NIST Update: What's new? What's going on?

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Norman, OK
November 19, 2019

What's new?

SRM 2391d: PCR-based DNA Profiling Standard

On sale as of July 9, 2019

2300 units were vialed

Components A-D are genomic DNA extracted from purchased blood

Different samples from 2391c

Component E consists of cells spotted onto FTA paper

Two 6 mm punches; approximately 75,000 cells per punch

Same cell line as used in 2391c (CRL-1486)

Materials

Table 1. Description of Components in SRM 2391d

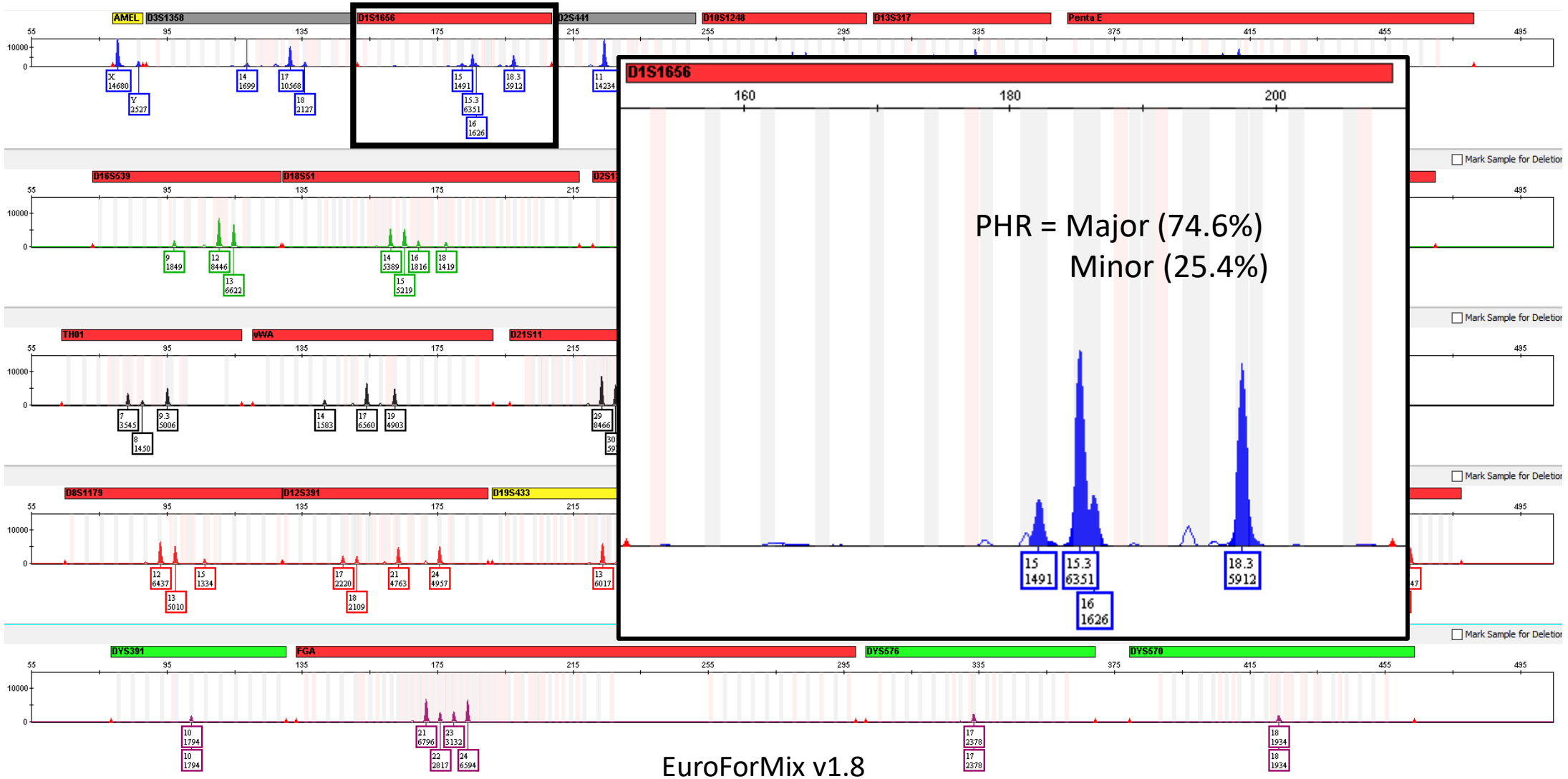
Assigned using digital PCR

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.

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



EuroForMix v1.8

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

Characterization of the material

Digital PCR
for quantification

QX200

Capillary Electrophoresis

3500xL

Next Generation Sequencing (NGS)

MiSeq FGx

Ion S5 XL

CE-based assays used in characterization

Table 2. Capillary Electrophoresis (CE) typing kits used for SRM 2391d testing

Thermo Fisher	Promega	Qiagen	InnoGenomics
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		

InnoGenomics
Innovation in Forensic Genetics

QIAGEN

34 Commercial CE-based assays

ThermoFisher SCIENTIFIC

Promega

In house CE-typing
D1S1677
D2S1776
D3S4529
D4S2408
D5S2800
D9S1122
D12ATA63
D14S14343
D17S1301
D20S482
DYS461
DYS505
DYS522
DYS612

NGS-based assays used in characterization

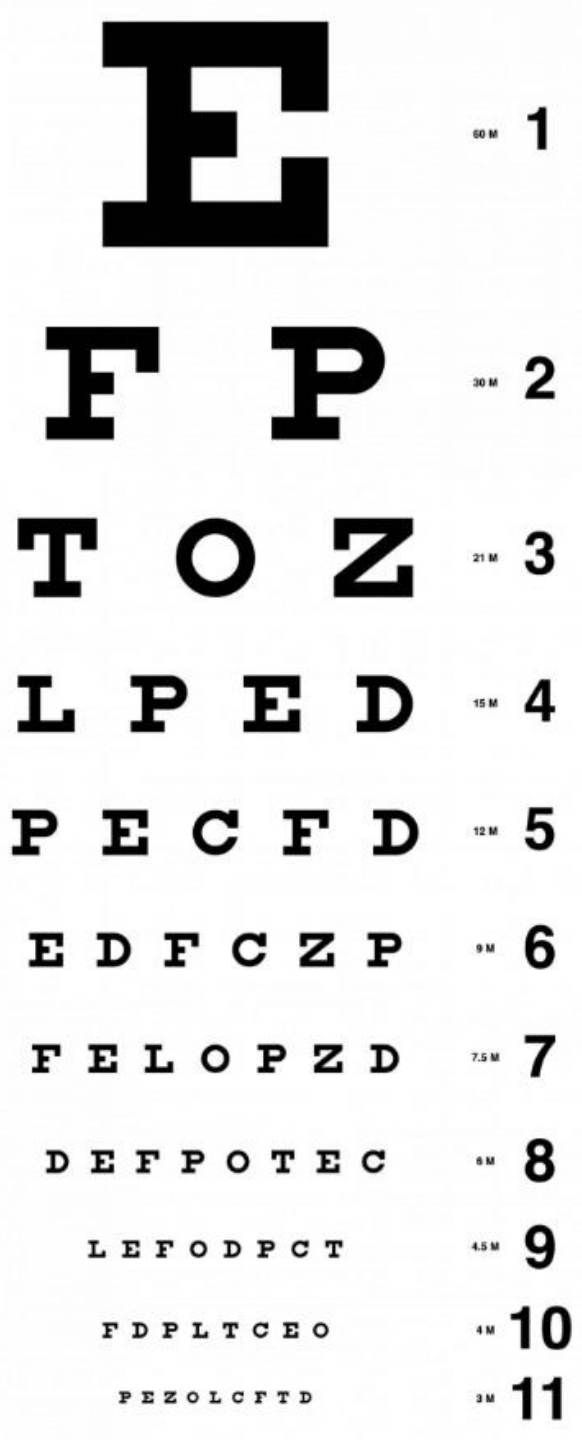
Table 3. Next Generation Sequencing (NGS) methods used for SRM 2391d testing

AFDIL MiSeq FGx	Verogen MiSeq FGx	Thermo Fisher Ion S5 XL	Promega MiSeq FGx	Qiagen MiSeq FGx
AFDIL mtGenome protocol [4] (mtDNAWhole Genome)	ForenSeq Signature Prep Kit	Precision ID GlobalFiler NGS STR Panel v2	PowerSeq 46GY System (prototype)	Human Mitochondrial Panel (mtDNA Whole Genome)
Literature-based assay	VEROGEN	Precision ID Ancestry Panel	PowerSeq CRM Nested System (mtDNA Control Region)	QIAGEN
		Precision ID Identity Panel	Promega	
		Ion Ampliseq DNA Phenotype Panel		
		Precision ID mtDNA Whole Genome Panel		

ThermoFisher SCIENTIFIC

Ring, J.D., et al., A performance evaluation of Nextera XT and KAPA HyperPlus for Rapid Illumina Library Preparation of Long-Range Mitogenome Amplicons; (2017) FSI G 29: 174–180

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	In-house Primers	ForenSeq	Precision ID GF	PowerSeq 46GY	CODIS 20	European Standard Set	Certified Value	Information Value	Total number Kits Tested	Total number (A-E extracted)	Total number E direct PCR
D1S1656					■	■	■	■	■	■	■						■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	■	X		21	18	3
D1S1677																													■		■			X		2	2	0
D2S1338	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■		X		26	21	5
D2S441					■	■	■	■	■	■	■						■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	■	X		20	17	3
D2S1360																										■										X	1	1	0
D2S1776																													■		■			X		2	2	0
D3S1358		■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	X		27	22	5
D3S1744																										■										X	1	1	0
D3S4529																													■		■			X		2	2	0
D4S2366																										■										X	1	1	0
D4S2408																													■	■	■	■			X		3	3	0
D5S818		■	■	■				■	■	■	■			■	■	■	■					■	■	■			■	■		■	■	■		X		19	14	5
D5S2500																										■										X	1	1	0
D5S2800																													■		■			X		2	2	0
D6S474																										■					■					X		2	2	0
D6S1043								■	■								■							■					■	■					X		6	5	1
D7S820	■	■	■	■				■	■	■	■			■	■	■	■					■	■	■			■	■		■	■	■		X		20	15	5
D7S1517																										■										X	1	1	0
D8S1132																										■										X	1	1	0
D8S1179		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	X		28	23	5
D9S1122																													■	■					X		2	2	0
D10S1248					■	■	■	■	■	■	■						■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	■	X		20	17	3
D10S2325																										■										X	1	1	0
D12S391					■	■	■	■	■	■	■						■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	■	X		22	19	3
D12ATA63																													■	■	■				X		2	2	0
D13S317	■	■	■	■				■	■	■	■			■	■	■	■					■	■	■			■	■		■	■	■		X		20	15	5
D14S1434																													■	■	■				X		2	2	0
D16S539	■	■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■		X		28	23	5
D17S1301																													■	■	■				X		2	2	0
D18S51	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	X		30	25	5
D19S433		■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■		X		25	20	5
D20S482																													■	■	■				X		2	2	0
D21S11	■	■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	X		28	23	5
D21S2055																										■										X	1	1	0
D22S1045					■	■	■	■	■	■	■							■	■	■	■	■	■	■	■		■	■		■	■	■	■	■	X		20	17	3
CSF1PO	■	■	■	■				■	■	■	■			■	■	■	■					■	■	■			■	■		■	■	■		X		20	15	5
F13A01													■																							X	1	1	0
F13B													■																							X	1	1	0
FESFPS													■																							X	1	1	0
FGA	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	X		29	24	5
LPL													■																							X	1	1	0
Penta C													■																							X	1	1	0
Penta D								■	■				■	■	■	■	■					■	■	■						■	■	■			X		13	11	2
Penta E								■	■				■	■	■	■	■					■	■	■						■	■	■			X		13	11	2
SE33						■	■			■	■							■	■	■	■		■		■	■	■	■							X		13	11	2
TH01		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	X		28	23	5
TPOX		■	■	■				■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	X		19	14	5
vWA		■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	X		27	22	5



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D1S1677																																		X		2	2	0
D2S1338																																		X		26	21	5
D2S441																																		X		20	17	3
D2S1360																																			X	1	1	0
D2S1776																																		X		2	2	0
D3S1358																																		X		27	22	5
D3S1744																																			X	1	1	0
D3S4529																																		X		2	2	0

18 CE-based typing kits

3 NGS-based typing kits

Component Example D1S1656	Genotype agreement observed for 18 CE-based typing kits
A	15.3, 18.3
B	13, 15.3
C	15, 16
D	15, 15.3, 16, 18.3
E	11, 16.3

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

			Coverage		ACR		Coverage		ACR		Coverage		ACR		Coverage		ACR	
	Locus	**2391d_A**	FS	ID	FS	ID	**2391d_B**	FS	ID	FS	ID	**2391d_C**	FS	ID	FS	ID	**2391d_E**	FS	ID	FS	ID
ForenSeq Only	rs1294331	CT					TT					CT					CT				
	rs13182883	GG					AG					AG					AG				
	rs1336071	CC					CC					CT					CC				
	rs2107612	AA					AG					AG					AA				
	rs2399332	GG					TT					GT					GT				
	rs279844	AT					AT					AA					AT				
	rs2920816	AG					AA					AG					AA				
	rs4606077	CC					CC					CT					CC				
	rs763869	AG					AG					AG					AG				
	rs8037429	CC					TT					CT					CC				
	rs8078417	CC					TT					CT					CT				

Coverage

	FS	ID
	> 2000	> 30,000
	2000 < = 1000	30,000 < = 20,000
	1000 < = 200	20,000 < = 10,000
	< 200	< 10,000

ACR
> 0.8
0.8 < = 0.6
0.6 < = 0.4
0.4 < = 0.2
< 0.2

Provided as information values

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

	Locus	2391d_A	Coverage FS	Coverage ID	ACR FS	ACR ID	2391d_B	Coverage FS	Coverage ID	ACR FS	ACR ID	2391d_C	Coverage FS	Coverage ID	ACR FS	ACR ID	2391d_E	Coverage FS	Coverage ID	ACR FS	ACR ID
FS	rs1919550	AA	●				AA	●				AA	●				AA	●			
	rs1042602	AC	●	●	●	●	CC	●	●			CC	●	●			CC	●	●		
	rs10497191	CC	●	●			TT	●	●			CT	●	●	◔	●	CT	●	●	●	●
	rs1079597	CC	●	●			TT	●	●			CC	●	●			CC	●	●		
	rs1110400	TT	●	●			TT	●	●			TT	●	●			TT	●	●		
	rs11547464	GG	●	●			GG	●	●			GG	●	●			GG	●	●		
	rs11652805	TT	●	●			CC	●	●			CT	●	●	●	●	TT	●	●		
	rs12203592	CC	●	●			CC	●	●			CC	●	●			CC	●	●		
	rs1229984	CC	●	●			CC	●	●			CC	●	●			CC	●	●		
	rs12439433	AA	●	●			AA	●	●			AA	●	●			AA	●	●		
	rs12498138	GG	●	●			GG	●	●			GG	●	●			GG	●	●		
	rs12821256	CC	●	●			TT	●	●			TT	●	●			TT	●	●		
	rs12896399	TT	●	●			GG	●	●			GT	●	●	●	●	GT	●	●	●	●
	rs12913832	AG	●	●	●	●	GG	●	●			AA	●	●			AG	●	●	◔	●
	rs1393350	GG	●	●			GG	●	●			GG	●	●			AG	●	●	◔	●

Coverage

	FS	ID
●	> 2000	> 20,000
●	2000 < = 1000	20,000 < = 10,000
●	1000 < = 200	10,000 < = 5,000
●	< 200	< 5,000

ACR	
●	> 0.8
◔	0.8 < = 0.6
◑	0.6 < = 0.4
◔	0.4 < = 0.2
○	< 0.2

Genotypes are reported not the application (eye color, ancestry)

Mitochondrial genome sequencing

NGS platform	Assay	Data Analysis Method
Ion Torrent S5XL	Precision ID mtDNA Whole Genome Panel	HID Genotyper Plugin v2.1 Converge v2.1
MiSeq FGx	Qiagen Human Mitochondrial Panel	GeneGlobe
MiSeq FGx	AFDIL mtGenome protocol	GeneMarker HTS v1.2.2 CLC bio Genomics Workbench v7/AQME
MiSeq FGx	Promega CRM Nested & WG	GeneMarker HTS v1.2.2

Heteroplasmy calling filter set at 5%

Can this material replace the SRM 2392 and 2392-I (Mitochondrial DNA standards?)

Your feedback is needed!

SRM 2391d Component E

Site	rCRS	Component E	Comment
73	A	G	
195	T	C	
263	A	G	
309.1	:	C	Insertion
315.1	:	C	Insertion
709	G	A	
750	A	G	
1438	A	G	
1888	G	A	
2706	A	G	
3107	C	:	Deletion
4216	T	C	
4464	G	A	
4769	A	G	
4917	A	G	
7028	C	T	
8697	G	A	
8860	A	G	
9100	A	G	
10463	T	C	
11251	A	G	
11719	G	A	
11812	A	G	
12408	T	C	
13368	G	A	
13965	T	C	
14233	A	G	
14766	C	T	
14905	G	A	
15326	A	G	
15452	C	A	
15607	A	G	
15928	G	A	
16126	T	C	
16294	C	T	
16519	T	C	

Mito Hg
A T2b3+151
B L1c1a+@198
C L1b1a
E T2a3

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

Material Details

SRM 2391d - PCR-Based DNA Profiling Standard

C - Certificate M - MSDS T - Table F - Data Files

Add Material to Cart

- C Certificate
- M Material Safety Data Sheet (MSDS)
- T Related Materials: 105.8 - DNA Profiling and Nucleic Acid Materials (solid forms)
- F Data and Information Files

Details	
Description:	PCR-Based DNA Profiling Standard
Lot:	N/A
Expiration Date:	6/4/2024
Unit Price * :	$945.00
Unit of Issue:	5 vials
Status:	Now Selling
Certificate Date:	6/21/2019
MSDS Date:	6/19/2019
Technical Contact:	Becky Steffen
Additional Information:	N/A

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 [9], 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.

Return to Material Details

Summary

The SRM 2391 series will continue to support the FBI-QAS and the validation and implementation of forensic marker systems

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

Certified allele calls supported by sequence data and CE-length based measurements

What else is going on?

SWGDAM Survey to determine the interest in eliminating the human DNA quantitation requirement for casework samples

SWGDAM survey to determine the interest in eliminating the DNA quantitation requirement for casework samples

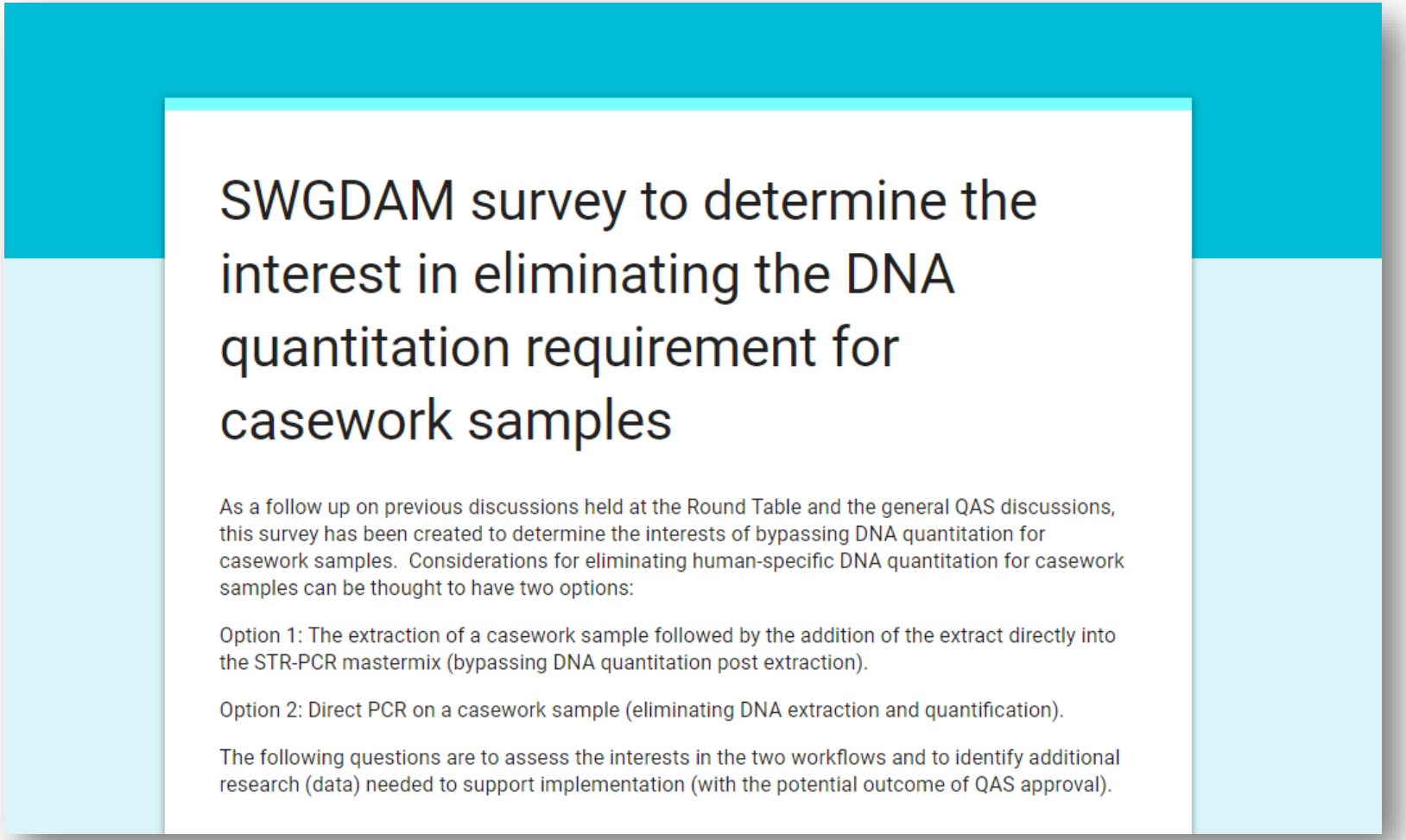
Why are we discussing elimination of quant for casework samples?

Follow up on previous discussions held at the SWGDAM Round Table and the general QAS discussions

Considerations for eliminating human-specific DNA quantitation for casework samples can be thought to have two options:

- **Option 1**: The extraction of a casework sample followed by the addition of the extract directly into the STR-PCR mastermix (eliminating DNA quantitation post extraction).

- **Option 2**: Direct PCR on a casework sample (eliminating DNA extraction and quantification).



Survey link was emailed to SWGDAM Members

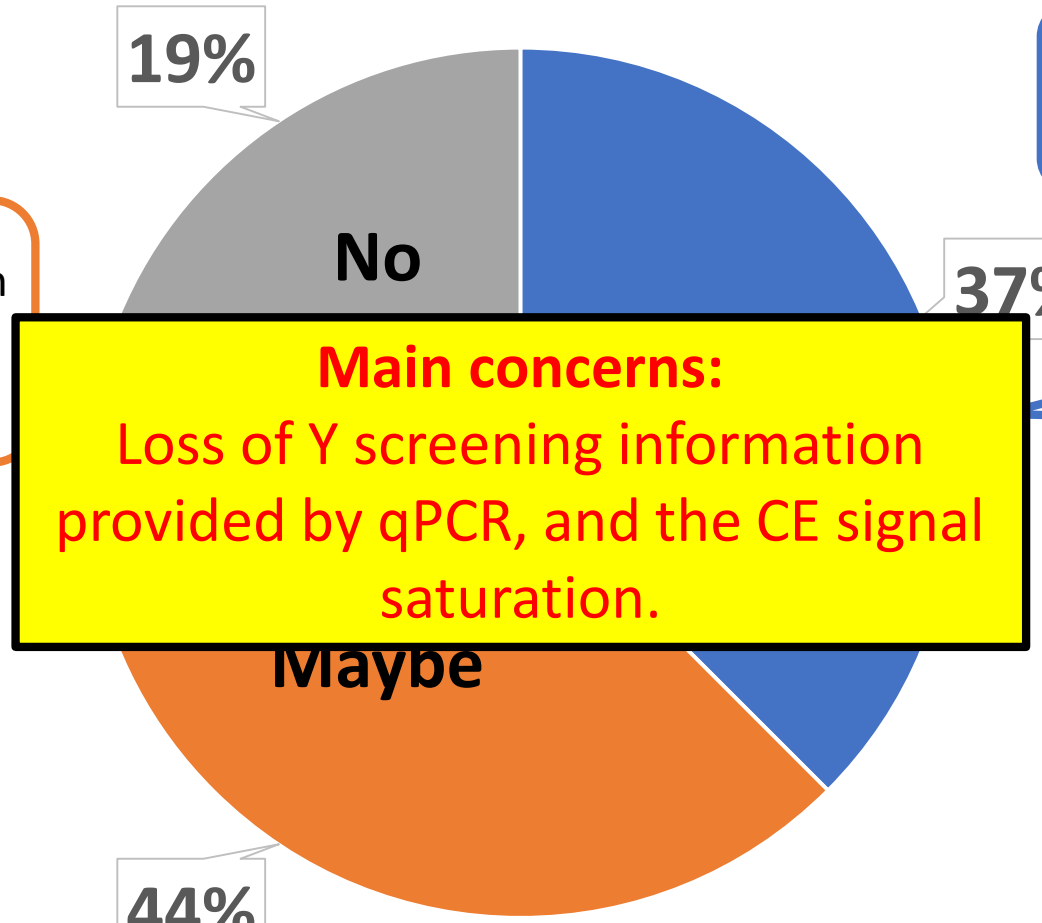
Members were able to respond from June 17th to July 8th.

Results were presented at the July SWGDAM Round Table Discussion

32 responses

n=32 responses

Does your lab have an interest in implementing Option 1 (extraction, but no quantitation)?



Yes, but only for certain sample types based upon validation.

Maybe skipping quant on samples you know are limited in quantity

Yes, on presumed single source samples with sufficient quantity of sample e.g. blood

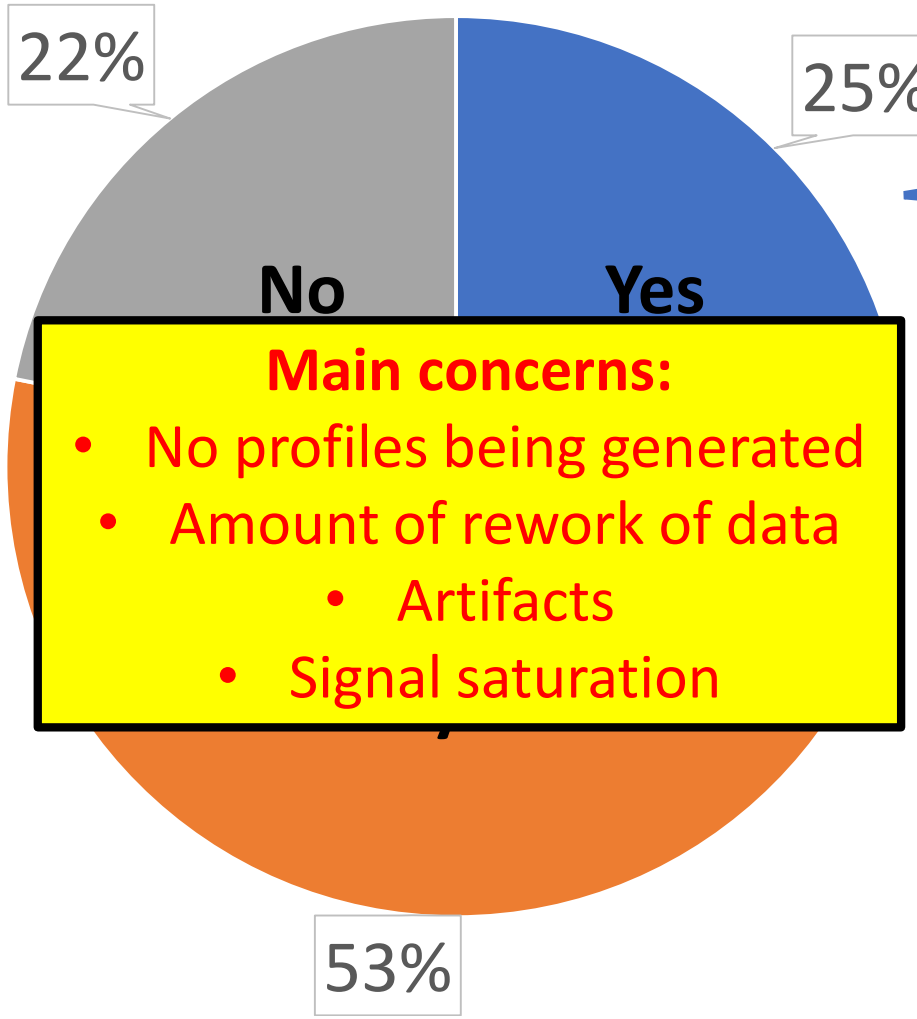
Main concerns:
Loss of Y screening information provided by qPCR, and the CE signal saturation.

Yes, for certain types of samples, that are allowed for in policy

Yes	12
Maybe	14
No	6

n=32 responses

Does your lab have an interest in implementing Option 2 (direct PCR)?



Currently perform LMD on sperm in SAKs

Main concerns:

- No profiles being generated
- Amount of rework of data
- Artifacts
- Signal saturation

Yes	8
Maybe	17
No	7

Differences from Option 1:

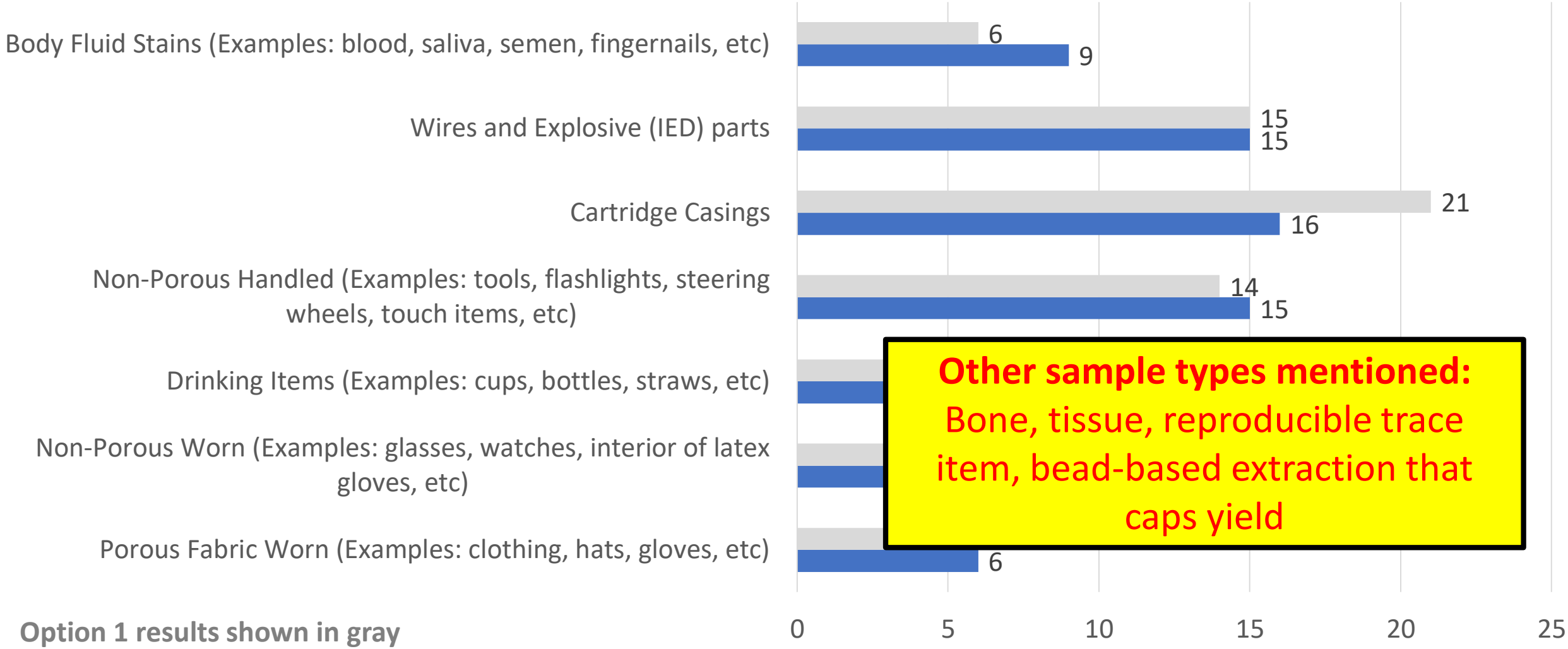
Yes to No: 4

Yes to Maybe: 2

Maybe to No: 2

n=26 responses

What type of casework samples would benefit? (Check all that apply)



Other sample types mentioned:
Bone, tissue, reproducible trace item, bead-based extraction that caps yield

Option 1 results shown in gray

Sample Loss

- False negatives
- Retesting

Artifacts

- Increase in artifacts
- Non-specific amp products

Additional Time

- Rework of samples
- Not stopping at quant

Data Reliability

- False negatives
- Inhibition
- Amp failures

Sample Usage

- Splitting between autosomal and YSTR typing

Blown Out Data

- Rework of samples
- Troubleshooting problems

Lack of Male Specific Info

- Affect SAK interpretation

Questions Raised:

What is the added value/gains from quant?
This seems to be the main question at hand.

Moving forward in the process?

Determining male DNA in sexual assault kits?

Identifying inhibition?

Are current chemistries for direct PCR optimized for casework samples?

Buccal sample processing =/= to casework.

Observations from the Round Table:

More comfortable as a group with Option 1

Those who were emphatic about eliminating quant want to be able to do direct PCR

- The **loss of DNA in extraction is the main problem**, not the loss of 2 µL into qPCR

Studies need to be done to move forward

- Start with review of existing casework data
- This allows for targeted path forward for study design

Moving Forward

NIST would like to collect data from you!

An excel template for data has been generated

- For consistency of data reported
- To make analysis easier

Collection	
Item Type (i.e. shirt, steering wheel)	DNA Source (touch or wearer)

Goal: use pre-existing casework data to identify trends in sample types

If willing to participate, please email me

Erica.Romsos@nist.gov and I will send the template

Moving Forward

NIST would like to collect data from you!

An excel template for data has been generated

- For consistency of data reported
- To make analysis easier

Collection		Quant Information				
Item Type (i.e. shirt, steering wheel)	DNA Source (touch or wearer)	Quant (total human)	Quant (Male)	Auto:Male Ratio	Degradation Index	Dropped at Quant?

Goal: use pre-existing casework data to identify trends in sample types

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Moving Forward

NIST would like to collect data from you!

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Collection		Quant Information					STR Typing			Results			
Item Type (i.e. shirt, steering wheel)	DNA Source (touch or wearer)	Quant (total human)	Quant (Male)	Auto:Male Ratio	Degradation Index	Dropped at Quant?	Amp volume	Interpretable results Y or N?	STR Chemistry	Results	total # called alleles	Calculated LR	CODIS eligible profile(s)?

Goal: use pre-existing casework data to identify trends in sample types

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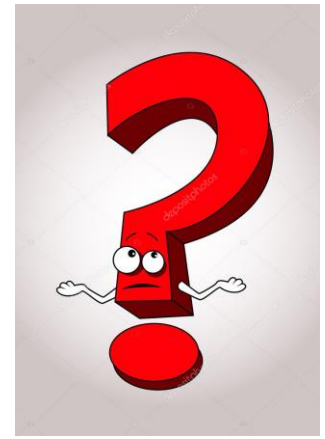
Summary

SRM 2391d: PCR-Based DNA Profiling Standard is the most ***comprehensive*** forensic SRM yet

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

NIST is asking for laboratory support in collecting data to examine a targeted path forward in the “no-quant” discussion.

Questions??



SHOULD YOU ASK A QUESTION IN LECTURE?

ARE YOU ACTUALLY INTERESTED TO KNOW THE ANSWER?
- NAH → SHUT UP
- I REALLY AM! → IS QUESTION REALLY DUMB?
  - PROBABLY → LOOK IT UP ON GOOGLE
  - NOT PARTICULARLY → DOES QUESTION INVOLVE PROFESSOR'S RESEARCH?
    - YES → IS PROFESSOR AN ARROGANT PRICK?
      - HE LOVES HIMSELF → IS THERE AT LEAST 30 MIN LEFT OF LECTURE?
        - YES → ASK AWAY!
        - NO → LET US LEAVE SO WE CAN EAT + PEE!
      - NO → ASK AWAY!
    - NO → IS THE LECTURE ALLOTTED TIME ALMOST OVER?
      - 30 SECONDS LEFT → LET US LEAVE SO WE CAN EAT + PEE!
      - NO → IS IT POSSIBLE THE QUESTION WAS ANSWERED WHEN YOU WERE SLEEPING 5 MIN AGO?
        - VERY LIKELY → WAIT FOR THE TRANSCRIPT
        - NO → I'VE HAD TOO MUCH COFFEE TO SLEEP → IS THE ANSWER TO THE QUESTION ONE OTHERS MIGHT CARE ABOUT?
          - MAYBE → DO YOU HAVE A QUESTION OR JUST A COMMENT TO MAKE YOU LOOK SMART?
            - WELL, HOW ELSE WILL HE KNOW? → ARE YOU ACTUALLY INTERESTED TO KNOW THE ANSWER?
            - NO → DO YOU REALLY LOVE HEARING YOURSELF TALK?
              - NO → GO BACK TO SLEEP
              - I DO! → AS IF ANYONE COULD STOP YOU, BRAD
          - UNLIKELY → ARE YOU ACTUALLY INTERESTED TO KNOW THE ANSWER?

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