

Standard Reference Material[®] 2391d

PCR-Based DNA Profiling Standard

CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are 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.

Description: 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 A1 in Appendix A. 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].

Table 1 lists Certified genotypes for 35 autosomal STR loci plus the sex-typing locus Amelogenin. Table 2 lists Certified haplotypes for 28 Y-STR loci. Table 3 lists Certified genotypes/haplotypes for seven X-STR loci.

Non-Certified Values: Non-certified values are provided in Appendix A.

Additional Information: Values of potential interest to users and additional information are provided in Appendix B.

Period of Validity: The certified values delivered by **SRM 2391d** are valid within the measurement uncertainty specified until **31 October 2027**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (<https://www.nist.gov/srm>) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

Table 1. Certified Genotypes for 35 Autosomal STR Loci and Amelogenin in SRM 2391d

Locus	Component				
	A	B	C	D	E
Amelogenin	X,X	X,Y	X,Y	X,Y	X,X
CSF1PO	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 references 3-5.

^(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 2. Certified Haplotypes for 28 Y-STR Loci in SRM 2391d

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 reference 6.

Table 3. Certified Genotypes/Haplotypes for 7 X-STR Loci in SRM 2391d

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.

Safety: 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 [7]. 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).

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: 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 Research Protections Office. This SRM was developed after an appropriate human subjects research determination by NIST. 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.

Location of Additional Data: The Certified STR sequences for Components A, B, C, and E, including NGS length-based allele calls, STRSeq ID [8], bracketed repeats, and full sequence strings (5' flank, repeat region, and 3' flank) are provided at https://shop.nist.gov/ccrz_ProductDetails?sku=2391d&cclcl=en_US in the "SRM 2391d_STRSeqID.xlsx" Excel file.

The STR sequences for Component D are not listed and should be inferred as a combination of Component A and Component C.

REFERENCES

- [1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf> (accessed May 2023).
- [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] 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).

- [4] 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).
- [5] QIAGEN Investigator HDplex Handbook, November 2012; available at <https://www.qiagen.com/us/resources/resourcedetail?id=7d1661bd-a47b-4b19-a882-357a61b48c64&lang=en> (accessed May 2023).
- [6] 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).
- [7] *Biosafety in Microbiological and Biomedical Laboratories*, 6th 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/labs/BMBL.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fbiosafety%2Fpublications%2Fbmb15%2Findex.htm (accessed May 2023).
- [8] 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).
- [9] 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).

Certificate Revision History: **12 May 2023** (Corrected reference numbers, editorial changes); **31 January 2023** (Change of period of validity, elevate information values to non-certified values, updated format, editorial changes); **21 June 2019** (Original certificate date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at <https://www.nist.gov/srm>.

* * * * * End of Certificate of Analysis * * * * *

APPENDIX A

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system. Non-certified values have been assigned to the DNA concentrations of Components A through D and the number of cells per paper punch of Component E (Table A1).

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 A1 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 A1. 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.

Table A1. Description of Components in SRM 2391d

Component	Description	Volume	Concentration ^(a)
A	Anonymous single-source female genomic DNA in TE^{-4} buffer	55 μL	$1.6 \pm 0.5 \text{ ng}/\mu\text{L}$
B	Anonymous single-source male genomic DNA in TE^{-4} buffer	55 μL	$1.7 \pm 0.5 \text{ ng}/\mu\text{L}$
C	Anonymous single-source male genomic DNA in TE^{-4} buffer	55 μL	$1.6 \pm 0.2 \text{ ng}/\mu\text{L}$
D	Mixed-source, 3:1 (3 parts Component A and 1 part Component C) genomic DNA in TE^{-4} buffer	55 μL	$1.5 \pm 0.4 \text{ ng}/\mu\text{L}$
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 non-certified 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.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

***** End of Appendix A *****

APPENDIX B

Table B1 lists the commercial CE multiplex kits used for SRM 2391d testing. Commercial companies include Thermo Fisher (Waltham, MA), Promega Corporation (Madison, WI), QIAGEN (Hilden, Germany), and InnoGenomics (New Orleans, LA). Table B2 lists the NGS methods used for SRM 2391d testing. Commercial companies include Thermo Fisher, Promega Corporation, QIAGEN, and Verogen (San Diego, CA). Additional mtDNA sequencing was carried out using a protocol published by the Armed Forces DNA Identification Laboratory (AFDIL) (Dover, DE) [9].

Table B1. Capillary Electrophoresis (CE) typing kits used for SRM 2391d testing

Thermo Fisher (15)	Promega (13)	QIAGEN (11)	InnoGenomics (1)
Minifiler	PowerPlex CS7	Investigator ESSplex SE Plus	InnoTyper 21
Identifiler	PowerPlex 16	Investigator ESSplex SE QS	
Identifiler Plus	PowerPlex 16 HS	Investigator IDplex Plus	
Identifiler Direct	PowerPlex 18D	Investigator IDplex GO!	
NGM	PowerPlex 21	Investigator HDplex	
NGM SElect	PowerPlex ESX 17	Investigator 24plex QS	
NGM SElect Express	PowerPlex ESX 17 Fast	Investigator 24plex GO!	
NGM Detect	PowerPlex ESI 17 Pro	Investigator 26plex QS	
Verifiler Express	PowerPlex ESI 17 Fast	Investigator Argus Y-28 QS	
Verifiler Plus	PowerPlex Fusion	Investigator Argus X-12 QS	
GlobalFiler	PowerPlex Fusion 6C	Investigator DIPplex	
GlobalFiler IQC	VersaPlex 27PY		
GlobalFiler Express	PowerPlex Y23		
Yfiler			
Yfiler Plus			

Table B2. Next Generation Sequencing (NGS) methods and kits used for SRM 2391d testing

AFDIL MiSeq FGx (1)	Verogen MiSeq FGx (4)	Thermo Fisher Ion S5 XL (9)	Promega MiSeq FGx (2)	QIAGEN MiSeq FGx (1)
AFDIL mtGenome protocol (mtDNA Whole Genome) [9]	ForenSeq Signature Prep Kit	Precision ID GlobalFiler NGS STR Panel v2	PowerSeq 46GY System	Human Mitochondrial Panel (mtDNA Whole Genome)
	ForenSeq MainstAY Kit	Precision ID Ancestry Panel	PowerSeq CRM Nested System, Custom (mtDNA Control Region)	
	ForenSeq Kintelligence Kit	Precision ID Identity Panel		
	ForenSeq mtDNA Control Region Kit	Precision ID mtDNA Whole Genome Panel		
		Precision ID mtDNA Control Region Panel		
		Ion Ampliseq DNA Phenotype Panel		
		Ion Ampliseq MH-74 Plex Panel		
		Ion Ampliseq VISAGE Panel		
		Ion Ampliseq Y-SNP Panel		

Values of potential interest to users are provided for markers that are typed exclusively with CE methods. The values of potential interest for users are provided for 13 autosomal STR loci in Table B3. The values of potential interest to users are provided for three Y-STR loci in Table B4. The values of potential interest to users are provided for five X-STR loci in Table B5. The values of potential interest to users are provided for 30 Insertion and Deletion (Indel) loci in Table B6. The values of potential interest to users are provided for 20 Insertion and Null allele (INNUL) loci in Table B7. The values of potential interest to users are provided for the GlobalFiler/GlobalFiler IQC Y-Indel in Table B8.

Table B3. Genotypes for 13 Autosomal STR Loci in SRM 2391d

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 B4. Haplotypes for 3 Y-STR Loci in SRM 2391d

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 B5. Genotypes/Haplotypes for 5 X-STR Loci in SRM 2391d

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 B6. Genotypes for 30 Insertion/Deletion (Indel) Loci^(a)
(QIAGEN Investigator DIPplex Kit) in SRM 2391d**

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 B7. Genotypes for 20 Insertion/Null Allele (INNUL) Loci^(a)
(Innogenomics InnoTyper 21 Human DNA Analysis Kit) in SRM 2391d**

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.

Table B8. Allele Call for the GlobalFiler and GlobalFiler IQC Y-Indel Locus in SRM 2391d

Locus	Component ^(a)		
	B	C	D
GlobalFiler and GlobalFiler IQC Y-Indel ^(b)	2	2	2

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

^(b) The allele call for this Y-Indel is designated as either a 1 or a 2.

Additional Values of Potential Interest Derived from NGS-methods

Values of potential interest to users are also assigned to 135 autosomal identity single nucleotide polymorphism (SNP) loci, 276 autosomal ancestry SNP loci, 17 autosomal phenotype SNP loci, 9,863 autosomal kinship SNP loci, 106 X-SNP loci, 930 Y-SNP loci, 74 microhaplotype (MH) loci, and whole mitochondrial genome DNA (mtDNA) sequences determined by sequencing.

Values of potential interest to users for SNP loci of forensic interest for Components A, B, C, and E are provided at https://shop.nist.gov/ccrz_ProductDetails?sku=2391d&cclcl=en_US in “SRM 2391d_SNP.xlsx” Excel files.

Values of potential interest to users for MH loci of forensic interest for Components A, B, C, and E are provided at https://shop.nist.gov/ccrz_ProductDetails?sku=2391d&cclcl=en_US in “SRM 2391d_MH.xlsx” Excel files.

Values of potential interest to users for the mtDNA whole genome sequences for Components A, B, C, and E are provided at https://shop.nist.gov/ccrz__ProductDetails?sku=2391d&cclcl=en_US 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.

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***** End of Appendix B *****