





Quality: Excellent Good Average Poor

Not your standard Standard:





NIST SRM 2391d: PCR-Based DNA Profiling Standard
Using it in YOUR lab



Becky Steffen
 National Institute of Standards and Technology
 Forensic DNA Typing Continuing Education Day 2020
 March 17, 2020

1

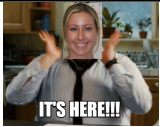
?  **Topics for Discussion**

- How do you make an SRM at NIST?
- What is new and different?
 - What markers, kits and instruments does it cover?
 - SRM 2391d data
- How can I use it in MY lab?
 - How do I make my own materials traceable to the SRM?
- Frequently Asked Questions (FAQs)


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SRM 2391d: PCR-Based DNA Profiling Standard

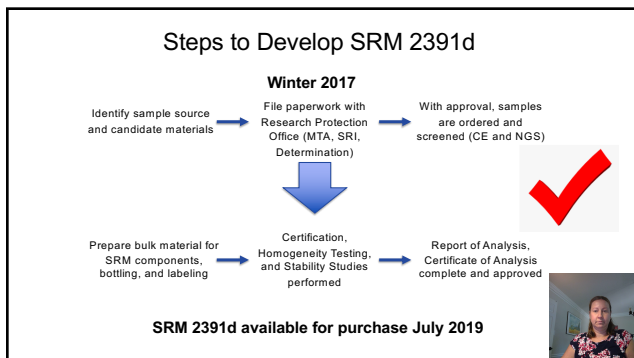


- Developed as a successor to SRM 2391c
 - SRM 2391c is no longer available for sale at NIST
 - The expiration date was recently extended by **2 years** to **Feb. 3, 2022**
 - We will not further extend the expiration date after this date
- **Next Generation Sequencing** is used for certification in 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**

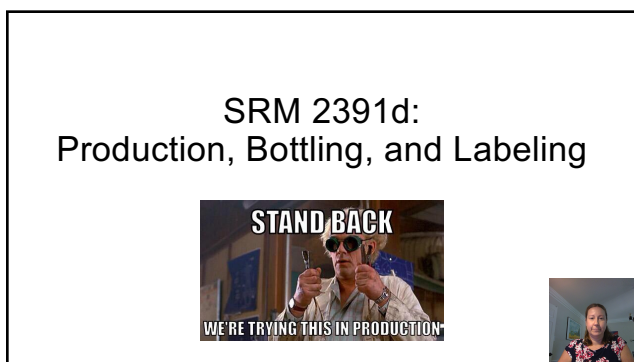
SRM 2391d is the most comprehensive NIST forensic SRM to date



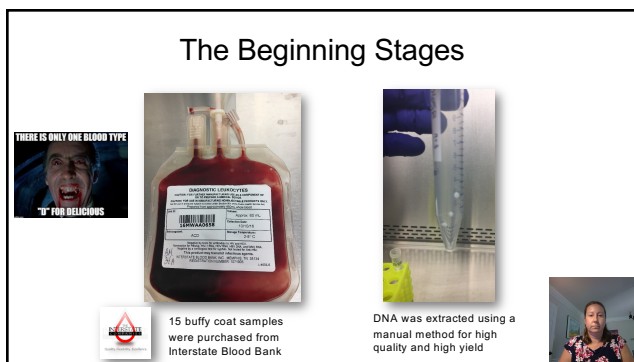
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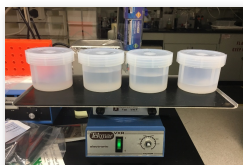


5



6

The Beginning Stages



DNA was allowed to solubilize and reconstitute in Teflon pots in TE⁻⁴

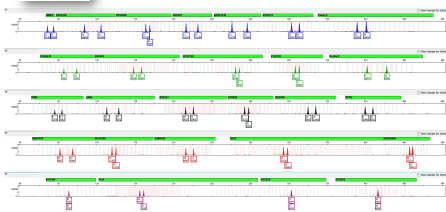


DNA was initially quality checked for purity and concentration on a NanoDrop



7

Preliminary Screening – CE & STR kits



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



8

Final Sample Selection and Certification

Final Set of Components Identified



Homogeneity


Value Assignment:
Certified, Reference,
and Information Values

Stability



9



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)



10

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



11

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



12

Completed Units for Testing

Homogeneity, Stability and Certification

-2200 units of each component



13

SRM 2391d: Value Assignment



14

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



15

Summary of Values Assigned

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



19



SRM 2391d: Certification Testing



20

What Platforms Were Used for Testing?

- **Capillary Electrophoresis (CE)** was performed with one instrument:
 - 3500xL Genetic Analyzer (ThermoFisher)
- **Next Generation Sequencing (NGS)** was performed with two different instruments:
 - MiSeq FGx (Verogen)
 - Ion S5 XL (ThermoFisher)



3500xl



MiSeq FGx

Ion S5 XL



21

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	VersaPlex 27PY		
	PowerPlex Y23		

22

Commercial NGS Kits that were tested (11 Kits Total)

AFDL/Seq (1)	Verogen/MISeq (1)	Thermo Fisher/Ion S5 XL (5)	Promega/MISeq (2)	Qiagen/MISeq (2)
miDNA Whole Genome	ForeSeq Signature Prep Kit	Precision ID GlobalFiler NGS STR Panel v2	PowerSeq 46GY (prototype)	QIAseq miDNA 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 miDNA Whole Genome Panel		

Ring et al. (2017)

23

Materials – Five Components

- Components A-D** are genomic DNA extracted from purchased blood:
 - Not from cell lines (challenges in obtaining permission from Coriell/NIH/GMS)
 - May be more commutable (similar to casework)
 - Different samples from 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 2391c

Component	Description	Volume	Information Values
A	Amplified high-molecular weight genomic DNA (HTA) (100 ng)	100 µL	10-12 STR loci
B	Amplified high-molecular weight genomic DNA (HTA) (100 ng)	100 µL	10-12 STR loci
C	Amplified high-molecular weight genomic DNA (HTA) (100 ng)	100 µL	10-12 STR loci
D	Amplified high-molecular weight genomic DNA (HTA) (100 ng)	100 µL	10-12 STR loci
E	Amplified high-molecular weight genomic DNA (HTA) (100 ng)	100 µL	10-12 STR loci

Concentrations were assigned using droplet digital PCR (ddPCR)

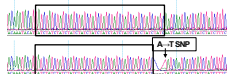
Components A-D have different profiles from SRM 2391c
Component E has the same profile as SRM 2391c

24

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



25

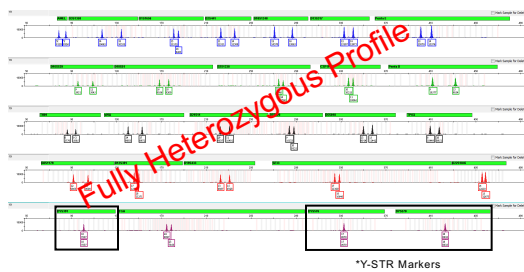
EXAMPLE

SRM 2391d Example Data: Component C

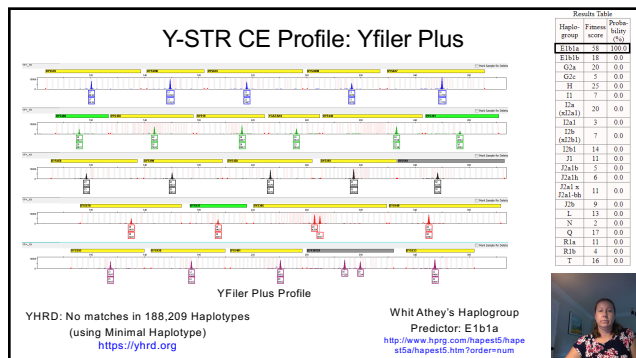


26

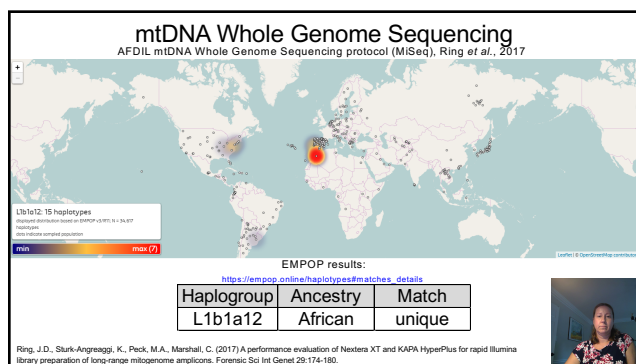
Autosomal STR CE Profile: PowerPlex Fusion 6C



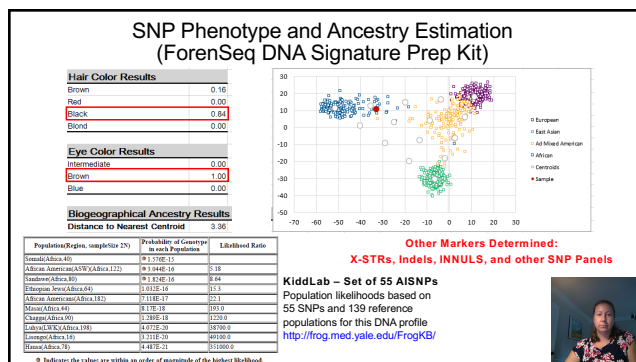
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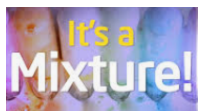


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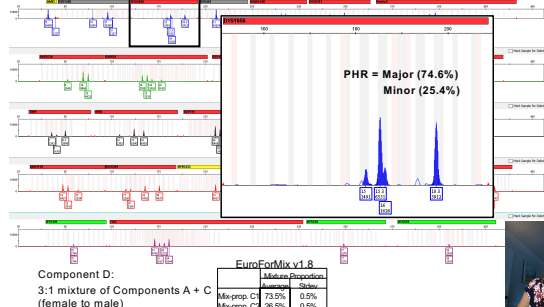


SRM 2391d: Component D (3:1 Mixture)



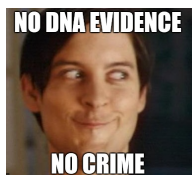
31

Autosomal STR CE Profile: PowerPlex Fusion 6C



32

SRM 2391d: Applications




33


How can SRM 2391d be used in YOUR lab?

- 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



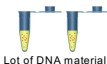
- Make **NIST traceable materials:**
<http://ts.nist.gov/traceability/>



34

Establishing Traceability to NIST SRM 2391d


- Traceability requires the establishment of an unbroken chain of comparisons to stated references: <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



Run against SRM 2391d

→

Everyday use calibrant



35

FAQ Frequently Asked Questions

- Can I have a free SRM?
 - Unfortunately we cannot give out free SRMs ☹️
 - We are not in charge of selling SRMs (Office of Reference Materials) but here is how you order it...
 - You can also request a quote with shipping included at srminfo@nist.gov



www.nist.gov/srm



36

Frequently Asked Questions

Standard Reference Materials
SRM Order Request System

Search Results

SRM Number	Description	Unit of Measure	Price
2391d	Pb in Glass	g	\$200.00

Add to Cart

37

Frequently Asked Questions

View Cart

SRM No.	Description	Status	Unit of Measure	Unit Price	Qty	Price
2391d	Pb in Glass	New Listing	g	\$200.00	1	\$200.00

Checkout **Empty Cart**

1. Log-in/Create account

2. Quote with shipping will be sent along with further instructions (pay.gov)

38

Frequently Asked Questions

- Where do I find SRM 2391d information?
 - Certificate of Analysis, expiration date, price, technical contact, and data files

www.nist.gov/srm

39

Frequently Asked Questions

Search Results

SRM Number: 2391d

Records Found: 1

Number	Description	Unit of Issue	Stock
2391d	PCR-Based DNA Profiling Standard	9 vials	\$966.00

40

Material Details
SRM 2391d - PCR-Based DNA Profiling Standard

Details

Description: PCR-Based DNA Profiling Standard

Lot: N/A

Expiration Date: 04/27/24

Unit Price: \$966.00

Unit of Issue: 9 vials

Status: Now Selling

Certificate Date: 02/1/2019

MDS Date: 01/16/2019

Technical Contact: Becky Steffen

Additional Information:

Shipping Information: Yes (Cold Pack)

Hazardous Material: No

Material Hazard Class: Not Hazardous to Ship

41

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2391d

PCR-Based DNA Profiling Standard

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 [5], 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](#)

42

Thank you for your attention!



Questions?
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A copy of this presentation is available at: <http://atbase.nist.gov/NISToub.htm#Presentations>
