Update of NIST Standard Reference Material (SRM) 2391c: PCR-Based DNA Profiling Standard What is new?

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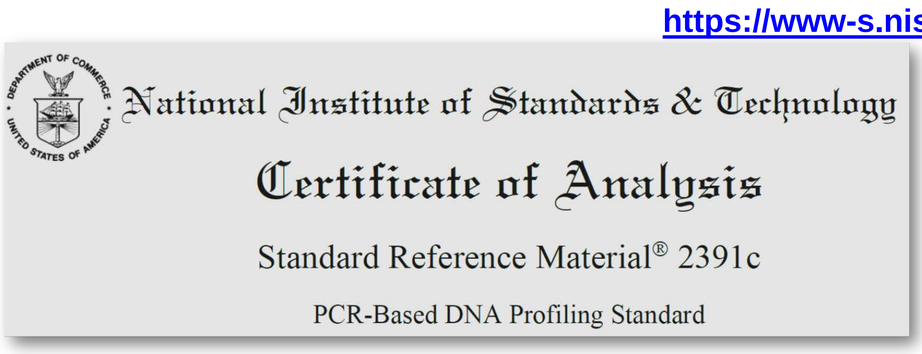
The NIST Standard Reference Material (SRM) 2391c: PCR-Based DNA Profiling Standard was updated in April 2015 to contain new information relevant to the forensic community. Previously, there were certified genotypes for 24 autosomal STR markers plus Amelogenin and 17 Y-STR markers. Due to the increase in markers present in larger commercial autosomal STR and Y-STR multiplex kits recently released, there is a need to add certified types for these new markers for each component of SRM 2391c (Components A-F). The updated Certificate of Analysis has certified values for 1 additional autosomal STR marker (D6S1043) and 12 additional Y-STR markers (29 total) as well as informational values for X-STR markers (12 total) and Insertions and Deletions (Indels) (30 total). Also, the number of STR multiplex assays tested increased from 24 to 43. Sanger sequencing was performed on Components A-C, E and F (Component D is a mixture of Components A and C) to determine the STR repeat motifs and to characterize adjacent flanking regions and underlying polymorphisms (sequence, insertion-deletion, variation in complex motifs) typically not detected by fragment-based typing. The sequenced regions include the commercial or known PCR binding sites commonly implemented in fragment-based typing. The certified, reference, and information values are presented. In addition, SRM 2391c can be used to establish traceability in a laboratory. The simple steps to achieve traceability with SRM 2391c will be explained.

SRM 2391c Certificate of Analysis was updated to include all commercially available autosomal STR, Y-STR, X-STR, and INDEL markers in April 2015:
https://www-s.nist.gov/srmors/certificates/view_certGIF.cfm?certificate=2391C



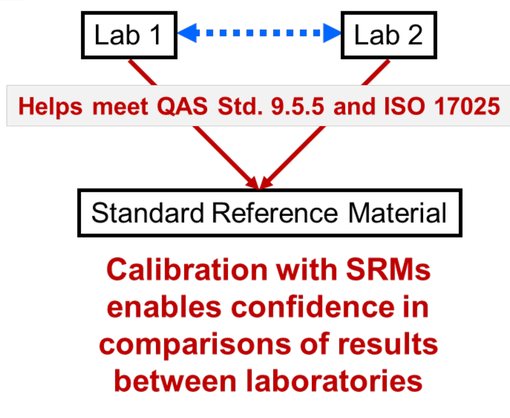
National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2391c

PCR-Based DNA Profiling Standard

Information updated April 2015



SRM 2391c is intended primarily for use:

- Standardization of forensic and paternity quality assurance procedures for PCR-based genetic testing
- Instructional law enforcement or non-clinical research purposes
- Quality assurance and traceability when assigning values to in-house control materials

SRM 2391c Components A-F:

- Components A through D are DNA extracts in liquid form (50 µL volume, 1.1 – 2.1 ng/µL)
- Components E and F are DNA spotted on 903 paper or FTA paper (Two 6 mm punches, 7.5 × 10^4 cells per punch)
- Component D is a 3:1 mixture of Components A and C DNA (50 µL volume, 1.1 – 2.1 ng/µL)

Table 1. Description of Components in SRM 2391c

Component		Description	Amount	Concentration[a]
A	Single-source	Anonymous single-source female genomic DNA in TE^{-4} buffer	50 µL	1.1 – 2.1 ng/µL
B		Anonymous single-source male genomic DNA in TE^{-4} buffer	50 µL	1.1 – 2.1 ng/µL
C		Anonymous single-source male genomic DNA in TE^{-4} buffer	50 µL	1.1 – 2.1 ng/µL
D	Mixture	Mixed-source (Components A and C) genomic DNA in TE^{-4} buffer	50 µL	1.1 – 2.1 ng/µL
E	Stain	Anonymous single-source female cells spotted on 903 paper	Two 6 mm punches	7.5×10^4 cells per punch
F	Stain	Anonymous single-source male cells spotted on FTA paper	Two 6 mm punches	7.5×10^4 cells per punch

[a]DNA concentrations and cell counts are nominal values and are not intended for use as quantitative standards.

Certified Values

- A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account.
- Certified genotypes/haplotypes have been assigned by electrophoretic base pair (bp) size matched to sequenced alleles and also confirmed by direct Sanger sequencing
- There are 25 certified autosomal STR plus the sex-typing marker Amelogenin genotypes and 29 certified Y-STR haplotypes of the six components

Reference Values

- A NIST reference value is a high-confidence estimate of the true value, but where all possible sources of bias have *not* been fully investigated by NIST
- Reference values have been assigned from repeat counts based on electrophoretic base pair size differences between non-sequenced alleles compared to sequenced alleles
- There are 26 reference autosomal STR genotypes of the six components

Information Values

- A NIST information value is data that may be of interest and use to the SRM user, but insufficient information is available to assess the confidence of the assignment
- Information values have been assigned to the DNA concentrations of Components A-D, the number of cells per paper punch of Components E and F, and the genotypes when typed with only one kit/set of primers
- There is one autosomal STR, 12 X-STR, and 30 Insertion/Deletion (Indel) information genotypes/haplotypes of the six components

STR Genotyping kits and Primer Mixes

Kit Provider			Primer Mixes
Thermo Fisher (14) *Foster City, CA*	Promega Corp. (16) *Madison, WI*	Qiagen Inc. (11) *Hilden, Germany*	NIST (2)
Identifiler	PowerPlex 16	ESSplex	26plex
Identifiler Plus	PowerPlex 16 HS	IDplex	miniSTRs
NGM	PowerPlex ESX 17	ESSplex SE	
NGM SElect	PowerPlex ESI 17	ESSplex SE Plus	
COfiler	PowerPlex ES	ESSplex SE GO!	
Profiler	PowerPlex S5	IDplex Plus	
Profiler Plus	PowerPlex Y	IDplex GO!	
Profiler Plus ID	FFFL	24plex	New kits added in update
SGM Plus	PowerPlex ESI 17 Pro	24plex GO!	
SEfiler	PowerPlex ESX 17 Fast	Argus X-12	
MiniFiler	PowerPlex ESI 17 Fast	DIPplex	
YFiler	PowerPlex 18D		
GlobalFiler	PowerPlex 21		
YFiler Plus	PowerPlex CS7		
	PowerPlex Fusion		
	PowerPlex Y23		

Updated Genotypes and Haplotypes for SRM 2391c

New information shaded in gray

Certified Autosomal STR Genotypes

Locus	Component					
	A	B	C	D	E	F
D1S1656	17.3,17.3	11,14	11,15	11,15,17.3	11,16.3	17.3,17.3
D2S1338	18,23	17,17	19,19	18,19,23	19,20	17,17
D2S441	10,10	10,14	10,10	10	10,10	14,14
D3S1358	15,16	15,19	16,18	15,16,18	14,15	16,17
D5S818	11,12	12,13	10,11	10,11,12	11,13	11,13
D6S1043	11,18	14,19	11,14	11,14,18	11,11	11,16
D7S820	11,11	10,10	10,12	10,11,12	8,10	8,12
D8S1179	13,14	10,13	10,17	10,13,14,17	11,13	10,13
D8S1115	15,16	15,17	9,9	9,15,16	9,16	9,17
D10S1248	15,16	13,13	12,16	12,15,16	14,14	14,15
D12S391	18.3,22	19,24	19,23	18.3,19,22,23	17,22	18,19
D13S317	8,8	9,12	11,11	8,11	8,12	8,11
D16S539	10,11	10,13	10,10	10,11	11,12	9,11
D18S51	12,15	13,16	16,19	12,15,16,19	14,17	17,22
D19S433	13,14	16,16.2	13.2,15.2	13,13.2,14,15.2	14,14	13,14
D21S11	28,32.2	32,32.2	29,30	28,29,30,32.2	29,30	29,32.2
D22S1045	15,15	15,17	16,16	15,16	16,17	11,15
CSF1PO	10,10	10,11	10,12	10,12	10,11	10,11
FGA	21,23	20,23	24,26	21,23,24,26	20,23	21,25
Penta D	9,13	8,12	10,11	9,10,11,13	14,14	9,10
Penta E	5,10	7,15	12,13	5,10,12,13	13,19	11,15
SE33	16,18	17,18	28.2,31.2	16,18,28.2,31.2	22,30.2	12,21
TH01	8,9.3	6,9.3	6,8	6,8,9.3	6,9.3	7,9.3
TPOX	8,8	8,11	11,11	8,11	8,11	8,8
vWA	18,19	17,18	16,18	16,18,19	17,18	16,18
Amelogenin	X,X	X,Y	X,Y	X,Y	X,X	X,Y

Certified Y-STR Haplotypes

Locus	Component*			
	B	C	D	F
DYS19	14	15	15	17
DYS385a	13	13	13	12
DYS385b	17	15	15	16
DYS389I	13	12	12	13
DYS389II	31	27	27	30
DYS390	23	24	24	24
DYS391	10	11	11	12
DYS392	11	13	13	11
DYS393	12	13	13	13
DYS437	14	16	16	15
DYS438	10	11	11	10
DYS439	11	12	12	11
DYS448	20	19	19	20
DYS449	26	29	29	30
DYS456	15	15	15	15
DYS458	17.2	17	17	18
DYS460	10	10	10	10
DYS481	25	26	26	25
DYS518	38	39	39	39
DYS533	11	10	10	11
DYS549	12	13	13	11
DYS570	18	20	20	17
DYS576	17	16	16	19
DYS627	22	21	21	21
DYS635	20	21	21	21
DYS643	9	12	12	13
DYF387S1a	35	36	36	35
DYF387S1b	38	38	38	39
Y GATA H4	11	11	11	11

*A and E are females are therefore not listed

Reference Autosomal STR Genotypes

Locus	Component					
	A	B	C	D	E	F
D1GATA113	12,12	12,12	7,12	7,12	12,12	7,13
D1S1627	13,14	11,14	14,14	13,14	13,14	13,14
D1S1677	13,15	12,13	14,15	13,14,15	14,16	15,15
D2S1776	12,12	9,12	12,13	12,13	9,11	11,11
D3S3053	9,11	11,12	9,11	9,11	9,11	11,11
D3S4529	14,16	13,14	13,15	13,14,15,16	13,16	12,15
D4S2364	9,10	8,9	9,9	9,10	9,10	10,10
D4S2408	8,9	9,10	8,8	8,9	8,8	8,11
D5S2500	18,18	17,17	14,14	14,18	17,17	17,17
D6S1017	8,10	8,10	8,10	8,10	10,13	12,12
D6S474	16,18	14,15	14,15	14,15,16,18	14,16	14,18
D9S1122	11,12	11,13	10,10	10,11,12	11,11	12,13
D9S2157	7,11	12,15	13,15	7,11,13,15	11,11	9,11
D10S1435	11,14	12,14	11,12	11,12,14	12,13	12,13
D11S4463	13,14	13,14	13,14	13,14	14,15	14,17
D12ATA63	13,15	15,17	12,12	12,13,15	12,17	12,15
D14S1434	10,14	10,14	13,14	10,13,14	10,14	13,14
D17S1301	11,13	10,10	12,12	11,12,13	11,14	12,12
D17S974	10,11	9,11	9,11	9,10,11	9,10	10,10
D18S853	11,13	11,14	11,15	11,13,15	11,14	11,12
D20S1082	11,14	11,15	11,15	11,14,15	11,15	11,15
D20S482	14,15	13,14	13,15	13,14,15	15,15	14,15
F13A01	4,5	3.2,7	5,6	4,5,6	5,7	5,6
F13B	8,9	9,10	10,10	8,9,10	9,10	8,10
FESFPS	12,12	11,11	11,13	11,12,13	11,12	10,11
LPL	10,11	10,10	10,12	10,11,12	10,11	10,12

Information Autosomal STR Genotypes

Locus	Component					
	A	B	C	D	E	F
Penta C	11,12	12,13	5,9	5,9,11,12	12,13	12,12

Information X-STR Genotypes

Locus	Component					
	A	B*	C*	D	E	F*
DXS7132	11,14	14	12	11,12,14	14,15	14
DXS7423	14,14	14	14	14	13,14	15
DXS8378	11,12	12	11	11,12	12,13	11
DXS10074	16,18	15	17	16,17,18	16,17	7
DXS10079	19,20	20	20	19,20	18,21	19
DXS10101	33.2,34	32	29.2	29.2,33.2,34	27.2,29.2	28.2
DXS10103	18,19	16	19	18,19	19,19	19
DXS10134	36,39.3	37	38	36,38,39.3	36,36	36
DXS10135	19,23	23	20	19,20,23	19,22	24
DXS10146	26,28	29	27	26,27,28	29,30	27
DXS10148	25.1,26.1	24.1	18	18,25.1,26.1	18,28.1	25.1
HPRTB	13,14	14	12	12,13,14	11,11	11

*B, C and F are males and do not have a second X chromosome

Information Insertion/Deletion Genotypes

Locus	Component					
	A	B	C	D	E	F
D6	+	+	-/+	-/+	-/+	+
D39	-	-	-	-	-/+	-
D40	-	-/+	-/+	-/+	-/+	+
D45	+	-/+	-	-/+	-	+
D48	-	-/+	-	-	-/+	+
D56	-/+	+	-	-/+	+	+
D58	-/+	-/+	-	-/+	+	-/+
D64	-/+	+	+	-/+	-	-/+
D67	-/+	-/+	+	-/+	+	-/+
D70	-/+	+	+	-/+	-	-/+
D77	-/+	-	-/+	-/+	-	-
D81	-	-	+	-/+	-	-
D83	-/+	-	-	-/+	-/+	-/+
D84	-/+	-/+	+	-/+	-	-/+
D88	-/+	-/+	-/+	-/+	-	-/+
D92	+	-/+	+	+	+	+
D93	+	+	+	+	-	-/+
D97	-	+	-	-	+	-/+
D99	+	+	+	+	-	+
D101	-/+	-	-/+	-/+	-	+
D111	+	-/+	-/+	-/+	-/+	+
D114	-/+	-/+	-	-/+	-/+	-
D118	-	-/+	-/+	-/+	-/+	+
D122	-/+	-/+	-	-/+	-/+	+
D124	-/+	-/+	-/+	-/+	-/+	+
D125	-	+	-/+	-/+	-	+
D128	+	+	+	+	-/+	-/+
D131	-/+	-/+	-/+	-/+	-/+	+
D133	-	-	-/+	-/+	-	-/+
D136	+	-/+	-/+	-/+	+	+

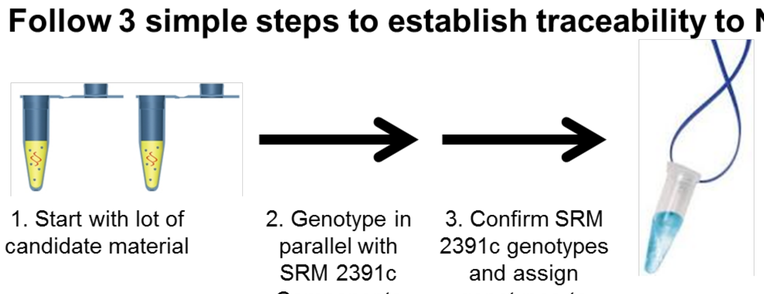
What is New?

- One autosomal STR marker was added (D6S1043) with **certified** genotypes
- 12 Y-STR markers were added (DYS449, DYS460, DYS481, DYS518, DYS533, DYS549, DYS570, DYS576, DYS627, DYS643, DYF387S1a and DYF387SIb) with **certified** haplotypes
- No new **reference** genotypes or haplotypes were added
- 12 X-STR markers were added with **information** genotypes/haplotypes
- 30 Indel markers were added with **information** genotypes
- 19 new STR multiplex kits were added

Establishing Traceability to NIST SRM 2391c

- 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 autosomal STR markers, the reference material is SRM 2391c
- Materials deemed traceable to NIST-created materials must have a record associated with them.

Follow 3 simple steps to establish traceability to NIST SRM 2391c



1. Start with lot of candidate material
2. Genotype in parallel with SRM 2391c Components
3. Confirm SRM 2391c genotypes and assign genotypes to candidate material

The in-house material is now NIST traceable

Sanger Sequencing for Certified Values

- Sanger sequencing was performed to determine the STR repeat regions and certify the genotypes and haplotypes of all commercially available autosomal STR and Y-STR markers (54 total) for Components A-C, E and F (Component D is a 3:1 mixture of A and C)
- Sequencing also characterized adjacent flanking regions and underlying polymorphisms (sequence, insertion-deletion, variation in complex motifs) typically not detected by fragment-based typing
- Sequence motifs for certified values of Components A-C, E and F are included in the Certificate of Analysis
- The Sanger sequencing data supports the high throughput next generation sequencing technologies at NIST for forensic typing applications

Poster available for download from STRBase
http://www.cstl.nist.gov/biotech/strbase/pub_pres/SteffenISFG2015SRM2391cposter.pdf