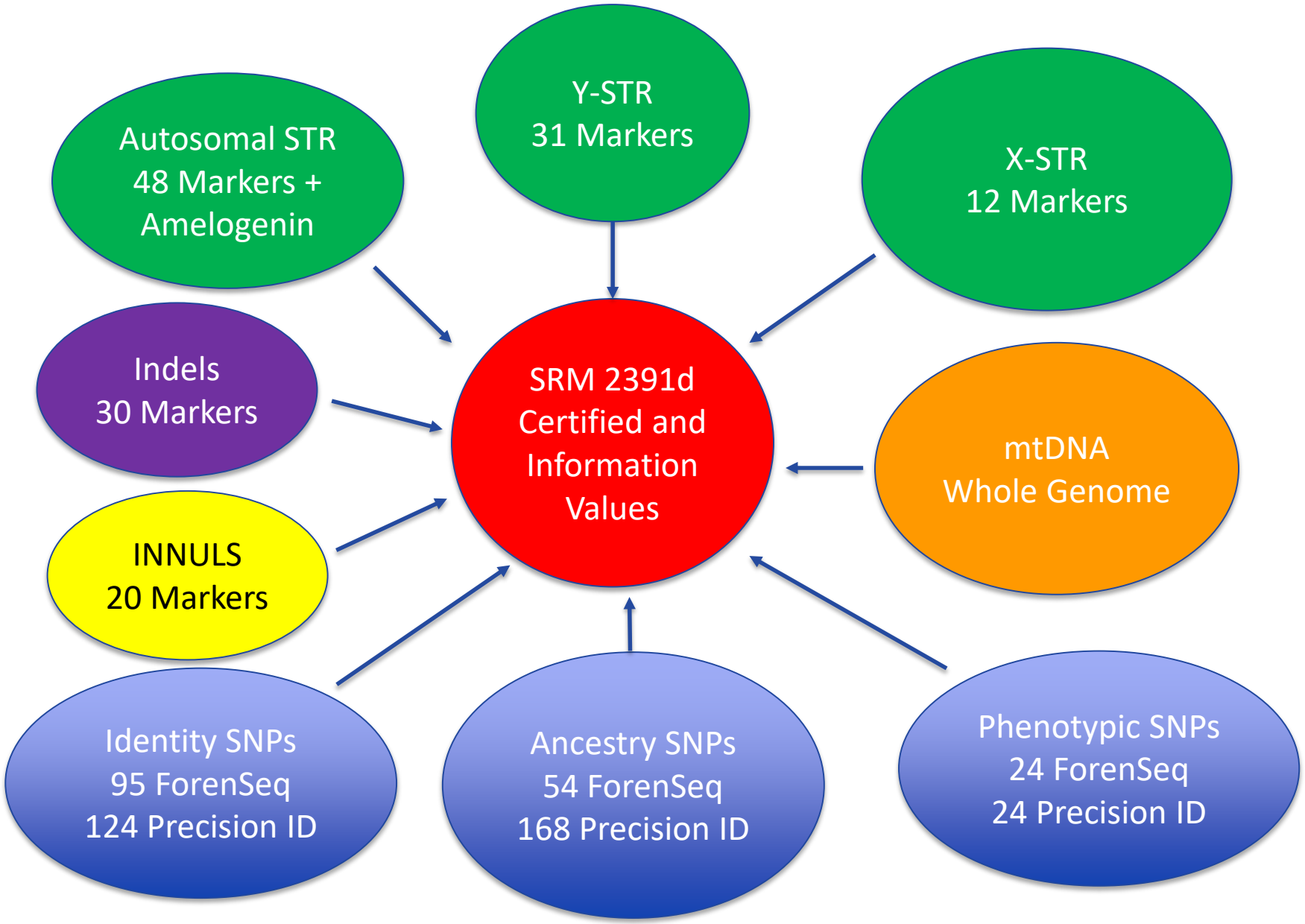
- **Certified Values** are assigned when there is a high coverage sequence string available for a marker

***Highest confidence*; all sources of uncertainty and bias examined**

- **Information Values** are assigned when only one primer set is used from CE testing and there is no sequence string to confirm

***For informational purposes*; no guarantees for uncertainty**

Markers included in the Certificate of Analysis



Which Autosomal STR Markers have Certified Values?

Autosomal STR Marker List	MiniFiler	Identifiler	Identifiler Plus	Identifiler Direct	NGM	NGM SElect	NGM Detect	Verifiler Plus	Verifiler Express	GlobalFiler	GlobalFiler Express	PP S5	PP CS7	PP 16	PP 16 HS	PP 18D	PP 21	PP ESX 17	PP ESX 17 Fast	PP ESI 17 Pro	PP ESI 17 Fast	PP Fusion	PP Fusion 6C	PP VersaPlex 27PY	ESSplex SE Plus	HDplex	24plex GO!	24plex QS	ForenSeq	Precision ID GF	PowerSeq 46GY	CODIS 20	European Standard Set	Certified Value	Information Value
D1S1656					■	■	■	■	■	■	■						■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	X	
D1S1677																		■	■	■	■	■	■	■			■	■	■	■	■			X	
D2S1338	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■		X	
D2S441					■	■	■	■	■	■	■							■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	X	
D2S1360																									■	■									X
D2S1776																													■					X	
D22S1045					■	■	■	■	■	■	■							■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	X	
CSF1PO	■	■	■	■				■	■	■	■			■	■	■	■						■	■			■	■						X	
F13A01													■																							X
F13B													■																							X
FESFPS													■																							X
FGA	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	X	
LPL													■																							X
Penta C													■																							X
Penta D								■	■				■	■	■	■	■						■	■					■	■	■			X	
Penta E								■	■				■	■	■	■	■						■	■					■	■	■			X	
SE33							■	■		■	■							■	■	■	■		■		■	■	■	■			■			X	
TH01		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	X	
TPOX		■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	X	
vWA		■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	X	

35 Certified Autosomal STR Markers
13 Information Autosomal STR Markers

Which Y-STR Markers have Certified Values?

Y-STR Markers
Thermo Fisher CE STR kits
Promega CE STR kits
Qiagen Investigator CE STR kits
Verogen NGS kit
Thermo Fisher NGS kits
Promega NGS kits

28 Certified Y-STR Markers
3 Information Y-STR Markers

Y-STR Marker List	GlobalFiler	GlobalFiler Express	Yfiler	Yfiler Plus	PP Fusion	PP Fusion 6C	PP VersaPlex 27PY	PowerPlex Y23	24plex GO!	24plex QS	ForenSeq	Precision ID GF	PowerSeq 46GY	Certified Value	Information Value
DYS19														X	
DYS385a/b														X	
DYS389I/II														X	
DYS390														X	
DYS391														X	
DYS392														X	
DYS393														X	
DYS437														X	
DYS438														X	
DYS439														X	
DYS448														X	
DYS449															X
DYS456														X	
DYS458														X	
DYS460														X	
DYS461														X	
DYS481														X	
DYS505														X	
DYS518															X
DYS522														X	
DYS533														X	
DYS549														X	
DYS570														X	
DYS576														X	
DYS612														X	
DYS627															X
DYS635														X	
DYS643														X	
Y-GATA-H4														X	
DYS387S1														X	

Which X-STR Markers have Certified Values?

X-STR Markers
Qiagen Investigator CE STR kit
Verogen NGS kit

7 Certified X-STR Markers
5 Information X-STR Markers

X-STR Marker List	Argus X-12	ForenSeq	Certified Value	Information Value
DXS7132			X	
DXS7423			X	
DXS8378			X	
DXS10074			X	
DXS10079				X
DXS10101				X
DXS10103			X	
DXS10134				X
DXS10135			X	
DXS10146				X
DXS10148				X
HPRTB			X	

Insertion/Deletion (Indel) Markers Tested

Insertion/Deletion (Indel) Markers

Qiagen Investigator CE kit

30 Information Indel Markers

Indel Marker List	Qiagen Investigator DIPplex CE Kit	Certified Value	Information Value
D6			X
D39			X
D40			X
D45			X
D48			X
D56			X
D58			X
D64			X
D67			X
D70			X
D77			X
D81			X
D83			X
D84			X
D88			X
D92			X
D93			X
D97			X
D99			X
D101			X
D111			X
D114			X
D118			X
D122			X
D124			X
D125			X
D128			X
D131			X
D133			X
D136			X

Insertion/Null (INNUL) Markers Tested

Insertion/Null Markers
InnoGenomics kit

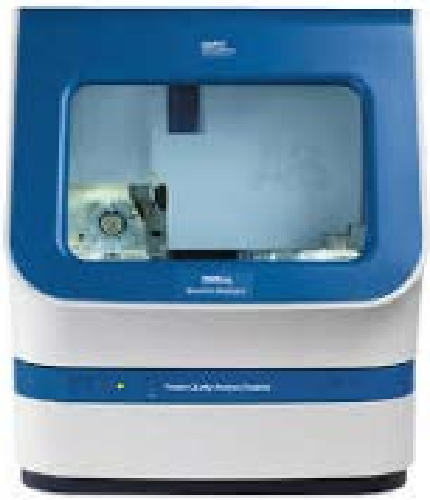
20 Information INNUL Markers

INNUL Marker List	InnoGenomics InnoTyper 21 CE Kit	Certified Value	Information Value
AC1141			X
AC2265			X
AC2305			X
AC4027			X
ACA1766			X
ALU79712			X
HS4.69			X
MLS09			X
MLS26			X
NBC10			X
NBC102			X
NBC106			X
NBC120			X
NBC13			X
NBC148			X
NBC216			X
NBC51			X
RG148			X
SB19.12			X
TARBP			X

SRM 2391d: Certification Testing

What Platforms Were Used for Testing?

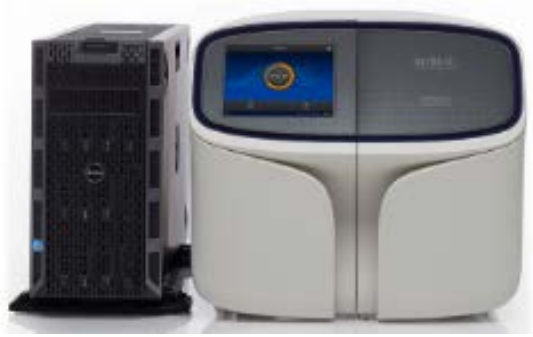
- **Capillary Electrophoresis (CE)** was performed with one instrument:
  - 3500xL Genetic Analyzer (ThermoFisher)



3500xl

- **Next Generation Sequencing (NGS)** was performed with two different instruments:
  - MiSeq FGx (Verogen)
  - Ion S5 XL (ThermoFisher)

MiSeq FGx



Ion S5 XL

Commercial CE Kits that were tested (34 Kits Total)

Thermo Fisher (13)	Promega (14)	Qiagen (6)	InnoGenomics (1)
Minifiler	PowerPlex S5	Investigator ESSplex SE Plus	InnoTyper 21
Identifiler	PowerPlex CS7	Investigator HDplex	
Identifiler Plus	PowerPlex 16	Investigator 24plex QS	
Identifiler Direct	PowerPlex 16 HS	Investigator 24plex GO!	
NGM	PowerPlex 18D	Investigator Argus X-12	
NGM SElect	PowerPlex 21	Investigator DIPplex	
NGM Detect	PowerPlex ESX 17		
Verifiler Express	PowerPlex ESX 17 Fast		
Verifiler Plus	PowerPlex ESI 17 Pro		
GlobalFiler	PowerPlex ESI 17 Fast		
GlobalFiler Express	PowerPlex Fusion		
Yfiler	PowerPlex Fusion 6C		
Yfiler Plus	PowerPlex VersaPlex 27PY		
	PowerPlex Y23		

ThermoFisher SCIENTIFIC

Promega

QIAGEN

InnoGenomics
Innovation in Forensic Genetics

Commercial NGS Kits that were tested (11 Kits Total)

AFDIL/MiSeq (1)	Verogen/MiSeq (1)	Thermo Fisher/Ion S5 XL (5)	Promega/MiSeq (2)	Qiagen/MiSeq (2)
mtDNA Whole Genome	ForenSeq Signature Prep Kit	Precision ID GlobalFiler NGS STR Panel v2	PowerSeq 46GY (prototype)	QIAseq mtDNA Whole Genome Panel
		Precision ID Ancestry Panel	PowerSeq CRM Nested System (mtDNA control region)	QIAseq SNP Panel
		Precision ID Identity Panel		
		Precision ID Phenotype Panel		
		Precision ID mtDNA Whole Genome Panel		

Ring *et al.* (2017)

What is Included in SRM 2391d?

- **Sample format**:
  - 4 extracted DNA samples
    - 3 single source and 1 mixed sample at a 3:1 ratio (female:male)
  - 1 cell line (female) spotted onto FTA paper as intact cells
  - 5 samples total: Components A-E

- **Concentration of the samples is ~1.5 ng/µL DNA for the extracted DNA (A-D) and 7.5 x 10^4 cells spotted on FTA paper (E)**
  - The concentrations were determined by droplet digital PCR (ddPCR)
  - The concentrations will NOT be certified values – they will be reported as information values

Component	ng/µL	U(ng/µL)
A	1.5	0.3
B	1.7	0.3
C	1.6	0.2
D	1.5	0.3

Components A-D have *different profiles* from SRM 2391c

Component E has *the same profile* as SRM 2391c

Where Are We Now?

- All components have been diluted (A-D), spotted (E), bottled and labeled
- Homogeneity Testing has been completed
- Stability Testing up to 30 weeks has been completed (4ºC, 22ºC, and 37ºC)

Statistical Engineering Division (SED) approved

- All CE testing has been completed (34 kits)
- NGS testing has been completed (11 kits)
- Data review process and concordance evaluations have been completed
- Reports of Analysis and Certificate of Analysis are in progress

SRM 2391d will be released in summer 2019

Autosomal STR Marker Certified Values (35 + Amelogenin)

	Component A		Component B		Component C		Component D				Component E	
Locus	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 3	Allele 4	Allele 1	Allele 2
AMEL	X	X	X	Y	X	Y	X	Y			X	X
CSF1PO	12	14	12	12	10	11	10	11	12	14	10	11
D10S1248	14	15	12	15	12	16	12	14	15	16	14	14
D12ATA63	13	17	17	18	13	15	13	15	17		12	17
D12S391	21	24	19	20	17	18	17	18	21	24	17	22
D13S317	9	12	11	11	12	14	9	12	14		8	12
D14S1434	11	13	13	14	10	14	10	11	13	14	10	14
D16S539	12	13	9	11	9	12	9	12	13		11	12
D17S1301	11	13	12	13	12	14	11	12	13	14	11	14
D18S51	14	15	17	18	16	18	14	15	16	18	14	17
D19S433	13	15	11	16.2	13	15	13	15			14	14
D1S1656	15.3	18.3	13	15.3	15	16	15	15.3	16	18.3	11	16.3
D1S1677	15	15	14	15	14	14	14	15			14	16
D20S482	13	14	15	16	14	15	13	14	15		15	15
D21S11	29	30	28	29	29	31	29	30	31		29	30
D22S1045	14	16	12	15	14	15	14	15	16		16	17
D2S1338	25	25	17	23	23	24	23	24	25		19	20
D2S1776	10	10	9	11	10	12	10	12			9	11
D2S441	11	11	11	11	11	14	11	14			10	10
D3S1358	17	17	15	17	14	18	14	17	18		14	15
D3S4529	13	15	13	14	16	16	13	15	16		13	16
D4S2408	9	9	10	10	8	10	8	9	10		8	8
D5S2800	14	17	14	17	14	18	14	17	18		17	17
D5S818	10	11	12	12	13	15	10	11	13	15	11	13
D6S1043	12	19	13	18	11	18	11	12	18	19	11	11
D6S474	16	18	14	16	14	18	14	16	18		14	16
D7S820	8	10	10	10	9	10	8	9	10		8	10
D8S1179	12	13	12	15	12	15	12	13	15		11	13
D9S1122	11	12	11	13	11	12	11	12			11	11
FGA	21	24	24	26	22	23	21	22	23	24	20	23
Penta D	8	9	11	13	9	13	8	9	13		14	14
Penta E	13	14	5	7	12	14	12	13	14		13	19
SE33	17	28.2	17	28.2	17	18	17	18	28.2		22	30.2
TH01	7	9.3	7	7	8	9.3	7	8	9.3		6	9.3
TPOX	8	9	8	12	8	10	8	9	10		8	11
vWA	17	19	15	17	14	17	14	17	19		17	18

***Interesting Example**

Y-STR Marker Certified Values (28)

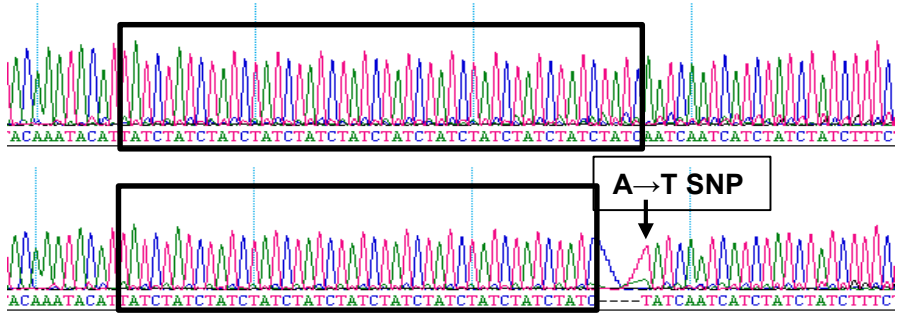
	Component B		Component C		Component D	
Locus	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
DYF387S1	36	38	36	39	36	39
DYS19	15		16		16	
DYS385	15	16	16	17	16	17
DYS389I	12		12		12	
DYS389II	30		31		31	
DYS390	21		21		21	
DYS391	11		10		10	
DYS392	11		11		11	
DYS393	13		13		13	
DYS437	14		14		14	
DYS438	11		11		11	
DYS439	13		12		12	
DYS448	21		22		22	
DYS456	15		15		15	
DYS458	17		18		18	
DYS460	10		10		10	
DYS461	13		13		13	
DYS481	26		28		28	
DYS505	13		12		12	
DYS522	11		11		11	
DYS533	11		11		11	
DYS549	11		12		12	
DYS570	20		18		18	
DYS576	15		17		17	
DYS612	34		34		34	
DYS635	21		21		21	
DYS643	15		14		14	
YGATAH4	13		12		12	

*Components A and E are females and therefore do not have a Y chromosome

What about Sanger Sequencing?

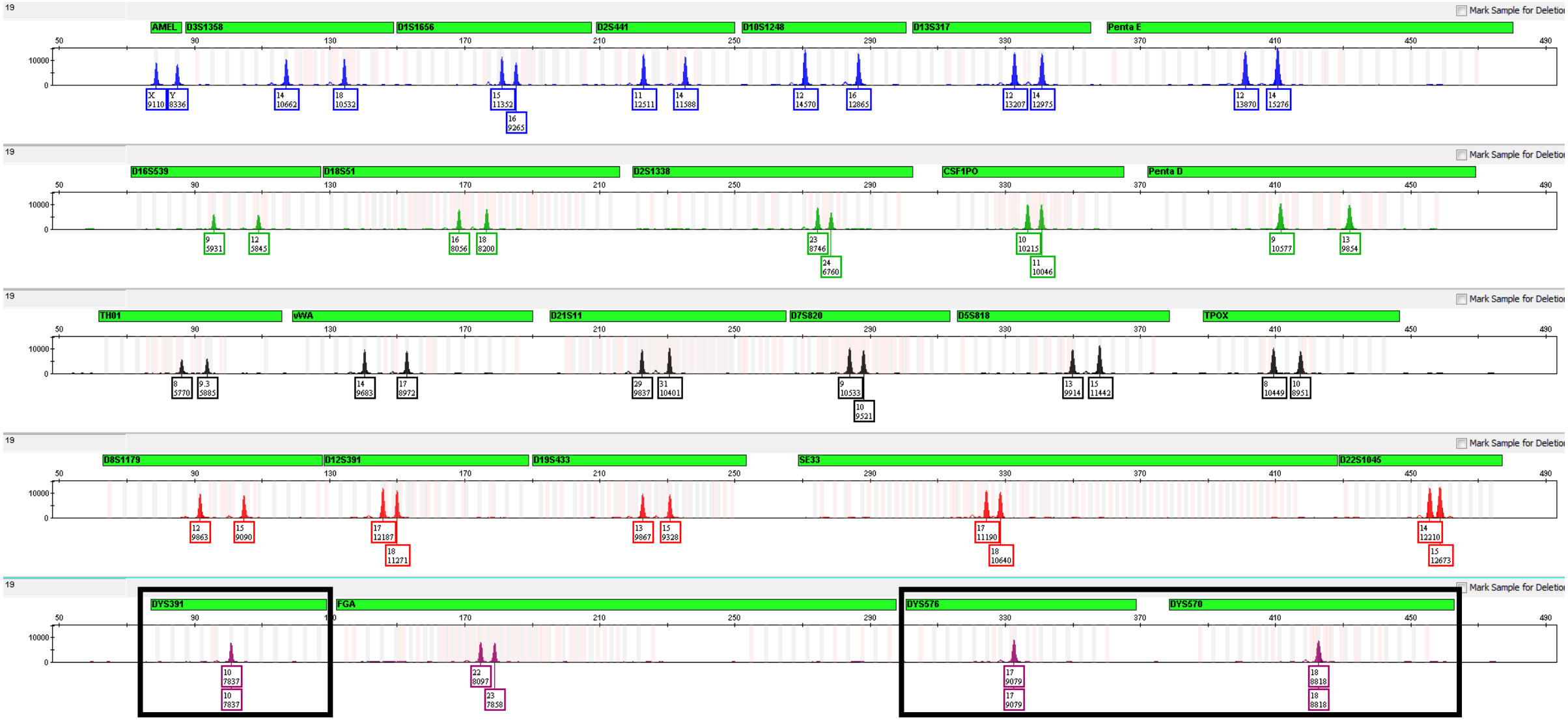
- We did not Sanger Sequence every certified type for the components of SRM 2391d
  - Sanger and NGS methods were used in parallel to characterize all STR alleles for SRM 2391c
    - All results were fully concordant
    - We established NGS as a primary method for certification
- However, if there were any issues, concerns or questionable results:
  - Discordant results between kits
  - Null alleles
  - Any other ambiguities that are observed

Sanger Sequencing was used to confirm results



SRM 2391d Example Data: Component C

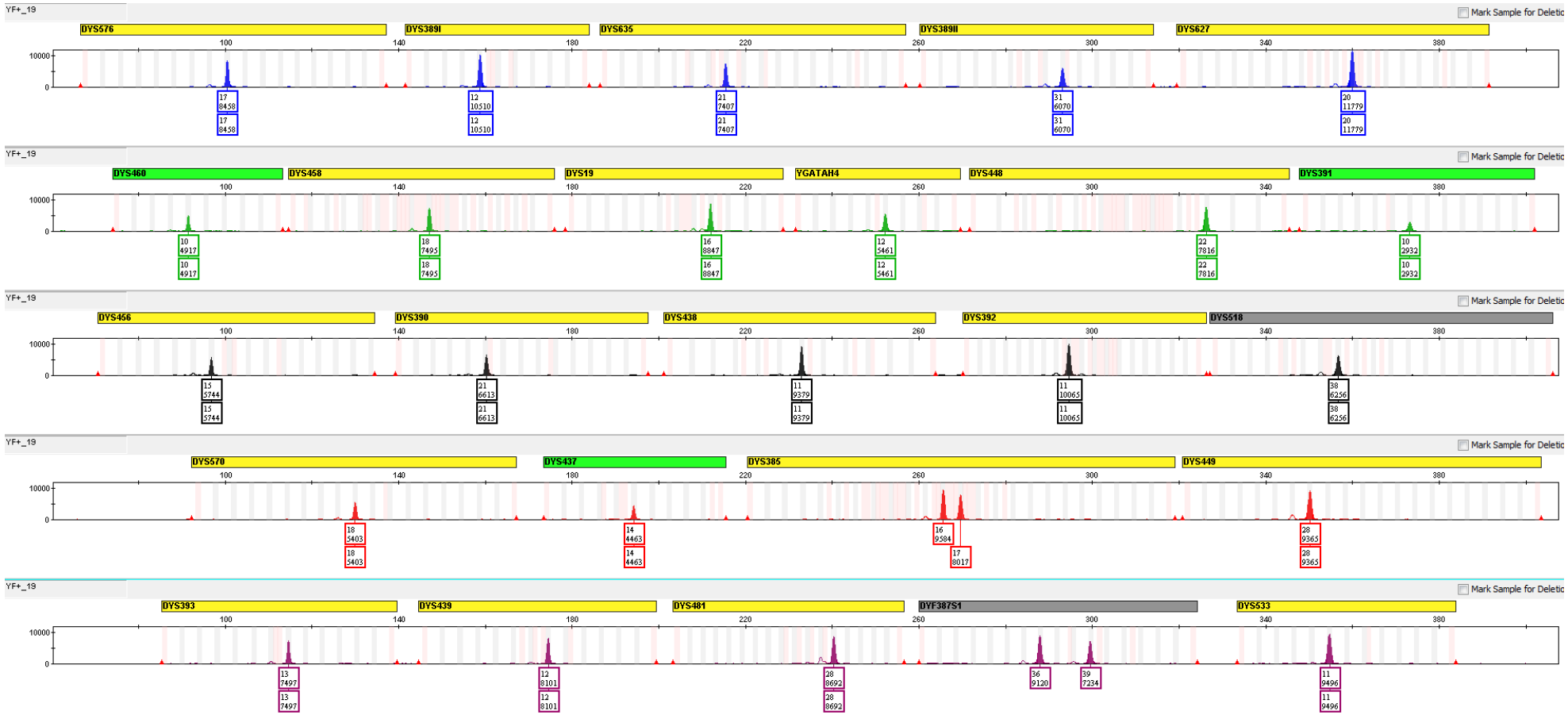
Autosomal STR CE Profile: PowerPlex Fusion 6C



Fully Heterozygous with PowerPlex Fusion 6C

*Y-STR Markers

Y-STR CE Profile: Yfiler Plus



YFiler Plus Profile

YHRD: No matches in 188,209 Haplotypes
(using Minimal Haplotype)
https://yhrd.org

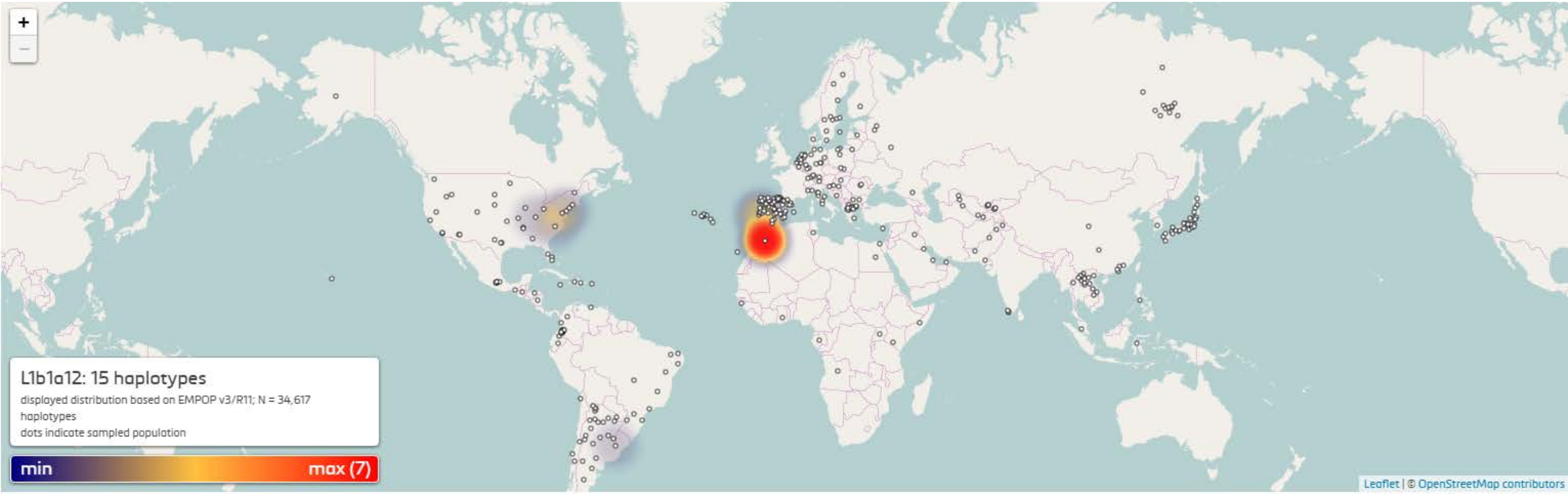
Whit Athey's Haplogroup Predictor: E1b1a
http://www.hprg.com/hapest5/hapest5a/hapest5.htm?order=num

Results Table

Haplo-group	Fitness score	Proba-bility (%)
E1b1a	58	100.0
E1b1b	18	0.0
G2a	20	0.0
G2c	5	0.0
H	25	0.0
I1	7	0.0
I2a (xI2a1)	20	0.0
I2a1	3	0.0
I2b (xI2b1)	7	0.0
I2b1	14	0.0
J1	11	0.0
J2a1b	5	0.0
J2a1h	6	0.0
J2a1 x J2a1-bh	11	0.0
J2b	9	0.0
L	13	0.0
N	2	0.0
Q	17	0.0
R1a	11	0.0
R1b	4	0.0
T	16	0.0

mtDNA Whole Genome Sequencing

AFDIL mtDNA Whole Genome Sequencing protocol (MiSeq), Ring *et al.*, 2017



EMPOP results:

https://empop.online/haplotypes#matches_details

Haplogroup	Ancestry	Match
L1b1a12	African	unique

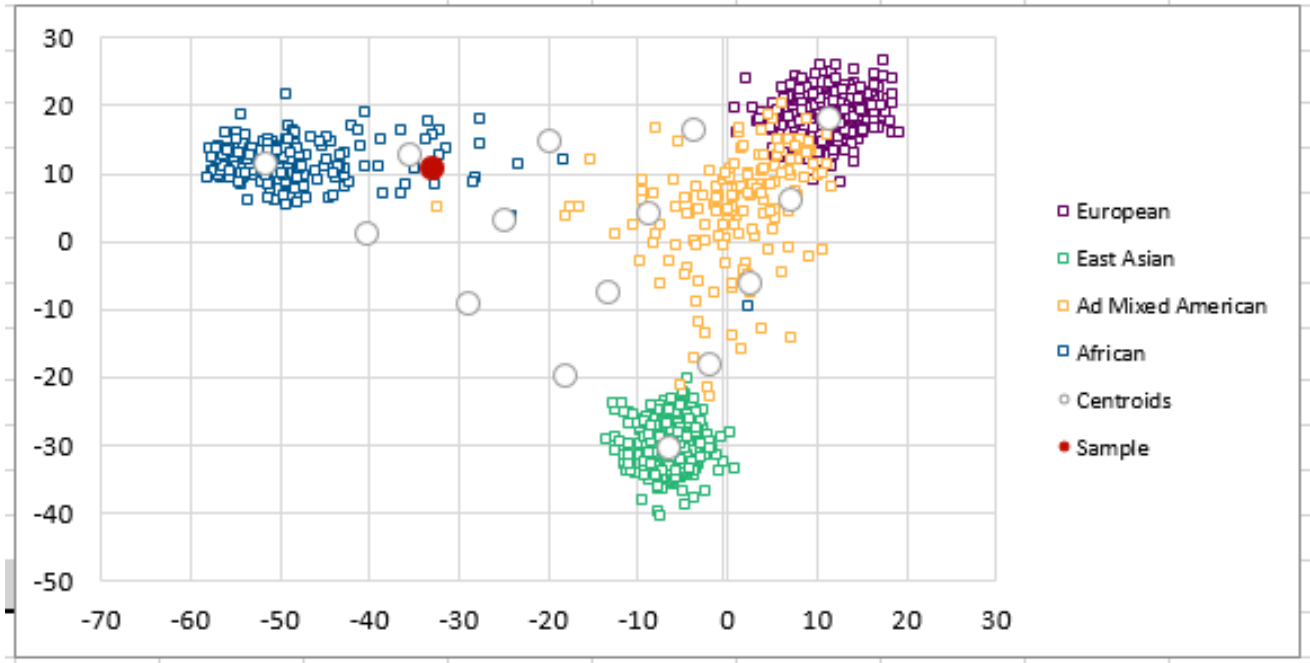
Ring, J.D., Sturk-Angreaggi, K., Peck, M.A., Marshall, C. (2017) A performance evaluation of Nextera XT and KAPA HyperPlus for rapid Illumina library preparation of long-range mitogenome amplicons. Forensic Sci Int Genet 29:174-180.

SNP Phenotype and Ancestry Estimation (ForenSeq DNA Signature Prep Kit)

Hair Color Results	
Brown	0.16
Red	0.00
Black	0.84
Blond	0.00

Eye Color Results	
Intermediate	0.00
Brown	1.00
Blue	0.00

Biogeographical Ancestry Results	
Distance to Nearest Centroid	3.36



Population(Region, sampleSize 2N)	Probability of Genotype in each Population	Likelihood Ratio
Somali(Africa,40)	● 1.576E-15	
African American(ASW)(Africa,122)	● 3.044E-16	5.18
Sandawe(Africa,80)	● 1.824E-16	8.64
Ethiopian Jews(Africa,64)	1.032E-16	15.3
African Americans(Africa,182)	7.118E-17	22.1
Masai(Africa,44)	8.17E-18	193.0
Chagga(Africa,90)	1.289E-18	1220.0
Luhya(LWK)(Africa,198)	4.072E-20	38700.0
Lisongo(Africa,16)	3.211E-20	49100.0
Hausa(Africa,78)	4.487E-21	351000.0

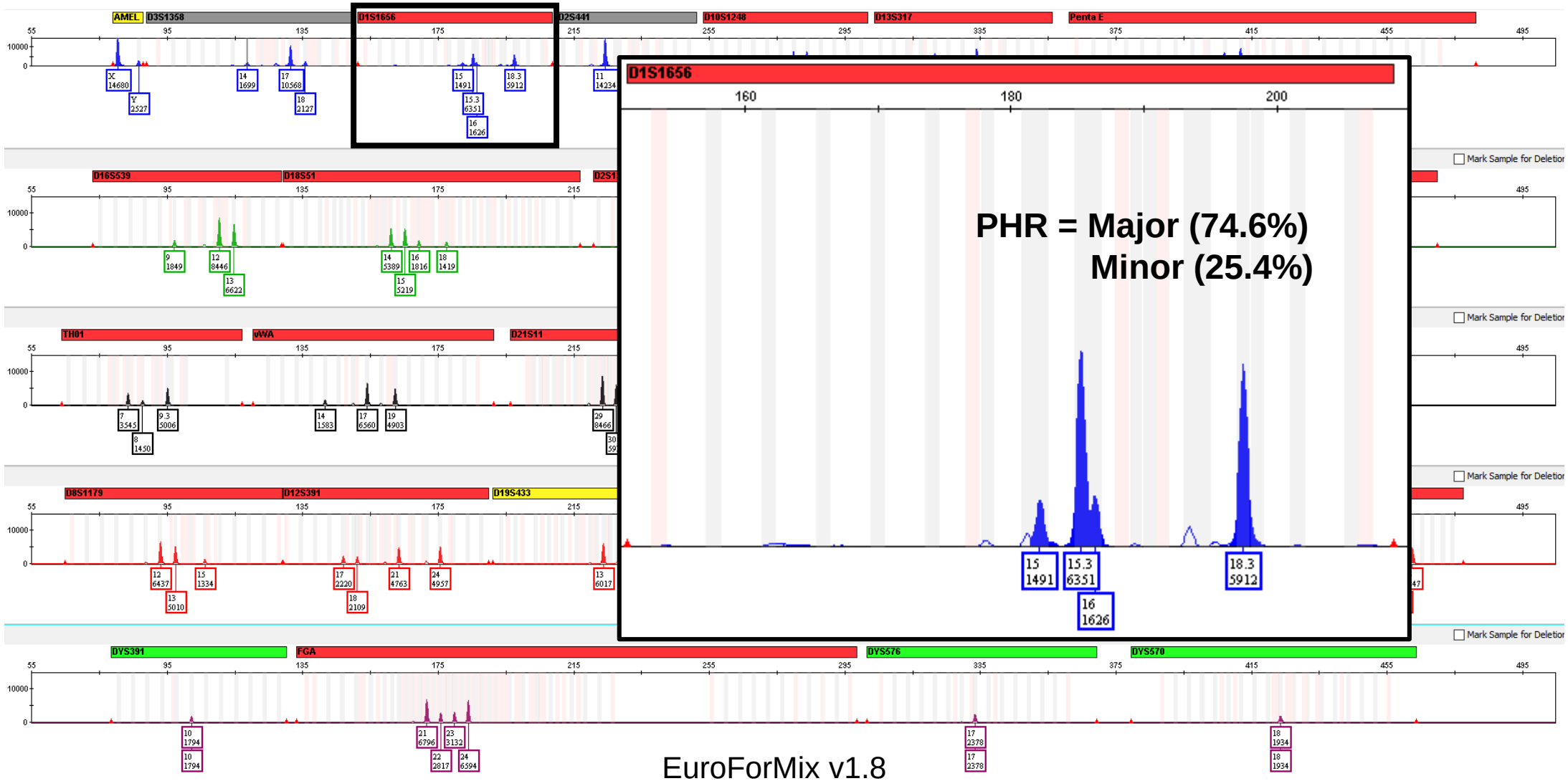
● Indicates the values are within an order of magnitude of the highest likelihood.

KiddLab – Set of 55 AISNPs
Population likelihoods based on 55 SNPs and 139 reference populations for this DNA profile
http://frog.med.yale.edu/FrogKB/

Other Markers Determined: X-STRs, Indels, INNULS, and other SNP Panels

SRM 2391d:
Component D (3:1 Mixture)

Autosomal STR CE Profile: PowerPlex Fusion 6C



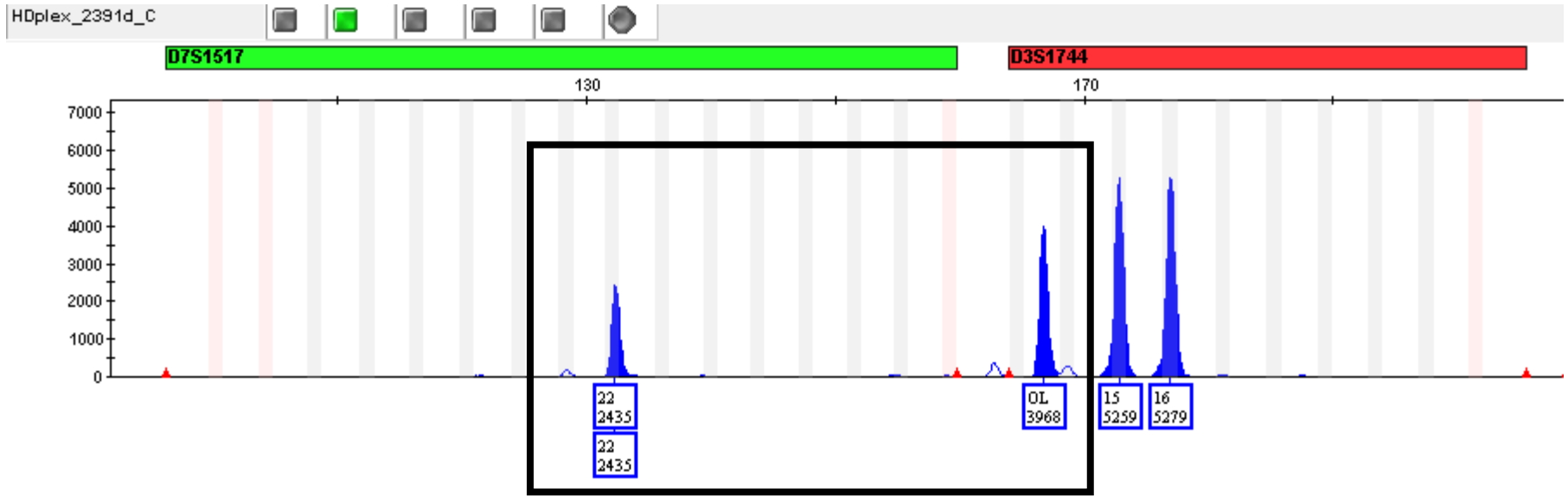
Component D:
3:1 mixture of Components A + C
(female to male)

EuroForMix v1.8

	Mixture Proportion	
	Average	Stdev
Mix-prop. C1	73.5%	0.5%
Mix-prop. C2	26.5%	0.5%

SRM 2391d:
Interesting Results

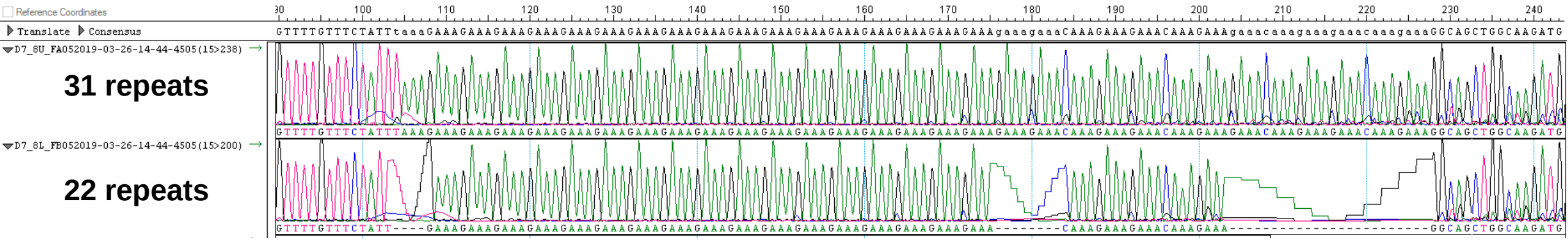
Qiagen Investigator HDplex – Component C



D7S1517 = (22,31)
D3S1744 = (15,16)

Sanger Sequencing confirmed these results

D7S1517 – Comp C (22,31) Sanger Sequencing

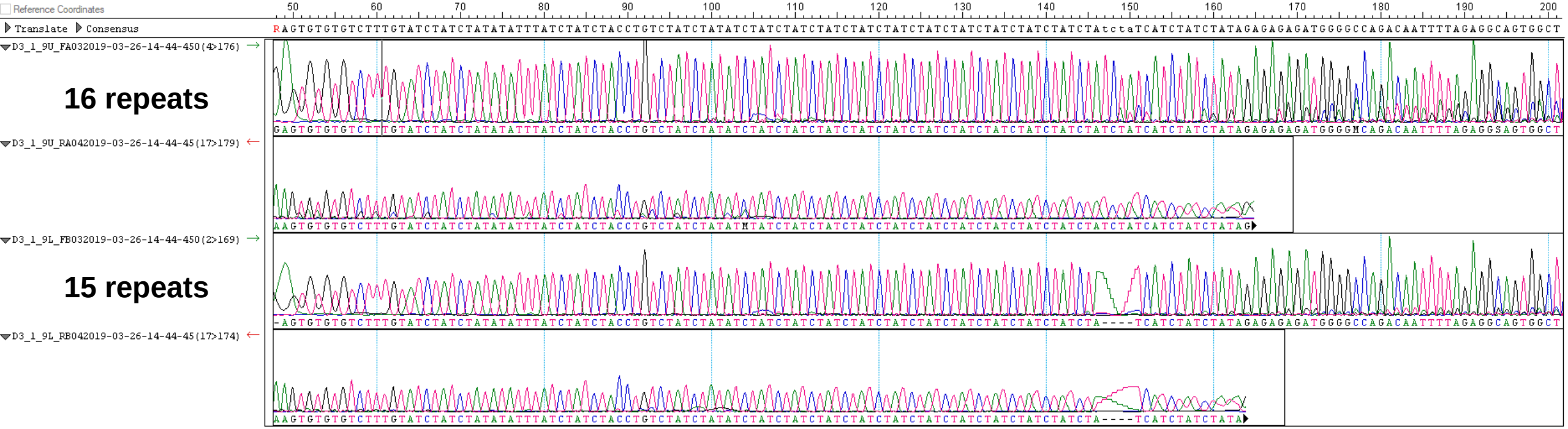


TAAA[GAAA]$_{19}$CAAA[GAAA]$_2$CAAA[GAAA]$_2$CAAA[GAAA]$_2$CAAA[GAAA] = 31 repeats

[GAAA]$_{17}$CAAA[GAAA]$_2$CAAA[GAAA] = 22 repeats

*TAAA is not listed in the repeat motif (Table 1, HDplex Handbook) but this seems to be variable and count towards the repeats for the larger allele

D3S1744 – Comp C (15,16) Sanger Sequencing

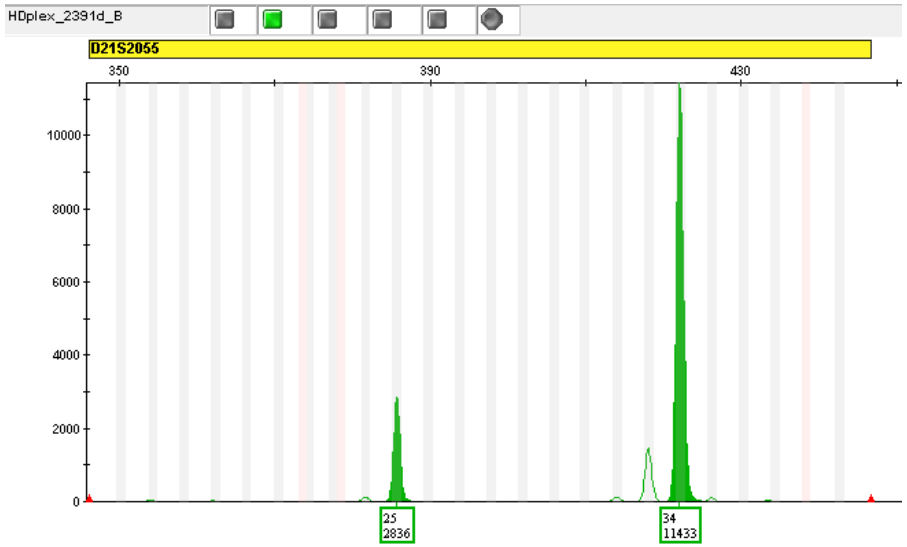


[TCTA]$_2$TA**[TCTA]$_{12}$**TCA**[TCTA]$_2$** = 16 repeats

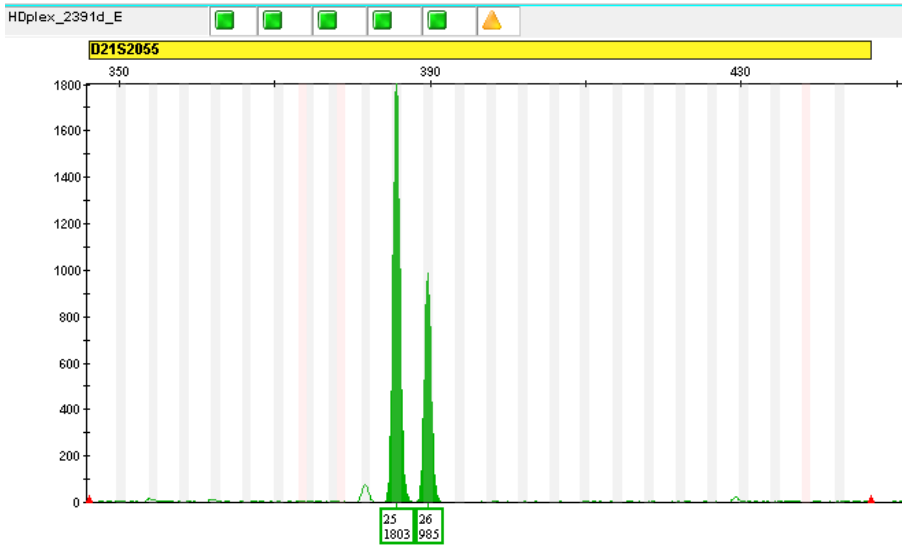
[TCTA]$_2$TA**[TCTA]$_{11}$**TCA**[TCTA]$_2$** = 15 repeats

Qiagen Investigator HDplex –D21S2055 Components B & E

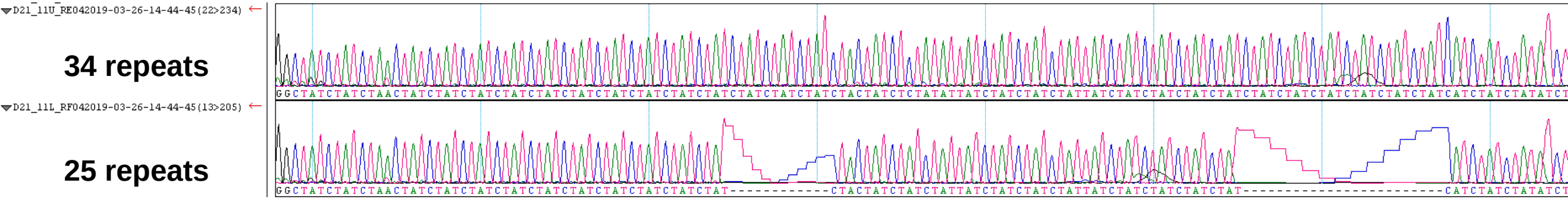
Peak Height imbalance for both Components
Component B = (25,34)
Component E = (25,26)



*There is no SNP or seq issue present in the sequence amped from these primers that may cause the PH imbalance. However, Sanger Sequencing confirmed that these are the correct types for both samples



D21S2055 – Comp B (25,34)
Sanger Sequencing

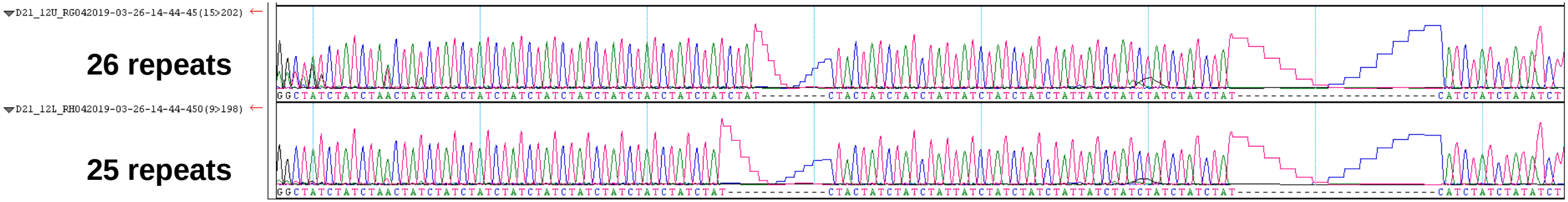


[CTAT]$_2$CTAA[CTAT]$_{13}$CTA**[CTAT]**[CTCT][ATAT]TAT**[CTAT]** $_3$TAT**[CTAT]$_{10}$**CAT**[CTAT]$_2$** = 34 repeats

[CTAT]$_2$CTAA[CTAT]$_{10}$CTA**[CTAT]$_3$**TAT**[CTAT]** $_3$TAT**[CTAT]$_4$**CAT**[CTAT]$_2$** = 25 repeats

*There is a repeat variation in the larger allele – instead of [CTAT]$_3$, it's broken up into [CTAT][CTCT][ATAT], but otherwise follows the same repeat pattern

D21S2055 – Comp E (25,26)
Sanger Sequencing

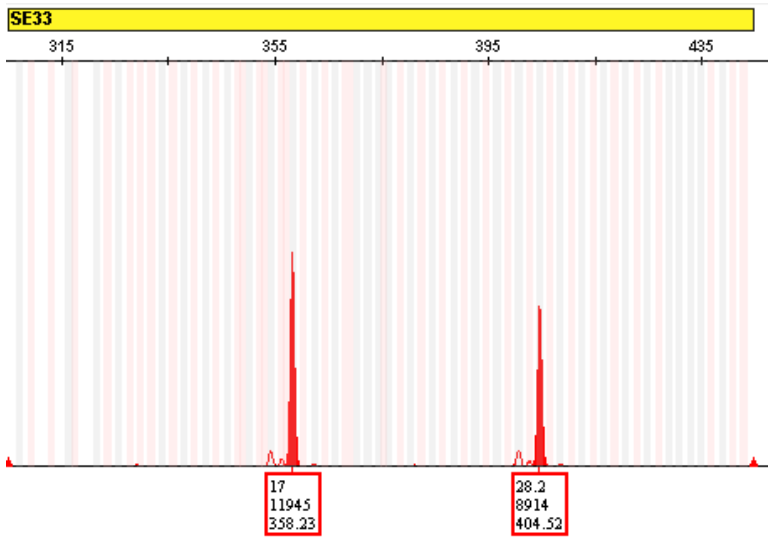


[CTAT]$_2$CTAA[CTAT]$_{11}$CTA**[CTAT]$_3$**TAT**[CTAT]** $_3$TAT**[CTAT]$_4$**CAT**[CTAT]$_2$** = 26 repeats

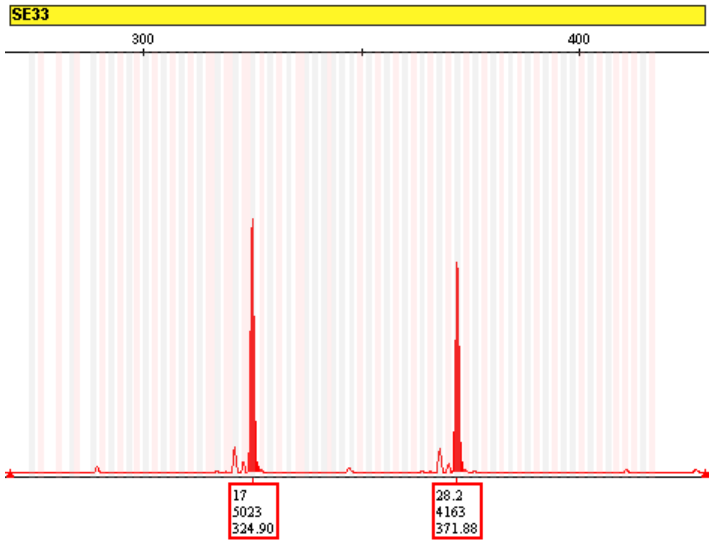
[CTAT]$_2$CTAA[CTAT]$_{10}$CTA**[CTAT]$_3$**TAT**[CTAT]** $_3$TAT**[CTAT]$_4$**CAT**[CTAT]$_2$** = 25 repeats

All CE Typing Kits – SE33 Component B (17,28.2)

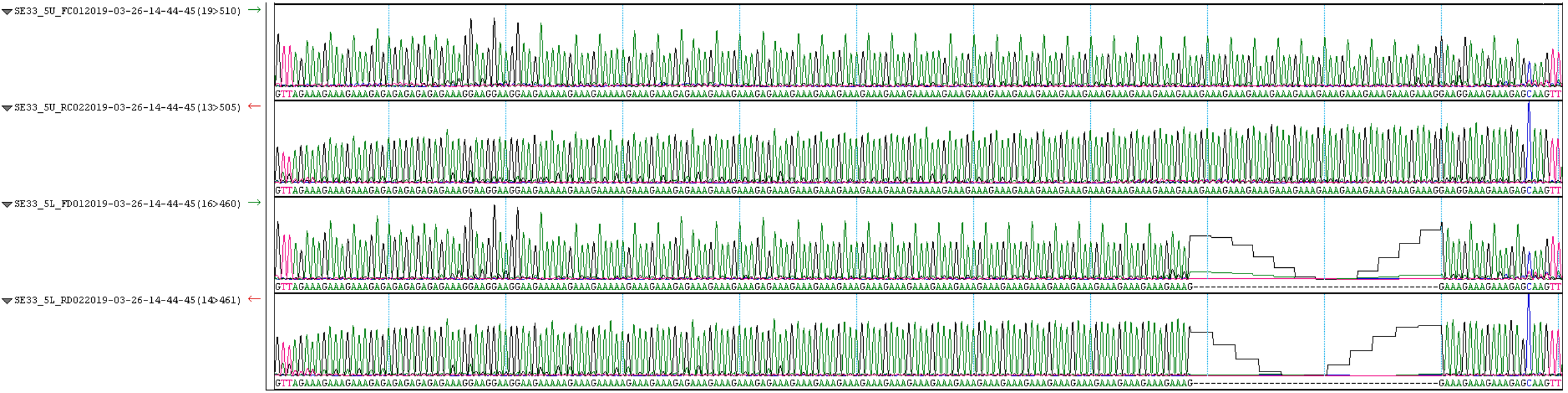
GlobalFiler



PP Fusion 6C



SE33 – Comp B (17,28.2)
Sanger Sequencing

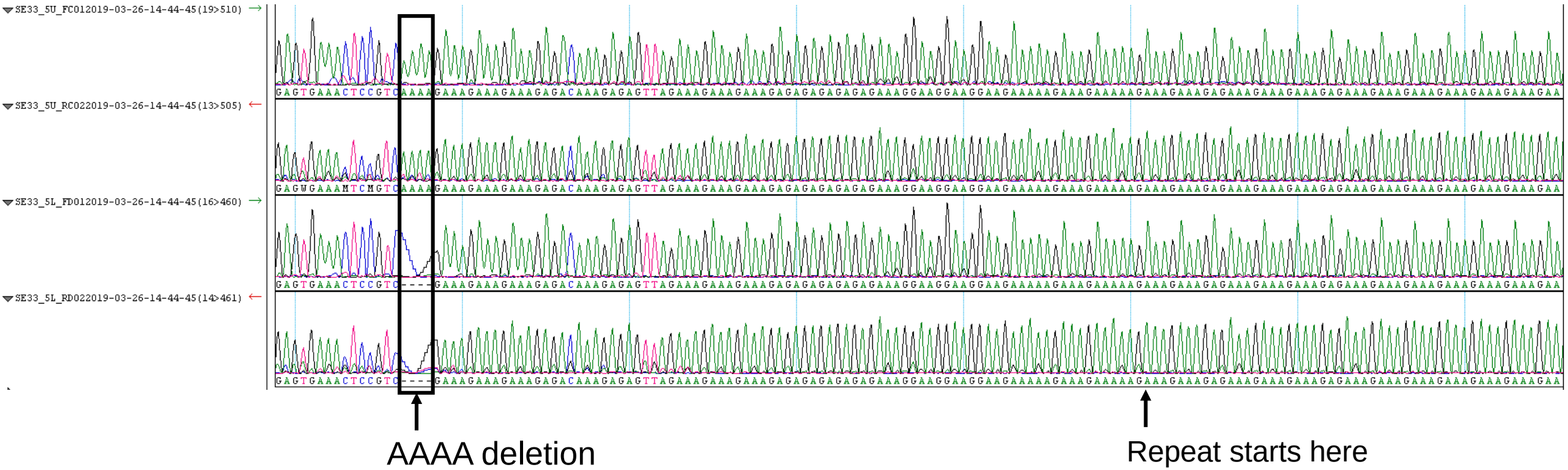


$[AAAG]_2$ AG $[AAAG]_3$ AG **$[AAAG]_6$ AA AAAG $[AAAG]_{21}$** G AAGG $[AAAG]_2$ AG = 28.2 repeats

$[AAAG]_2$ AG $[AAAG]_3$ AG $[AAAG]_{18}$ G $[AAAG]_3$ AG - **del AAAA 85 bp US** = 18 repeats, CE calls 17 repeats

*Deletion illustrated on next slide

SE33 – Comp B 18 allele Seq, 17 allele CE
AAAA deletion 85 bp upstream from the repeat



SRM 2391d: Applications

Applications of SRM 2391d

- To meet the FBI Quality Assurance Standards: QAS 9.5.5

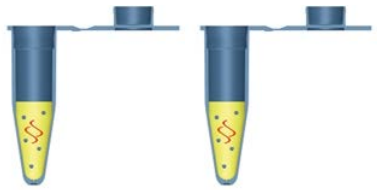
> 9.5.5 The laboratory shall check its DNA procedures annually or whenever substantial changes are made to a procedure against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

- Validation Studies: instrument, commercial kit, and software
  - Developmental and Internal Validations
  - Known, ***well-characterized*** samples for all systems commercially available

- Make NIST traceable materials (see http://ts.nist.gov/traceability/)

Establishing Traceability to NIST SRM 2391d

- Traceability requires the establishment of an unbroken chain of comparisons to stated references (see http://ts.nist.gov/traceability/)
- In the case of DNA testing with STR markers, the reference material is SRM 2391d
- Materials deemed traceable to NIST-created materials must have a record associated with them.



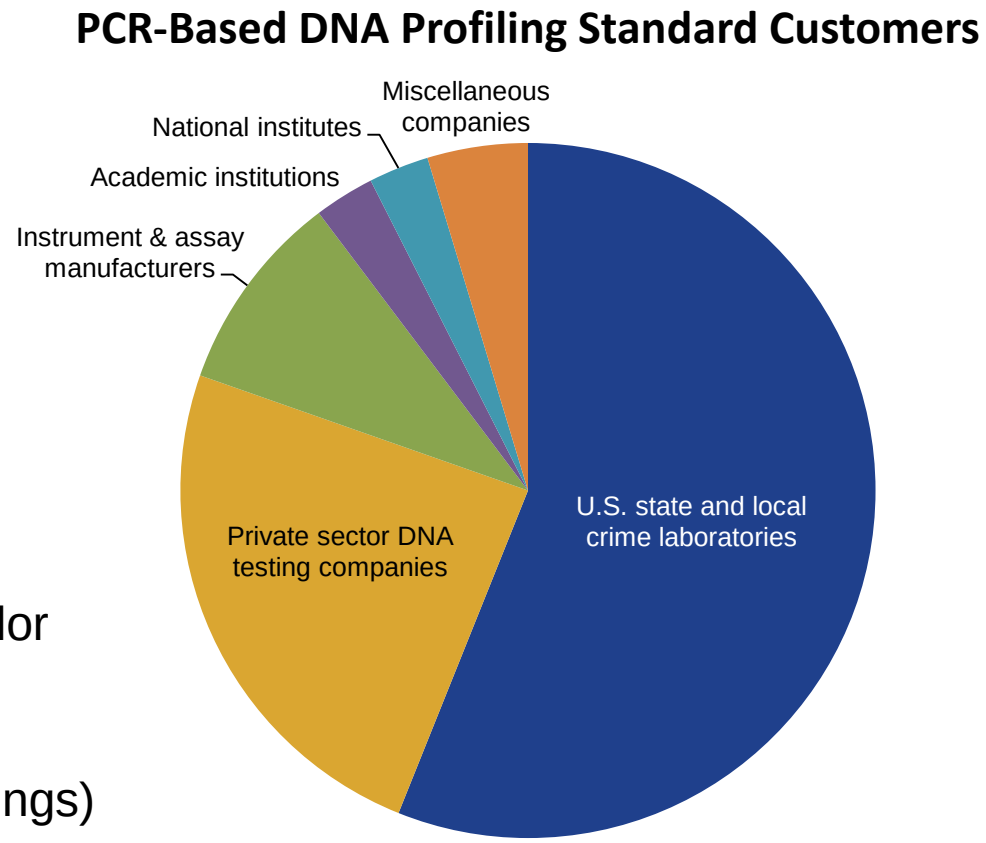
Lot of DNA material

Run against SRM 2391d

Everyday use calibrant

Support to the Forensic Community

- PCR-Based DNA Profiling Standard Customers
  - U.S. state and local crime laboratories
  - Private sector DNA testing companies
  - Instrument and assay manufacturers
  - Academic institutions
  - National institutes
  - Miscellaneous companies/industry



- **Emerging Forensic Technology**
  - New Markers
    - CODIS 13 → CODIS 20: January 1, 2017
    - New SNP markers for ancestry and eye/hair color predictions
  - New Methods
    - Next Generation Sequencing (full sequence strings)
    - New CE instruments and STR kits

Summary and Final Thoughts

- The next PCR-Based DNA Profiling Standard is being developed as the most ***comprehensive*** forensic SRM yet
  - STR genotypes and haplotypes
  - Information from commercially available forensic markers beyond the STR markers

- Capillary Electrophoresis and Next Generation Sequencing have been performed to assign certified and information values to the final components

- SRM 2391d can be used for validation studies and to support the forensic community as new technologies emerge

- SRM 2391d will be available in summer 2019

Thank you for your attention!

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Questions?

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