



Certificate of Analysis

Standard Reference Material[®] 2391d

PCR-Based DNA Profiling Standard

This Standard Reference Material (SRM) is intended primarily for use in the standardization of forensic and paternity quality assurance procedures for polymerase chain reaction (PCR)-based genetic testing, for instructional law enforcement or non-clinical research purposes, and for quality assurance when assigning values to in-house control materials. It is not intended for any human or animal clinical diagnostic use. This SRM is composed of well-characterized human deoxyribonucleic acid (DNA) in two forms; genomic DNA (Components A through D) and DNA to be extracted from cells that have been spotted onto FTA paper (Component E). The complete listing of Components is included in Table 1. A unit of SRM 2391d is composed of one vial of each of five components packaged together in one box.

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 [1]. The certified values for the components in SRM 2391d were derived from a combination of Capillary Electrophoresis (CE) and Next Generation Sequencing (NGS)-based characterizations that allowed for the counting and direct sequencing of short tandem repeats (STRs) at a locus. High confidence allele calls were established by using multiple PCR-based STR typing kits and NGS-based kits and technologies. The STR allele calls certified in SRM 2391d are traceable to the natural unit count one by virtue of counting the number of repeat units through the analysis of CE and NGS data [2]. Tables 2 and 3 list CE and NGS-based methods used for characterization of the autosomal STR loci, Y-STR loci, and X-STR loci.

Table 4 lists genotypes for 35 Certified autosomal STR loci plus the sex-typing locus Amelogenin. Table 5 lists Certified haplotypes for 28 Certified Y-STR loci. Table 6 lists Certified genotypes/haplotypes for seven X-STR loci.

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 highest confidence of the assignment [1]. Information Values have been assigned to the DNA concentrations of Components A through D (Table 1), the number of cells per paper punch of Component E (Table 1), and the genotypes/haplotypes (when typed exclusively with CE methods) of the five Components at 13 autosomal STR loci (Table 7), three Y-STR loci (Table 8), five X-STR loci (Table 9), 30 Insertion and Deletion (Indel) loci (Table 10), and 20 Insertion and Null allele (INNUL) loci (Table 11). Information Values are also assigned to 101 autosomal identity single nucleotide polymorphism (SNP) loci, 34 Y-SNP loci, 188 autosomal ancestry and phenotype SNP loci, and whole mitochondrial genome DNA (mtDNA) sequences determined by sequencing.

Expiration of Certification: The certification of **SRM 2391d** is valid, within the measurement uncertainty specified, until **04 June 2024**, provided the SRM is handled and stored in accordance with the instructions given in this certification (see “Instructions for Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Overall direction and coordination of the technical activities leading to certification were under the leadership of C.R. Steffen of the NIST Biomolecular Measurement Division.

Analytical determinations and technical measurements leading to the certification of this SRM were performed by E.L. Romsos, A. Tona, L.A. Borsuk, K.M. Kiesler, S. Riman, and K.B. Gettings of the NIST Biomolecular Measurement and Biosystems and Biomaterials Divisions.

Statistical consultation was provided by H.K. Iyer of the NIST Statistical Engineering Division.

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Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNINGS TO USER

Warning: SRM 2391d components are human source materials. The suppliers of the source materials used to prepare this product found the materials to be non-reactive when tested for hepatitis B surface antigen (HBsAg), human immunodeficiency virus (HIV), hepatitis C virus (HCV), and human immunodeficiency virus 1 antigen (HIV-1Ag) by Food and Drug Administration (FDA) licensed tests. However, because no test method can offer complete assurance that HIV, hepatitis viruses, or other infectious agents are absent, this SRM should be handled at the Biosafety Level 1 for any potentially infectious human serum or blood specimen [3]. SRM 2391d components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

Storage: Store refrigerated at a temperature range of 2 °C to 8 °C (do not freeze).

INSTRUCTIONS FOR USE

Vials for Components A through D should be briefly vortexed and centrifuged prior to opening. After opening the vials, sample aliquots for analysis should be withdrawn immediately and processed without delay for the certified values to be applicable. Component E, cells on FTA paper, should be washed to reduce PCR inhibitors and salts prior to PCR amplification.

SOURCE AND ANALYSIS⁽¹⁾

Source of Material: The human genomic DNA extracts prepared at NIST for Components A through C were derived from buffy coat white blood cells from single source anonymous donors under the approval of the NIST Human Subjects Protection Office. The cell line used for Component E was obtained from American Type Culture Collection (Manassas, VA) under license and material transfer agreements. All source materials have been tested and found negative for HBsAg, HIV, HCV, and HIV-1Ag before use.

Interlaboratory Study: Four laboratories participated in the characterization of the material comprising SRM 2391d including Promega Corporation (Madison, WI), Qiagen (Germantown, MD), Verogen (San Diego, CA), and the Armed Forces DNA Identification Laboratory (AFDIL) (Dover, DE).

Description of Components: Five components are included in each unit. Components A through D each contain 55 µL of extracted genomic DNA in TE⁻⁴ buffer, pH 8.0, and are packaged in perfluoroalkoxy fluoropolymer (PFA) vials. Table 1 lists the DNA concentration and expanded uncertainty for the Components based upon results from droplet digital polymerase chain reaction (ddPCR). Component E contains two 6 mm punches of FTA paper. Each punch was prepared to hold approximately 7.5×10^4 cells. Component E is packaged in sterile 0.5 mL polypropylene vials. A detailed description of the individual components in SRM 2391d is listed in Table 1. Note that SRM 2391d is modified from SRM 2391c in that Components A through D are different samples with different profiles; however, Component E remains the same.

⁽¹⁾Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1. Description of Components in SRM 2391d

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.

Table 2 lists the commercial CE multiplex kits used for SRM 2391d testing. Commercial companies include Thermo Fisher (Waltham, MA), Promega Corporation, Qiagen, and InnoGenomics (New Orleans, LA). Table 3 lists the NGS methods used for SRM 2391d testing. Commercial companies include Thermo Fisher, Promega Corporation, Qiagen, and Verogen. Additional mtDNA sequencing was carried out using a protocol published by AFDIL [4].

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 SSelect	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		

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] (mtDNA Whole Genome)	ForenSeq Signature Prep Kit	Precision ID GlobalFiler NGS STR Panel v2	PowerSeq 46GY System (prototype)	Human Mitochondrial Panel (mtDNA Whole Genome)
		Precision ID Ancestry Panel	PowerSeq CRM Nested System (mtDNA Control Region)	
		Precision ID Identity Panel		
		Ion Ampliseq DNA Phenotype Panel		
		Precision ID mtDNA Whole Genome Panel		

The genotypes/haplotypes for this SRM are listed in Tables 4 through 11. The Certified Values for 35 autosomal STR loci plus Amelogenin are provided in Table 4. The Certified Values for 28 Y-STR loci are provided in Table 5. The Certified Values for seven X-STR loci are provided in Table 6. The Information Values for 13 autosomal STR loci are provided in Table 7. The Information Values for three Y-STR loci are provided in Table 8. The Information Values for five X-STR loci are provided in Table 9. The Information Values for 30 Indels are provided in Table 10. The Information Values for 20 INNULS are provided in Table 11.

Location of Data: The Certified STR sequences for Components A, B, C, and E, including NGS length-based allele calls, STRSeq ID [9], bracketed repeats, and full sequence strings (5' flank, repeat region, and 3' flank) are provided at https://www-s.nist.gov/srmors/view_detail.cfm?srm=2391D under Material Details, Data and Information Files in the "SRM 2391d_STRSeqID.xlsx" Excel file.

The Information Values for SNP loci of forensic interest for Components A, B, C, and E are provided at https://www-s.nist.gov/srmors/view_detail.cfm?srm=2391D under Material Details, Data and Information Files in "SRM 2391d_AISNP-PISNP.xlsx" and "SRM 2391d_IISNP.xlsx" Excel files.

The Information Values for the mtDNA whole genome sequences for Components A, B, C, and E are provided at https://www-s.nist.gov/srmors/view_detail.cfm?srm=2391D under Material Details, Data and Information Files in the "SRM 2391d_mtDNA.xlsx" Excel file.

The sequences for STR alleles, SNP alleles, and mtDNA whole genomes for Component D are not listed and should be inferred as a combination of Component A and Component C.

Table 4. Certified Genotypes, 35 Autosomal STR Loci and Amelogenin

Locus	Component				
	A	B	C	D	E
AMEL	X,X	X,Y	X,Y	X,Y	X,X
CSFIPO	12,14	12,12	10,11	10,11,12,14	10,11
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
D2S441	11,11	11,11	11,14	11,14	10,10
D2S1338	25,25	17,23	23,24	23,24,25	19,20
D2S1776	10,10	9,11	10,12	10,12	9,11
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
D5S818	10,11	12,12	13,15	10,11,13,15	11,13
D5S2800	14,17	14,17	14,18	14,17,18	17,17
D6S474 ^(a)	16,18	14,16	14,18	14,16,18	14,16
D6S1043	12,19	13,18	11,18	11,12,18,19	11,11
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
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
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
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 ^(b) ,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

^(a) When typing D6S474 with the Investigator HDplex kit from Qiagen the resulting allele calls are reported to be one repeat unit less (i.e. Component A (15,17), Component B (13,15), Component C (13,17), Component D (13,15,17), and Component E (13,15)) due to a difference in the nomenclature as described in [5-7].

^(b) A 17 allele is reported for Component B at SE33 using all commercial CE multiplex kits tested; however, the certified sequence reveals 18 repeats with a 4 base pair (AAAA) deletion 85 base pairs upstream from the repeat.

Table 5. Certified Haplotypes, 28 Y-STR Loci

Locus	Component ^(a)		
	B	C	D
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^(b)	34	34	34
DYS635	21	21	21
DYS643	15	14	14
DYF387S1	36,38	36,39	36,39
YGATAH4	13	12	12

^(a) Components A and E do not have a Y-chromosome (female) and are not included in this table.

^(b) When typing DYS612 for Components B, C, and D with the ForenSeq DNA Signature Prep Kit from Verogen the resulting allele call is reported to be six repeat units less (i.e. 28) due to a difference in the nomenclature as described in [8].

Table 6. Certified Genotypes/Haplotypes, 7 X-STR Loci

Locus	Component				
	A	B ^(a)	C ^(a)	D	E
DXS7132	14,14	15	14	14,14	14,15
DXS7423	14,15	14	15	14,15	13,14
DXS8378	11,12	10	11	11,12	12,13
DXS10074	7,19	11	18	7,18,19	16,17
DXS10103	18,19	19	19	18,19	19,19
DXS10135	21.1,23	25	18	18,21.1,23	19,22
HPRTB	12,13	12	13	12,13	11,11

^(a) Components B and C are males and do not have a second X chromosome.

Table 7. Information Genotypes, 13 Autosomal STR Loci

Locus	Component				
	A	B	C	D	E
D2S1360	22,22	22,3,25	20,22,3	20,22,22,3	22,26
D3S1744	16,19	16,17	15,16	15,16,19	16,18
D4S2366	10,14	10,10	10,10	10,14	9,9
D5S2500	12,15	10,16	9,15	9,12,15	12,12
D7S1517	19,24	17,20	22,31	19,22,24,31	23,24
D8S1132	17,22	20,24	18,22	17,18,22	18,20
D10S2325	7,12	11,14	7,11	7,11,12	10,10
D21S2055	25,26	25,34	16.1,25	16.1,25,26	25,26
F13A01	6,12	3,2,5	5,7	5,6,7,12	5,7
F13B	6,10	6,6	8,10	6,8,10	9,10
FESFPS	10,11	10,13	10,11	10,11	11,12
LPL	12,13	10,12	10,10	10,12,13	10,11
Penta C	9,11	7,9	11,13	9,11,13	12,13

Table 8. Information Haplotypes, 3 Y-STR Loci

Locus	Component ^(a)		
	B	C	D
DYS449	32	28	28
DYS518	37	38	38
DYS627	18	20	20

^(a) Components A and E do not have a Y-chromosome (female) and are not included in this table.

Table 9. Information Genotypes/Haplotypes, 5 X-STR Loci

Locus	Component				
	A	B ^(a)	C ^(a)	D	E
DXS10079	19,23	21	21	19,21,23	18,21
DXS10101	28.2,29	26	29	28.2,29	27.2,29.2
DXS10134	35,39	35	37	35,37,39	36,36
DXS10146	29,30	28	23	23,29,30	29,30
DXS10148	25.1,28.1	40.1	22.1	22.1,25.1,28.1	18,28.1

^(a) Components B and C are males and do not have a second X chromosome.

Table 10. Information Genotypes, 30 Insertion/Deletion (Indel) Loci^(a)

Locus	Component				
	A	B	C	D	E
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	-	+	+	-/+	+

^(a) Length Variation for indels: + homozygous insertion; - homozygous deletion; +/- heterozygous deletion/insertion.

Table 11. Information Genotypes, 20 Insertion/Null Allele (INNUL) Loci^(a)

Locus	Component				
	A	B	C	D	E
AC1141	I,N	N,N	N,N	I,N	I,I
AC2265	I,N	I,N	I,N	I,N	I,N
AC2305	I,N	N,N	I,N	I,N	N,N
AC4027	I,N	I,N	I,N	I,N	I,I
ACA1766	I,N	I,I	I,I	I,N	N,N
ALU79712	N,N	N,N	I,N	I,N	I,I
HS4.69	I,N	I,I	I,N	I,N	I,I
MLS09	I,I	I,I	I,N	I,N	I,I
MLS26	N,N	I,N	N,N	N,N	I,I
NBC10	I,I	I,I	N,N	I,N	I,N
NBC13	I,N	N,N	N,N	I,N	N,N
NBC51	N,I	N,I	N,I	N,I	N,I
NBC102	N,I	N,I	I,I	N,I	N,I
NBC106	N,N	I,I	I,N	I,N	N,N
NBC120	I,I	I,N	I,I	I,I	I,I
NBC148	I,I	I,N	I,I	I,I	I,I
NBC216	N,N	I,I	I,I	I,N	I,I
RG148	N,N	I,N	I,N	I,N	N,N
SB19.12	I,N	N,N	I,I	I,N	N,N
TARBP	I,N	N,N	N,N	I,N	N,N

^(a) Length Variation for INNULS: I,I homozygous insertion; N,N homozygous null allele; I,N heterozygous insertion/null allele; N,I heterozygous null allele/insertion.

REFERENCES

- [1] May, W.E.; Gills, T.E.; Parris, R.; Beck, II, C.M.; Fassett, J.D.; Gettings, R.J.; Greenberg, R.R.; Guenther, F.R.; Kramer, G.; MacDonald, B.S.; Wise, S.A.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); <https://www.nist.gov/sites/default/files/documents/srm/SP260-136.PDF> (accessed June 2019).
- [2] De Bidvre, P.; Dybkaer, R.; Fajgelj, A.; Hibbert, D.B. *Metrological Traceability of Measurement Results in Chemistry: Concepts and implementation*; Pure Appl. Chem., Vol. 83 Issue 10, pp. 1873–1935 (2011).
- [3] *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed.; HHS publication No. (CDC) 21-1112; Chosewood, LC; Wilson, DE, Eds.; US Government Printing Office: Washington, D.C. (2009); available at <https://www.cdc.gov/biosafety/publications/bmbl5/index.htm> (accessed June 2019).
- [4] Ring, J.D., Sturk-Andreaggi, K., Peck, M.A., Marshall, C. *A performance evaluation of Nextera XT and KAPA HyperPlus for Rapid Illumina Library Preparation of Long-Range Mitogenome Amplicons*; Forensic Sci. Int. Genet., Vol. 29, pp. 174–180 (2017).
- [5] Hill, C.R.; Butler, J.M.; Vallone, P.M.; *A 26Plex Autosomal STR Assay to Aid Human Identity Testing*; J. Forensic Sci., Vol. 54 Issue 5, pp. 1008–1015 (2009).
- [6] Hill, C.R.; Kline, M.C.; Coble, M.D.; Butler, J.M.; *Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples*; J. Forensic Sci., Vol. 53 Issue 1, pp. 73–80 (2008).
- [7] Qiagen Investigator HDplex Handbook, November 2012; available at <https://www.qiagen.com/us/resources/resourcedetail?id=7d1661bd-a47b-4b19-a882-357a61b48c64&lang=en> (accessed June 2019).
- [8] Ballantyne, K.N.; Ralf, A.; Aboukhalid, R.; et al. *Toward Male Individualization with Rapidly Mutating Y-Chromosomal Short Tandem Repeats*; Human Mutat., Vol. 35, pp. 1021–1032 (2014).
- [9] Gettings, K.B.; Borsuk, L.A.; Ballard, D.; Bodner, M.; Budowle, B.; Devesse, L.; King, J.; Parson, W.; Phillips, C.; Vallone, P.M.; *STRSeq: A Catalog of Sequence Diversity at Human Identification Short Tandem Repeat Loci*; Forensic Sci Int Genet., Vol. 31, pp. 111–117 (2017).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at <https://www.nist.gov/srm>.