NIST PCR-Based DNA Profiling Standard (SRM 2391d): What is New and Different?

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Poster # 48

Poster available for download from STRBase: https://strbase.nist.gov/NISTpub.htm#Presentations

The fifth installment of the NIST Standard Reference Material (SRM) 2391 series was recently released as SRM 2391d: PCR-based DNA Profiling Standard. This set of highly characterized DNA samples comprise the most comprehensive forensic DNA standard yet and varies from the previous versions in many ways. These differences include unique samples and profiles for four of the five components, as well as providing more information to the end-user. The DNA forensic community has progressed greatly in the 24 years since the first release of SRM 2391 in 1995, which was certified initially for variable number tandem repeat (VNTR) and dot-blot hybridization markers and was later updated with autosomal short tandem repeat (STR) markers. The subsequent two iterations, 2391a and 2391b, were certified for only autosomal STR markers, but it wasn't until SRM 2391c was released in 2011 and later updated in 2015 and 2018 [1] that other forensically-relevant markers were added to the Certificate of Analysis (COA). These included Y-STR markers, X-STR markers, insertion/deletion (indel) markers, and single nucleotide polymorphism (SNP) markers. The goal when developing SRM 2391d was to provide a highly characterized set of genomic DNA samples for all commercially available forensic DNA markers at the time of certification. This includes of all the markers previously covered in the updated version of SRM 2391c as well as whole mitochondrial genome DNA (mtDNA) sequences, insertion/null allele (INNUL) markers, and Y-SNP markers. High confidence allele calls were established by using multiple polymerase chain reaction (PCR) length-based STR typing kits and sequence-based kits and technologies. The range of certified and information values that are associated with this SRM, how these values were assigned [2], as well as the various methods used to obtain these values including capillary electrophoresis (CE) and next generation sequencing (NGS) is presented. An additional feature of SRM 2391d is that concentrations of each sample are provided in the COA as an information value, whereas previously it was provided as a range. The concentrations were determined by droplet digital PCR (ddPCR) and are listed with an expanded uncertainty. There are several interesting and unique characteristics and nomenclature discrepancies of the components that are highlighted and discussed. SRM 2391d enables the standardization and calibration of the typing process for STR and other sequence-based markers for human identity testing. Other important uses such as quality control, traceability and validation of new technologies in the forensic community are highlighted.

Released for Sale: July 9, 2019

Materials (Five Components)

Components A-D are genomic DNA extracted from purchased blood

- Not from cell lines (challenges in obtaining permission from Coriell/NIGMS)
- *May* be more commutable (similar to casework)
- **Different samples from 2391c**

Components A-D have *different profiles* from SRM 2391c
Component E has *the same profile* as SRM 2391c

Component E consists of cells spotted onto FTA paper

- Two 6 mm punches; approximately 75,000 cells per punch
- Toward the end of SRM 2391c profile degradation was observed for cells stored on 903 paper (cells on 903 paper not included in SRM 2391d)
- **Same cell line as used in 2391c (CRL-1486)**

Table 1. Description of Components in SRM 2391d — **Information Values**

Component	Description	Volume	Concentration[(a)]
A	Anonymous single-source female genomic DNA in TE^{-4} buffer	55 µL	1.6 ± 0.5 ng/µL
B	Anonymous single-source male genomic DNA in TE^{-4} buffer	55 µL	1.7 ± 0.5 ng/µL
C	Anonymous single-source male genomic DNA in TE^{-4} buffer	55 µL	1.6 ± 0.2 ng/µL
D	Mixed-source, 3:1 (3 parts Component A and 1 part Component C) genomic DNA in TE^{-4} buffer	55 µL	1.5 ± 0.4 ng/µL
E	Anonymous single-source female cells spotted on FTA paper[(b)]	Two 6 mm punches	7.5 × 10^4 cells per punch

(a) DNA concentrations and cell counts are provided as Information Values.
(b) FTA paper cards contain chemicals that lyse cells, denature proteins and protect nucleic acids from nucleases, oxidation and UV damage. FTA cards rapidly inactivate organisms, including blood-borne pathogens, and prevent the growth of bacteria and other microorganisms.

Concentrations were assigned using droplet digital PCR (ddPCR)

Applied Genetics SRM 2391d Team

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How are SRM 2391d values assigned?

Certified Values are assigned when there is high coverage sequencing data available for a marker

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

Information Values are assigned when only one primer PCR set is used from CE testing and there is no supporting sequencing data

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

What Platforms Were Used for Testing?

Capillary Electrophoresis (CE) was performed with one instrument:

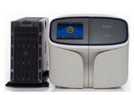
- 3500xL Genetic Analyzer (Thermo Fisher)

3500xl

Next Generation Sequencing (NGS) was performed with two different instruments:

- MiSeq FGx (Verogen)
- Ion S5 XL (Thermo Fisher)

MiSeq FGx



Ion S5 XL

Commercial CE Kits 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

Commercial NGS Kits/Methods 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)

References:
[1] SRM 2391d: PCR-Based DNA Profiling Standard Certificate of Analysis (2019). Available online at https://www-s.nist.gov/srmors/certificates/2391d.pdf. Accessed September 16, 2019.
[2] Thompson, A.; Taylor, B.N.; Guide for the Use of the International System of Units (SI); NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/physical-measurement-laboratory/special-publication-811. Accessed September 17, 2019.

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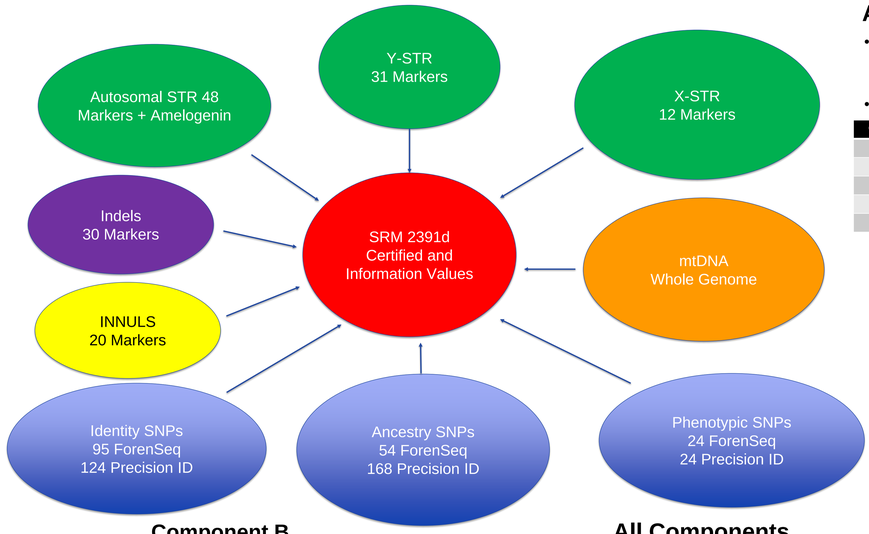
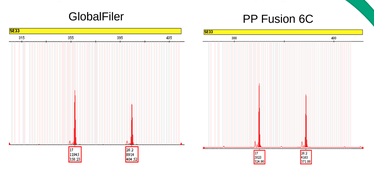
Markers Included in the Certificate of Analysis & Interesting Results



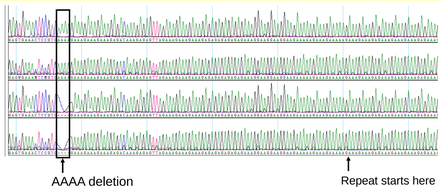
Component B
SE33 (17*,28.2)
Agreement across 13 CE-based typing kits



Sequencing revealed 18 repeats with a 4 bp AAAA deletion 85 bp upstream from the repeat and is noted in the COA

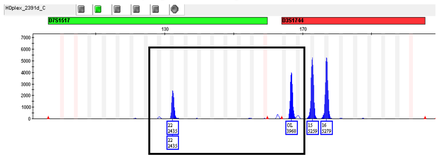
Component B
SE33 (18,28.2)
Sequencing Results

[AAAG]$_2$ AG [AAAG]$_3$ AG **[AAAG]$_{18}$** G [AAAG]$_3$ AG = 18 repeats



***A 17 allele is reported in the Certificate of Analysis (COA) for Component B at SE33 using all commercial CE multiplex kits tested**

Component C
D7S1517/D3S1744
Qiagen Investigator HDplex



D7S1517 = (22,31)
D3S1744 = (15,16)
Sanger Sequencing confirmed these results

All Components
D6S474
Qiagen Investigator HDplex

Component	SRM 2391d	Investigator HDplex kit
A	16,18	15,17
B	14,16	13,15
C	14,18	13,17
D	14,16,18	13,15,17
E	14,16	13,15

- Complex motif [**AGAT**][**GATA**] as described in Hill et al. 2008
- Reported as such in SRM2391b and 2391c
- Precision ID GlobalFiler NGS reports [**AGAT**][**GATA**]
- Investigator HDplex reports **one repeat less** [**TAGA**]TGA[**TAGA**]
- The **resulting allele calls** are **reported** in the COA as above (the **one repeat unit less** HDplex allele calls are also noted in the COA)

Identity SNP markers

- 101 autosomal SNPs reported
  - ForenSeq (94)
  - Precision ID Identity Panel (90) + 34 Y-SNPs

83 identity autosomal SNPs in common

- Forward strand genotype reported

Ancestry and Phenotype SNP markers

- Ancestry/Phenotype SNPs 188 total
  - ForenSeq (78)
  - Precision ID Ancestry (165) and Phenotype Panel (24)

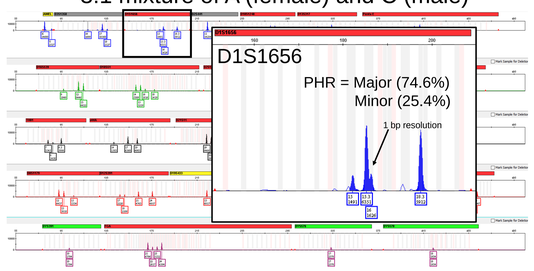
77 SNPs in common

- Forward strand genotype reported

Component	ForenSeq			Precision ID				Mito	Y SNP
	Ancestry	*Hair*	*Eye*	*Ancestry*	*Hair*	*Hair*	*Eye*		
A	European	0.68	0.66	European	0.66	1.00 light	0.67	T2b3	-
B	African	0.69	0.86	African	0.66	0.93 light	0.85	L1c1a	E
C	African	0.84	1.00	African	0.68	1.00 dark	1.00	L1b1a	E
E	European	0.61	0.71	European/SW Asian	0.69	0.72 light	0.72	T2a3	-

Predictions made using vendor tools for autosomal SNP markers

Component D
3:1 mixture of A (female) and C (male)



Component A & B
DYS612
ForenSeq Signature Prep Kit

Component	SRM 2391d	ForenSeq
A	34	28
B	34	28

Six repeat difference

Calls Reported in SRM 2391d Certificate of Analysis (COA)

- Kayser, et al. Am. J. Hum. Genet. 2004
  First described: **[CCT]$_5$[CTT]$_1$[TCT]$_4$[CCT]$_1$[TCT]$_n$**
- D'Amato, et al. FSI Genetics 2010 (ForenSeq)
  Proposed nomenclature: **[CCT]$_5$[CTT]$_1$[TCT]$_4$[CCT]$_1$[TCT]$_n$**
- Ballantyne et al. Human Mutation 2014 (NIST SRM 2391d)
  Compliance with ISFG guidelines: **[CCT]$_5$[CTT]$_1$[TCT]$_4$[CCT]$_1$[TCT]$_n$**
- The **resulting allele call** is **reported** in the COA as above (the **six repeat units less** ForenSeq allele call is also noted in the COA)

Where do I find SRM 2391d information?

https://www-s.nist.gov/srmors/view_detail.cfm?srm=2391d

Technical Contact: Becky Steffen

Data and Information Files

SRM 2391d - PCR-Based DNA Profiling Standard

Certified STR sequences for Components A, B, C, and E, including NGS length-based allele call, STRSeq ID, bracketed repeats, and full sequence strings (5' flank, repeat region, and 3' flank): **SRM 2391d_STRSeqID.xlsx**

The Information Values for SNP loci of forensic interest for Components A, B, C, and E: **SRM 2391d_AISNP-PISNP.xlsx** and **SRM 2391d_IISNP.xlsx**

The Information Values for the mtDNA whole genome sequences for Components A, B, C, and E: **SRM 2391d_mtDNA.xlsx**

Unit Price: $945.00

Expiration Date: 6/4/2024

Marker Type	Number of Certified Loci	Number of Information Loci
Autosomal STR	35	13
Y-STR	28	3
X-STR	7	5
Mitochondrial DNA	-	Full mtGenome
Indel/Innuls	-	50
SNPs	-	323

The SRM 2391 series will continue to support the FBI-QAS and the validation and implementation of forensic marker systems. Certified allele calls are supported by sequence data and CE-length based measurements.