National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2391c

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 F). The complete listing of Components is included in Table 1. A unit of SRM 2391c 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]. Table 2 lists 25 certified autosomal STR plus the sex-typing marker Amelogenin genotypes and Table 3 lists 29 genetic loci certified Y-STR haplotypes of the Components using commercially available PCR multiplex kits of forensic interest at the time of the SRM production and update. These genotypes/haplotypes have been assigned by electrophoretic base pair (bp) size match to sequenced alleles and also confirmed by direct sequencing.

Component D contains a mixture of female DNA (Component A) and male DNA (Component C). The certified ratio of the mass of Component A DNA relative to that of Component C, in Component D is:

Component A DNA/Component C DNA: 3.1 ± 0.1

The uncertainty in the value, calculated according to the method described in the ISO/JCGM Guide [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty and the coverage factor $k = 2.6$ corresponds to approximately 95 % confidence. The mass ratio composition of Component D is traceable to the SI unit of count (1).

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 [1]. Table 4 lists the genotypes for loci of forensic interest but for which the reference values have been assigned from repeat counts based on electrophoretic base pair size differences between non-sequenced alleles compared to sequenced alleles. The SRM 2391c genotypes as determined by the methods used, are traceable to the SI unit of count (1).

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 [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 F (Table 1), and the genotypes (when typed with only one kit/set of primers) of the five Components at the Penta C locus (Table 5), X-STR loci (Table 6), and Insertions and Deletions (Indels) (Table 7).

Expiration of Certification: The certification of **SRM 2391c** is valid, within the measurement uncertainties specified, until **03 February 2022**, 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 M.C. Kline of the NIST Biomolecular Measurement Division.

Michael J. Tarlov, Chief
Biomolecular Measurement Division

Gaithersburg, MD 20899
Certificate Issue Date: 09 October 2020
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

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.

On January 16, 2018, NIST confirmed evidence of degradation in Component E and therefore it was removed along with all associated data from the SRM 2391c unit and Certificate of Analysis.

Analytical determinations and technical measurements leading to the certification of this SRM were performed by M.C. Kline, J.L. Almeida, C.R. Steffen E.L. Romsos, and K.B. Gettings of the NIST Biomolecular Measurement and Biosystems and Biomaterials Divisions.

Statistical consultation was provided by J.H. Yen of the NIST Statistical Engineering Division and D.L. Duewer of the NIST Chemical Sciences Division.

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

Preparation of this SRM was supported in part by the National Institute of Justice, U.S. Department of Justice.

NOTICE AND WARNINGS TO USER

Warning: SRM 2391c 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 2 for any potentially infectious human serum or blood specimen [3]. SRM 2391c 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 ºC to 8 ºC (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 F, cells on FTA paper, should be washed prior to PCR amplification.

SOURCE AND ANALYSIS[(1)]

Source of Material: The DNA extract for Component A was derived from Buffy coat white blood cells. DNA extracts and cells used for Components B and C were derived from cell lines obtained from Coriell Cell Repositories (Camden, NJ). The cell line used for Component F was obtained from American Type Culture Collection (Manassas, VA). All source materials had been tested and found negative for HBsAg, HIV, HCV, and HIV-1Ag before use.

Interlaboratory Commutability Study: Four laboratories were selected to establish the commutability of the material comprising SRM 2391c, they were Palm Beach Sheriff's Office (Palm Beach, FL), Promega Corporation (Madison, WI), Life Technologies (Foster City, CA) and Bode Technology Group (Lorton, VA). All data was concordant across all kits used by these laboratories.

Description of Components: Five components are included in each unit. Components A through D each contain 50 µL of extracted genomic DNA in TE^{-4} buffer, pH 8.0, which consists of deionized water with 10 mmol/L 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) and 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA) and are packaged in perfluoroalkoxy fluoropolymer (PFA) vials. These four components were prepared to have similar UV optical densities at 260 nm. Table 1 lists the range in DNA concentration for the components based upon results from several quantitative PCR (qPCR)-based methods calibrated to Component A of SRM 2372 *Human DNA Quantitation Standard*. Component F contains two 6 mm punches of FTA paper. Each punch was prepared to hold approximately 7.5×10^4 cells. Component F is packaged in sterile 0.5 mL polypropylene vials. A detailed description of the individual components in SRM 2391c is listed in Table 1. Note that SRM 2391c is modified from SRM 2391b

[(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.

in that Component D is a mixture of Components A and C; Component F is a "stain" spotted on paper discs; and Components B, C, D, and F are characterized at 29 Y-chromosome short tandem repeat (STR) loci [4].

Table 1. Description of Components in SRM 2391c

Component	Description	Amount	Concentration[(a)]
A	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	Mixed-source (Components A and C) genomic DNA in TE^{-4} buffer	50 µL	1.1 – 2.1 ng/µL
F	Anonymous single-source male cells spotted on FTA paper[(b)]	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.

[(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.

Description of Analyses: Component D is a mixture prepared gravimetrically by combining Component A and Component C. The composition of this mixture was verified using results from the TrueAllele electropherogram deconvolution software package [5] and the electrophoretic peak-height ratios of alleles sets where alleles can be uniquely assigned to DNA from Component A and C sources. Figure 1 summarizes these results.

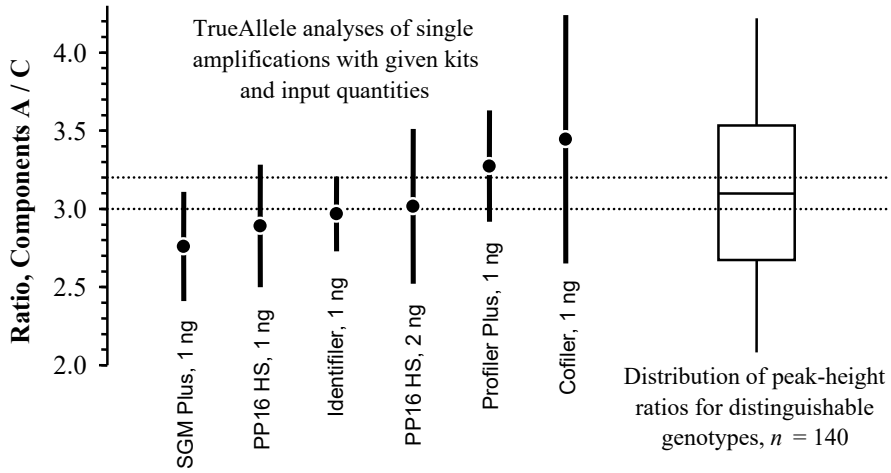

Figure 1. Verification of Component A/Component C Mass Ratio. The horizontal lines bracket the certified 95 % level of confidence uncertainty interval; the vertical dot & bars represent the mean ± one standard deviation TrueAllele results for six independent amplifications; and the box & whisker diagram represents the median, central 50 %, and central 95 % of the peak-height ratios for 140 unique sets of alleles.

The types for this SRM are listed in Tables 2 through 7. The certified values for 25 autosomal STR loci plus Amelogenin are provided in Table 2. The certified values for 29 Y-STR loci are provided in Table 3. The reference values for 26 autosomal loci are provided in Table 4. Table 5 lists information values for a single locus. Table 6 lists information values for 12 X-STR loci. Table 7 lists information values for 30 insertions and deletions (indels). The results are concordant across all kits and all laboratories. Table 8 lists the STR kits and primer mixes used at NIST and other laboratories to assign the certified, reference and information genotypes. Table 9 lists the autosomal STR sequencing for Component A. Table 10 lists the autosomal STR sequencing for Component B. Table 11 lists the Y-STR sequencing for Component B. Table 12 lists the autosomal STR sequencing for Component C. Table 13 lists the Y-STR sequencing for Component C. Table 14 lists the autosomal STR sequencing for Component F. Table 15 lists the Y-STR sequencing for Component F. Component D was not sequenced because it is a mixture of Components A and C. Table 16 lists the GlobalFiler Y-Indel information values for Components B, C, D, and F. The information values for SNP loci of forensic interest for Components A, B, C, and F can be found in Supplemental Table 1 provided on https://strbase.nist.gov/srm_tab.htm. These values were obtained from the Illumina ForenSeq System and the Thermo Fisher Precision ID Identity and Ancestry Panels.

Table 2. Certified Genotypes, 25 Autosomal STR Loci and Amelogenin

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

Table 3. Certified Haplotypes, 29 Y-STR Loci

Locus	Component[a]			
	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] Component A does not have a Y-chromosome and thus has no type at this locus

Table 4. Reference Genotypes, 26 Autosomal STR Loci

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

Table 5. Information Genotypes, 1 Autosomal STR Locus

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

Table 6. Information Genotypes, 12 X-STR Loci

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

[(a)]Components B, C, and F are males and do not have a second X chromosome.

Table 7. Information Genotypes, 30 Insertion/Deletion (Indel) Loci[(a)]

Locus	Component				
	A	B	C	D	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	+	–/+	–/+	–/+	+

[(a)]Length Variation for indels: + homozygous insertion; – homozygous deletion; –/+ heterozygous deletion/insertion.

Table 8. STR Genotyping kits and Primer Mixes used by NIST Researchers for Certification of SRM 2391c

Kit Provider			Primer Mixes
Thermo Fisher Foster City, CA	Promega Corp Madison, WI	Qiagen Inc. Hilden, Germany	NIST
Identifiler	PowerPlex 16	ESSplex	26plex [6]
Identifiler Plus	PowerPlex 16 HS	IDplex	miniSTRs [7,8]
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	
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		
Yflier Plus	PowerPlex CS7		
	PowerPlex Fusion		
	PowerPlex Fusion 6C		
	PowerPlex Y23		

Table 9. Autosomal STR Sequencing for Component A

SRM 2391c – Component A				
Marker	**Length-based Types**	**Sanger Result**	**Repeat Structure –Allele 1**	**Repeat Structure –Allele 2**
D1S1656	17.3, 17.3	17.3, 17.3	[TAGA]$_4$ TGA [TAGA]$_{12}$ TAGG [TG]$_5$	[TAGA]$_4$ TGA [TAGA]$_{12}$ TAGG
D2S1338	18, 23	18, 23	[TGCC]$_6$ [TTCC]$_{12}$	[TGCC]$_7$ [TTCC]$_{13}$ GTCC [TTCC]$_2$
D2S441	10, 10	10, 10	[TCTA]$_{10}$	[TCTA]$_8$ TCTG [TCTA]$_1$
D3S1358	15, 16	15, 16	TCTA [TCTG]$_2$ [TCTA]$_{12}$	TCTA [TCTG]$_3$ [TCTA]$_{12}$
D5S818	11, 12	11, 12	[AGAT]$_{11}$	[AGAT]$_{12}$
D6S1043	11, 18	11, 18	[AGAT]$_{11}$	[AGAT]$_{12}$ ACAT [AGAT]$_5$
D7S820	11, 11	11, 11	[GATA]$_{11}$	[GATA]$_{11}$
D8S1179	13, 14	13, 14	[TCTA]$_{13}$	[TCTA]$_2$ TCTG [TCTA]$_{11}$
D8S1115	15, 16	15, 16	[ATT]$_{15}$	[ATT]$_{16}$
D10S1248	15, 16	15, 16	[GGAA]$_{15}$	[GGAA]$_{16}$
D12S391	18.3, 22	18.3, 22	AGAT GAT [AGAT]$_9$ [AGAC]$_7$ AGAT	[AGAT]$_{13}$ [AGAC]$_8$ AGAT
D13S317	8, 8	8, 8	[TATC]$_8$	[TATC]$_8$
D16S539	10, 11	10, 11	[GATA]$_{10}$	[GATA]$_{11}$
D18S51	12, 15	12, 15	[AGAA]$_{12}$	[AGAA]$_{15}$
D19S433	13, 14	13, 14	[AAGG] AAAG [AAGG] TAGG [AAGG]$_{11}$	[AAGG] AAAG [AAGG] TAGG [AAGG]$_{12}$
D21S11	28, 32.3	28, 32.3	[TCTA]$_4$ [TCTG]$_6$ {[TCTA]$_3$ TA [TCTA]$_3$ TCA [TCTA]$_2$ TCCATA} [TCTA]$_{10}$	[TCTA]$_5$ [TCTG]$_6$ {[TCTA]$_3$ TA [TCTA]$_3$ TCA [TCTA]$_2$ TCCATA} [TCTA]$_{12}$ TA TCTA
D22S1045	15, 15	15, 15	[ATT]$_{12}$ ACT [ATT]$_2$	[ATT]$_{12}$ ACT [ATT]$_2$
CSF1PO	10, 10	10, 10	[AGAT]$_{10}$	[AGAT]$_{10}$
FGA	21, 23	21, 23	[TTTC]$_3$TTTT TTCT [CTTT]$_{13}$ CTCC [TTCC]$_2$	[TTTC]$_3$TTTT TTCT [CTTT]$_{15}$ CTCC [TTCC]$_2$
Penta D	9, 13	9, 13	[AAAGA]$_9$	[AAAGA]$_{13}$
Penta E	5, 10	5, 10	[AAAGA]$_5$	[AAAGA]$_{10}$
SE33	16, 18	16, 18	[AAAG]$_2$ AG [AAAG]$_3$ AG [AAAG]$_{16}$ G [AAAG]$_3$ AG	[AAAG]$_2$ AG [AAAG]$_3$ AG [AAAG]$_{18}$ G [AAAG]$_3$ AG
TH01	8, 9.3	8, 9.3	[AATG]$_8$	[AATG]$_6$ ATG [AATG]$_3$
TPOX	8, 8	8, 8	[AATG]$_8$	[AATG]$_8$
vWA	18, 19	18, 19	TCTA [TCTG]$_4$ [TCTA]$_{13}$	TCTA [TCTG]$_4$ [TCTA]$_{14}$

Note: Sequence information in gray indicates bases that are not counted toward the length-based genotype designation.

Table 10. Autosomal STR Sequencing for Component B

SRM 2391c – Component B[a]				
Marker	**Length-based Types**	**Sanger Result**	**Repeat Structure –Allele 1**	**Repeat Structure –Allele 2**
D1S1656	11, 14	11, 14	[TAGA]$_{11}$ [TG]$_5$	[TAGA]$_{14}$ [TG]$_5$
D2S1338	17, 17	17, 17	[TGCC]$_6$ [TTCC]$_{11}$	[TGCC]$_6$ [TTCC]$_{11}$
D2S441	10, 14	10, 14	[TCTA]$_{10}$	[TCTA]$_{11}$ TTTA [TCTA]$_2$
D3S1358	15, 19	15, 19	TCTA [TCTG]$_3$[TCTA]$_{11}$	TCTA [TCTG]$_3$[TCTA]$_{15}$
D5S818	12, 13	12, 13	[AGAT]$_{12}$	[AGAT]$_{13}$
D6S1043	14, 19	14, 19	[AGAT]$_{14}$	[AGAT]$_{13}$ ACAT [AGAT]$_5$
D7S820	10, 10	10, 10	[GATA]$_{10}$	[GATA]$_{10}$
D8S1179	10, 13	10, 13	[TCTA]$_{10}$	[TCTA]$_{13}$
D8S1115	15, 17	15, 17	[ATT]$_{15}$	[ATT]$_{17}$
D10S1248	13, 13	13, 13	[GGAA]$_{13}$	[GGAA]$_{13}$
D12S391	19, 24	19, 24	[AGAT]$_{12}$ [AGAC]$_6$ AGAT	[AGAT]$_{15}$ [AGAC]$_9$
D13S317	9, 12	9, 12	[TATC]$_9$	[TATC]$_{12}$
D16S539	10, 13	10, 13	[GATA]$_{10}$	[GATA]$_{13}$
D18S51	13, 16	13, 16	[AGAA]$_{13}$	[AGAA]$_{16}$
D19S433	16, 16.2	16, 16.2	[AAGG] AAAG [AAGG] TAGG [AAGG]$_{14}$	[AAGG] AA-- [AAGG] TAGG [AAGG]$_{15}$[a]
D21S11	32, 32.2	32, 32.2	[TCTA]$_4$ [TCTG]$_6$ {[TCTA]$_3$ TA [TCTA]$_3$ TCA [TCTA]$_2$ TCCATA} [TCTA]$_{14}$	[TCTA]$_5$ [TCTG]$_6$ {[TCTA]$_3$ TA [TCTA]$_3$ TCA [TCTA]$_2$ TCCATA} [TCTA]$_{12}$ TA TCTA
D22S1045	15, 17	15, 17	[ATT]$_{12}$ ACT [ATT]$_2$	[ATT]$_{14}$ ACT [ATT]$_2$
CSF1PO	10, 11	10, 11	[AGAT]$_{10}$	[AGAT]$_{11}$
FGA	20, 23	20, 23	[TTTC]$_3$ TTTT TTCT [CTTT]$_{12}$ CTCC [TTCC]$_2$	[TTTC]$_3$ TTTT TTCT [CTTT]$_{15}$ CTCC [TTCC]$_2$
Penta D	8, 12	8, 12	[AAAGA]$_8$	[AAAGA]$_{12}$
Penta E	7, 15	7, 15	[AAAGA]$_7$	[AAAGA]$_{15}$
SE33	17, 18	17, 18	[AAAG]$_2$ AG [AAAG]$_3$ AG [AAAG]$_{17}$ G [AAAG]$_3$ AG	[AAAG]$_2$ AG [AAAG]$_3$ AG [AAAG]$_{18}$ G [AAAG]$_3$ AG
TH01	6, 9.3	6, 9.3	[AATG]$_6$	[AATG]$_6$ ATG [AATG]$_3$
TPOX	8, 11	8, 11	[AATG]$_8$	[AATG]$_{11}$
vWA	17, 18	17, 18	TCTA [TCTG]$_4$ [TCTA]$_{12}$	TCTA [TCTG]$_4$ [TCTA]$_{13}$

[a] Deletion of 2 bp in an uncounted repeat unit results in the 16.2 designation.

Note: Sequence information in gray indicates bases that are not counted toward the length-based genotype designation.

Table 11. Y-STR Sequencing for Component B

SRM 2391c – Component B			
Marker	**Length-based Types**	**Sanger Result**	**Repeat Structure –Allele 1**
DYS19	14	14	[TAGA]$_3$ TAGG [TAGA]$_{11}$
DYS385a	13	13	[GAAA]$_{13}$
DYS385b	17	17	[GAAA]$_{17}$
DYS389I	13	13	[TCTG]$_3$ [TCTA]$_{10}$
DYS389II	31	31	[TCTG]$_6$ [TCTA]$_{12}$ N$_{48}$ [TCTG]$_3$ [TCTA]$_{10}$
DYS390	23	23	[TCTG]$_8$ [TCTA]$_{10}$ TCTG [TCTA]$_4$
DYS391	10	10	[TCTA]$_{10}$
DYS392	11	11	[TAT]$_{11}$
DYS393	12	12	[AGAT]$_{12}$
DYS437	14	14	[TCTA]$_8$ [TCTG]$_2$ [TCTA]$_4$
DYS438	10	10	[TTTTC]$_{10}$
DYS439	11	11	[AGAT]$_{11}$
DYS448	20	20	[AGAGAT]$_{12}$ N$_{42}$ [AGAGAT]$_8$
DYS449	26	26	[TTTC]$_{11}$ N$_{50}$ [TTTC]$_{15}$
DYS456	15	15	[AGAT]$_{15}$
DYS458	17.2	17.2	[GAAA]$_{15}$ AA [GAAA]$_2$
DYS460	10	10	[ATAG]$_{10}$
DYS481	25	25	[CTT]$_{25}$
DYS518	38	38	[AAAG]$_3$ GAAG [AAAG]$_{14}$ GGAG [AAAG]$_4$ N$_6$ [AAAG]$_{15}$
DYS533	11	11	[ATCT]$_{11}$
DYS549	12	12	[GATA]$_{12}$
DYS570	18	18	[TTTC]$_{18}$
DYS576	17	17	[AAAG]$_{17}$
DYS627	22	22	[AGAG]$_3$[AAAG]$_{19}$
DYS635	20	20	[TCTA]$_4$ [TGTA]$_2$ [TCTA]$_2$ [TGTC]$_2$ [TCTA]$_{10}$
DYS643	9	9	[CTTTT]$_9$
DYF387S1a	35	35	[AAAG]$_3$ GTAG [GAAG]$_4$ N$_{20}$ [GAAG]$_9$ [AAAG]$_{13}$
DYF387S1b	38	38	[AAAG]$_3$ GTAG [GAAG]$_4$ N$_{20}$ [GAAG]$_{10}$ [AAAG]$_{15}$
Y GATA H4	11	11	[TAGA]$_{11}$

Note: Sequence information in gray indicates bases that are not counted toward the length-based genotype designation.

Table 12. Autosomal STR Sequencing for Component C

SRM 2391c – Component C				
Marker	**Length-based Types**	**Sanger Result**	**Repeat Structure –Allele 1**	**Repeat Structure –Allele 2**
D1S1656	11, 15	11, 15	[TAGA]$_{11}$ [TG]$_{5}$	[TAGA]$_{14}$ TAGG [TG]$_{5}$
D2S1338	19, 19	19, 19	[TGCC]$_{7}$ [TTCC]$_{12}$	[TGCC]$_{7}$ [TTCC]$_{12}$
D2S441	10, 10	10, 10	[TCTA]$_{8}$ TCTG [TCTA]$_{1}$	[TCTA]$_{8}$ TCTG [TCTA]$_{1}$
D3S1358	16, 18	16, 18	TCTA [TCTG]$_{3}$ [TCTA]$_{12}$	TCTA [TCTG]$_{3}$ [TCTA]$_{14}$
D5S818	10, 11	10, 11	[AGAT]$_{10}$	[AGAT]$_{11}$
D6S1043	11, 14	11, 14	[AGAT]$_{11}$	[AGAT]$_{14}$
D7S820	10, 12	10, 12	[GATA]$_{10}$	[GATA]$_{12}$
D8S1179	10, 17	10, 17	[TCTA]$_{10}$	[TCTA]$_{2}$ TCTG [TCTA]$_{14}$
D8S1115	9, 9	9, 9	[ATT]$_{9}$	[ATT]$_{9}$
D10S1248	12, 16	12, 16	[GGAA]$_{12}$	[GGAA]$_{16}$
D12S391	19, 23	19, 23	[AGAT]$_{13}$ [AGAC]$_{5}$ AGAT	[AGAT]$_{12}$ [AGAC]$_{11}$
D13S317	11, 11	11, 11	[TATC]$_{11}$, A→T SNP 1 bp ds from repeat	[TATC]$_{11}$, A→T SNP 1 bp ds from repeat
D16S539	10, 10	10, 10	[GATA]$_{10}$	[GATA]$_{10}$
D18S51	16, 19	16, 19	[AGAA]$_{16}$	[AGAA]$_{19}$
D19S433	13.2, 15.2	13.2, 15.2	[AAGG] AA-- [AAGG] TAGG [AAGG]$_{12}$[(a)]	[AAGG] AA-- [AAGG] TAGG [AAGG]$_{14}$[(a)]
D21S11	29, 30	29, 30	[TCTA]$_{4}$ [TCTG]$_{6}$ {[TCTA]$_{3}$ TA [TCTA]$_{3}$ TCA [TCTA]$_{2}$ TCCATA} [TCTA]$_{11}$	[TCTA]$_{6}$ [TCTG]$_{5}$ {[TCTA]$_{3}$ TA [TCTA]$_{3}$ TCA [TCTA]$_{2}$ TCCATA} [TCTA]$_{11}$
D22S1045	16, 16	16, 16	[ATT]$_{13}$ ACT [ATT]$_{2}$	[ATT]$_{13}$ ACT [ATT]$_{2}$
CSF1PO	10, 12	10, 12	[AGAT]$_{10}$	[AGAT]$_{12}$
FGA	24, 26	24, 26	[TTTC]$_{3}$ TTTT TTCT [CTTT]$_{16}$ CTCC [TTCC]$_{2}$	[TTTC]$_{3}$ TTTT TTCT [CTTT]$_{18}$ CTCC [TTCC]$_{2}$
Penta D	10, 11	10, 11	[AAAGA]$_{10}$	[AAAGA]$_{11}$
Penta E	12, 13	12, 13	[AAAGA]$_{12}$	[AAAGA]$_{13}$
SE33	28.2, 31.2	28.2, 31.2	[AAAG]$_{2}$ AG [AAAG]$_{3}$ AG [AAAG]$_{10}$ AAAAAG [AAAG]$_{17}$ GAAGG [AAAG]$_{2}$ AG	[AAAG]$_{2}$ AG [AAAG]$_{3}$ AG [AAAG]$_{9}$ AAAAAG [AAAG]$_{21}$ GAAGG [AAAG]$_{2}$ AG
TH01	6, 8	6, 8	[AATG]$_{6}$	[AATG]$_{8}$
TPOX	11, 11	11, 11	[AATG]$_{11}$	[AATG]$_{11}$
vWA	16, 18	16, 18	TCTA [TCTG]$_{4}$ [TCTA]$_{11}$	TCTA [TCTG]$_{4}$ [TCTA]$_{13}$

[(a)] Deletion of 2 bp in an uncounted repeat unit results in the 13.2 and 15.2 designations.
Note: Sequence information in gray indicates bases that are not counted toward the length-based genotype designation.
Abbreviations: SNP = single nucleotide polymorphism, bp = base pairs, ds = downstream from the repeat structure.

Table 13. Y-STR Sequencing for Component C

SRM 2391c – Component C			
Marker	**Length-based Types**	**Sanger Result**	**Repeat Structure –Allele 1**
DYS19	15	15	[TAGA]$_3$ TAGG [TAGA]$_{12}$
DYS385a	13	13	[GAAA]$_{13}$
DYS385b	15	15	[GAAA]$_{15}$
DYS389I	12	12	[TCTG]$_3$ [TCTA]$_9$
DYS389II	27	27	[TCTG]$_5$ [TCTA]$_{10}$ N$_{48}$ [TCTG]$_3$ [TCTA]$_9$
DYS390	24	24	[TCTG]$_8$ [TCTA]$_{11}$ TCTG [TCTA]$_4$
DYS391	11	11	[TCTA]$_{11}$
DYS392	13	13	[TAT]$_{13}$
DYS393	13	13	[AGAT]$_{13}$
DYS437	16	16	[TCTA]$_{10}$ [TCTG]$_2$ [TCTA]$_4$
DYS438	11	11	[TTTTC]$_{11}$
DYS439	12	12	[AGAT]$_{12}$
DYS448	19	19	[AGAGAT]$_{11}$ N$_{42}$ [AGAGAT]$_8$
DYS449	29	29	[TTTC]$_{14}$ N$_{50}$ [TTTC]$_{15}$
DYS456	15	15	[AGAT]$_{15}$
DYS458	17	17	[GAAA]$_{17}$
DYS460	10	10	[ATAG]$_{10}$
DYS481	26	26	[CTT]$_{26}$
DYS518	39	39	[AAAG]$_3$ GAAG [AAAG]$_{16}$ GGAG [AAAG]$_4$ N$_6$ [AAAG]$_{14}$
DYS533	10	10	[ATCT]$_{10}$
DYS549	13	13	[GATA]$_{13}$
DYS570	20	20	[TTTC]$_{20}$
DYS576	16	16	[AAAG]$_{16}$
DYS627	21	21	[AGAG]$_3$[AAAG]$_{18}$
DYS635	21	21	[TCTA]$_4$ [TGTA]$_2$ [TCTA]$_2$ [TGTA]$_2$ [TCTA]$_{11}$
DYS643	12	12	[CTTTT]$_{12}$
DYF387S1a	36	36	[AAAG]$_3$ GTAG [GAAG]$_4$ N$_{20}$ [GAAG]$_9$ [AAAG]$_{14}$
DYF387S1b	38	38	[AAAG]$_3$ GTAG [GAAG]$_4$ N$_{20}$ [GAAG]$_{11}$ [AAAG]$_4$ ACAG [AAAG]$_9$
Y GATA H4	11	11	[TAGA]$_{11}$

Note: Sequence information in gray indicates bases that are not counted toward the length-based genotype designation.

Table 14. Autosomal STR Sequencing for Component F

SRM 2391c – Component F				
Marker	**Length-based Types**	**Sanger Result**	**Repeat Structure –Allele 1**	**Repeat Structure –Allele 2**
D1S1656	17.3, 17.3	17.3, 17.3	[TAGA]$_4$ TGA [TAGA]$_{12}$ TAGG [TG]$_5$	[TAGA]$_4$ TGA [TAGA]$_{12}$ TAGG [TG]$_5$
D2S1338	17, 17	17, 17	[TGCC]$_6$ [TTCC]$_{11}$	[TGCC]$_6$ [TTCC]$_{11}$
D2S441	14, 14	14, 14	[TCTA]$_{11}$ TTTA [TCTA]$_2$	[TCTA]$_{11}$ TTTA [TCTA]$_2$
D3S1358	16, 17	16, 17	TCTA [TCTG]$_2$ [TCTA]$_{13}$	TCTA [TCTG]$_3$ [TCTA]$_{13}$
D5S818	11, 13	11, 13	[AGAT]$_{11}$	[AGAT]$_{13}$
D6S1043	11, 16	11, 16	[AGAT]$_{11}$	[AGAT]$_{14}$ ACAT [AGAT]$_1$
D7S820	8, 12	8, 12	[GATA]$_8$	[GATA]$_{12}$
D8S1179	10, 13	10, 13	[TCTA]$_{10}$	[TCTA] TCTG [TCTA]$_{11}$
D8S1115	9, 17	9, 17	[ATT]$_9$	[ATT]$_{17}$
D10S1248	14, 15	14, 15	[GGAA]$_{14}$	[GGAA]$_{15}$
D12S391	18, 19	18, 19	[AGAT]$_{11}$ [AGAC]$_6$ AGAT	[AGAT]$_{12}$ [AGAC]$_6$ AGAT
D13S317	8, 11	8, 11	[TATC]$_8$	[TATC]$_{11}$
D16S539	9, 11	9, 11	[GATA]$_9$	[GATA]$_{11}$
D18S51	17, 22	17, 22	[AGAA]$_{17}$	[AGAA]$_{22}$
D19S433	13, 14	13, 14	[AAGG] AAAG [AAGG] TAGG [AAGG]$_{11}$	[AAGG] AAAG [AAGG] TAGG [AAGG]$_{12}$
D21S11	29, 32.2	29, 32.2	[TCTA]$_4$ [TCTG]$_6$ {[TCTA]$_3$ TA [TCTA]$_3$ TCA [TCTA]$_2$ TCCATA} [TCTA]$_{11}$	[TCTA]$_5$ [TCTG]$_6$ {[TCTA]$_3$ TA [TCTA]$_3$ TCA [TCTA]$_2$ TCCATA} [TCTA]$_{12}$ TA [TCTA]
D22S1045	11, 15	11, 15	[ATT]$_8$ ACT [ATT]$_2$	[ATT]$_{12}$ ACT [ATT]$_2$
CSF1PO	10, 11	10, 11	[AGAT]$_{10}$	[AGAT]$_{11}$
FGA	21, 25	21, 25	[TTTC]$_3$ TTTT TTCT [CTTT]$_{13}$ CTCC [TTCC]$_2$	[TTTC]$_3$ TTTT TTCT [CTTT]$_{17}$ CTCC [TTCC]$_2$
Penta D	9, 10	9, 10	[AAAGA]$_9$	[AAAGA]$_{10}$
Penta E	11, 15	11, 15	[AAAGA]$_{11}$	[AAAGA]$_{15}$
SE33	12, 21	12, 21	[AAAG]$_2$ AG [AAAG]$_3$ AG [AAAG]$_{12}$ G [AAGG]$_3$ AG	[AAAG]$_2$ AG [AAAG]$_3$ AG [AAAG]$_{21}$ G [AAAG]$_3$ AG
TH01	7, 9.3	7, 9.3	[AATG]$_7$	[AATG]$_6$ ATG [AATG]$_3$
TPOX	8, 8	8, 8	[AATG]$_8$	[AATG]$_8$
vWA	16, 18	16, 18	TCTA [TCTG]$_4$ [TCTA]$_{11}$	TCTA [TCTG]$_4$ [TCTA]$_{13}$

Note: Sequence information in gray indicates bases that are not counted toward the length-based genotype designation.

Table 15. Y-STR Sequencing for Component F

SRM 2391c – Component F			
Marker	**Length-based Types**	**Sanger Result**	**Repeat Structure –Allele 1**
DYS19	17	17	[TAGA]$_3$ TAGG [TAGA]$_{14}$
DYS385a	12	12	[GAAA]$_{12}$
DYS385b	16	16	[GAAA]$_{16}$
DYS389I	13	13	[TCTG]$_3$ [TCTA]$_{10}$
DYS389II	30	30	[TCTG]$_5$ [TCTA]$_{12}$ N$_{48}$ [TCTG]$_3$ [TCTA]$_{10}$
DYS390	24	24	[TCTG]$_8$ [TCTA]$_{11}$ TCTG [TCTA]$_4$
DYS391	12	12	[TCTA]$_{12}$
DYS392	11	11	[TAT]$_{11}$
DYS393	13	13	[AGAT]$_{13}$
DYS437	15	15	[TCTA]$_9$ [TCTG]$_2$ [TCTA]$_4$
DYS438	10	10	[TTTTC]$_{10}$
DYS439	11	11	[AGAT]$_{11}$
DYS448	20	20	[AGAGAT]$_{12}$ N$_{42}$ [AGAGAT]$_8$
DYS449	30	30	[TTTC]$_{16}$ N$_{50}$ [TTTC]$_{14}$
DYS456	15	15	[AGAT]$_{15}$
DYS458	18	18	[GAAA]$_{18}$
DYS460	10	10	[ATAG]$_{10}$
DYS481	25	25	[CTT]$_{25}$
DYS518	39	39	[AAAG]$_3$ GAAG [AAAG]$_{18}$ GGAG [AAAG]$_4$ N$_6$ [AAAG]$_{12}$
DYS533	11	11	[ATCT]$_{11}$
DYS549	11	11	[GATA]$_{11}$
DYS570	17	17	[TTTC]$_{17}$
DYS576	19	19	[AAAG]$_{19}$
DYS627	21	21	[AGAG]$_3$ [AAAG]$_{18}$
DYS635	21	21	[TCTA]$_4$ [TGTA]$_2$ [TCTA]$_2$ [TGTA]$_2$ [TCTA]$_{11}$
DYS643	13	13	[CTTTT]$_{13}$
DYF387S1a	35	35	[AAAG]$_3$ GTAG [GAAG]$_4$ N$_{20}$ [GAAG]$_9$ [AAAG]$_{13}$
DYF387S1b	39	39	[AAAG]$_3$ GTAG [GAAG]$_4$ N$_{20}$ [GAAG]$_{10}$[AAAG]$_{16}$
Y GATA H4	11	11	[TAGA]$_{11}$

Note: Sequence information in gray indicates bases that are not counted toward the length-based genotype designation

Table 16. GlobalFiler Y-Indel Information Values

Locus	**Component[(a)]**			
GlobalFiler Y-Indel	**B**	**C**	**D**	**F**
	2	2	2	2

[(a)] Component A does not have a Y-chromosome and thus has no type at this locus.

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