The New Standard Reference Material® 2391c: PCR-based DNA Profiling Standard

National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

Email:
margaret.kline@nist.gov

Margaret C. Kline, Erica L.R. Butts, Carolyn (Becky) R. Hill, Michael D. Coble, David L. Duewer, and John M. Butler

U.S. National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8314

P-348

Standard Reference Material® 2391c (SRM 2391c) is the fourth generation certified reference material for PCR-based DNA profiling. The first generation SRM 2391 was released in 1995; all subsequent generations have had minor modifications in the type and number of loci certified but always used DNA from the same donors**. SRM 2391c has been produced with an entirely new set of genomic DNA samples.** In addition to four liquid samples, SRM 2391c has two dry storage matrices including FTA paper as well as 903 paper.

SRM 2391c consists of six components: three are single source genomic DNA samples that are labeled A, B, and C, **with the fourth genomic sample (component D) as a mixture of components A and C (≈3:1 ratio).** Component E consists of two 6 mm punches of 903 paper that have been spotted with approximately 75,000 cells / spot. Component F consists of two 6 mm punches of FTA paper that have been spotted with approximately 75,000 cells / spot of a different cell line.

The six components representing 5 different DNA samples plus the mixture component have been analyzed using 22 commercially available STR typing kits, obtained from three different vendors, as well as the 26plex STR multiplex developed at NIST. **In total there is data for 51 autosomal STRs and 17 Y-STRs included in the certificate of analysis.**

SRM 2391c Component Descriptions

Component A is extracted genomic DNA from Buffy coat white blood cells from an anonymous female.

Components B and C are two cell line DNAs purchased from Coriell Cell Repositories (Camden, NJ): NA03469 and NA10451.

Component D is a 3:1 mixture of the Components A and C materials.

Components A,B,C and D are solubilized in TE^{-4} buffer (10 mmol/L Tris HCl, 0.1 mmol/L EDTA, pH 8.0)

Components E and F are "stains" created by depositing cells on to pre-punched paper spots that had been loaded into 96-well plates.

Component E is a "stain" on 903 paper of a female cell line (CRL-1486).

Component F is a "stain" on FTA paper of a male cell line (HTB-157).

These two cell lines were purchased from the American Type Culture Collection (Manassas, VA) and grown at NIST.

"Conventional" DNA concentrations of the materials used to prepare the SRM 2391c components A, B, and C were estimated from optical densities at 260 nm determined using the BioCary 100 spectrophotometer. The conventional assertion is that an aqueous solution of 50 ng/µL of double-stranded DNA in a 1.0-cm pathlength cuvette has at 260 nm an optical density, OD_{260}, of 1.0 [1]. Dilutions of these materials were made based on the OD_{260} measurements.

The DNA concentrations for the final dilutions of components A, B, C, and D were verified by qPCR using three commercial quantification kits and one in-house kit. The three commercial kits include Quantifiler Human DNA Quantification Kit (Life Technologies), Quantifiler Duo DNA Quantification Kit (Life Technologies), and Plexor HY System (Promega). SRM 2372: Human DNA Quantitation Standard component A was used to calibrate each assay. The range of the qPCR results are displayed in Table 1.

Table 1. Description of Components in SRM 2391c

Component	Description	Quantity [a]
A	50 µL of anonymous female genomic DNA	1.4 – 1.9 ng DNA/µL
B	50 µL of anonymous male genomic DNA	1.3 – 1.5 ng DNA/µL
C	50 µL of anonymous male genomic DNA	1.3 – 2.0 ng DNA/µL
D	50 µL of mixed-source (Components A and C)	1.4 – 2.0 ng DNA/µL
E	Two 6 mm punches of CRL-1486 cells spotted on 903 paper	7.5 x10^4 cells per punch
F	Two 6 mm punches of HTB-157 cells, spotted on FTA paper	7.5 x10^4 cells per punch

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

The STR genotypes for this SRM result from analyses performed at NIST; Palm Beach Sheriff's Office (West Palm Beach, FL); Bode Technology Group (Lorton, VA); Promega Corp. (Madison, WI); and Life Technologies (Foster City, CA).

All results are concordant across all kits and all laboratories.

In order to avoid any potential null alleles, the SRM 2391c components were tested with 22 different genotyping kits found in Table 2. **There were no discordant results observed in the tested loci with these materials.**

Table 2. STR Genotyping kits and primer mixes used at NIST to certify SRM 2391c

Kit Provider			Primer Mixes
Life Technologies	*Promega*	*Qiagen*	*NIST*
Identifiler	Powerplex 16	ESSplex	26plex [2]
Identifiler Plus	Powerplex 16 HS	IDplex	miniSTRs [3,4]
NGM	Powerplex ESX 17		
NGM SElect	Powerplex ESI 17		
COfiler	Powerplex ES		
Profiler	Powerplex S5		
Profiler Plus	Powerplex Y		
Profiler Plus ID	FFFL		
SGM Plus			
SEfiler			
MiniFiler			
Yfiler			

References:

[1] Sambrook, J. and Russell D.W. (2001) Molecular Cloning a Laboratory Manual, Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York**.**

[2] Hill, C.R., Butler, J.M., Vallone, P.M. (2009) A 26plex autosomal STR assay to aid human identity testing. *J. Forensic Sci.* 54(5): 1008-1015.

[3] Hill, C.R., Kline, M.C., Coble, M.D., Butler, J.M. (2008) Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. *J. Forensic Sci*. 53(1):73-80.

[4] Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. *J. Forensic Sci.* 50: 43-53.

[5] Perlin MW and Sinelnikov A. (2009) An information gap in DNA evidence interpretation. *PLoS One* 4(12):e8327; http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0008327

[6] JCGM 100:2008. Guide to the Expression of Uncertainty in Measurement (GUM). BIPM, Sèvres, France; http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf.

[7] Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results;* NIST Technical Note 1297, U.S. Government Printing Office, Washington, DC (1994); http://physics.nist.gov/Pubs/guidelines/TN1297/tn1297s.pdf.

Poster available for download from STRBase
http://www.cstl.nist.gov/biotech/strbase/pub_pres/KlineISFG2011poster.pdf

The SRM delivers Certified Values for genotypes at loci when two or more kits use different primers to generate the Polymerase Chain Reaction (PCR) products or when the results obtained with a single set of primers have been confirmed by sequencing. It also delivers a Certified Value for the relative composition of Component D that was prepared from the source materials used for two of the other components.

Table 3. Certified Genotypes of 41 STR Loci and Amelogenin

	Component					
Locus	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
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
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
DYS456		15	15	15		15
DYS458		17.2	17	17		18
DYS635		20	21	21		21
Y GATA H4		11	11	11		11

The SRM delivers Reference Values for loci assigned from repeat counts based on electrophoretic base pair (bp) size differences between non-sequenced alleles compared to sequenced alleles.

NIST Information Value is data that may be of interest and use to the SRM user, but insufficient information is available to access the confidence of the assignment (Penta C)

Table 4. Genotypes of 26 Reference STR Loci and 1 Information STR Locus

	Component					
Locus	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
Penta C	11,12	12,13	5,9	5,9,11,12	12,13	12,12

Funding This project was supported by 2008-DN-R-121, which is an interagency agreement between NIJ and the NIST Office of Law Enforcement Standards, awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice. Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

SRM 2391c Component D: The Mixture

Component D is a mixture prepared by gravimetrically combining three parts of Component A with one part of Component C. The preparative composition of this mixture was verified using peak height ratios from the multiplex assays used to assign genotypes and results from the TrueAllele electropherogram deconvolution software package [5].

The certified ratio for Component D, the mass of Component A relative to that of Component C, is

3.1 ± 0.1 Component A / Component C.

The uncertainty in the value, calculated according to the method described in the *Guide to the Expression of Uncertainty in Measurement* (GUM) [6, 7], is expressed as an expanded uncertainty, *U*. The expanded uncertainty is calculated as $U = k \times u_c$, where u_c is the combined uncertainty and the coverage factor k=2.6 corresponds to approximately 95 % confidence.

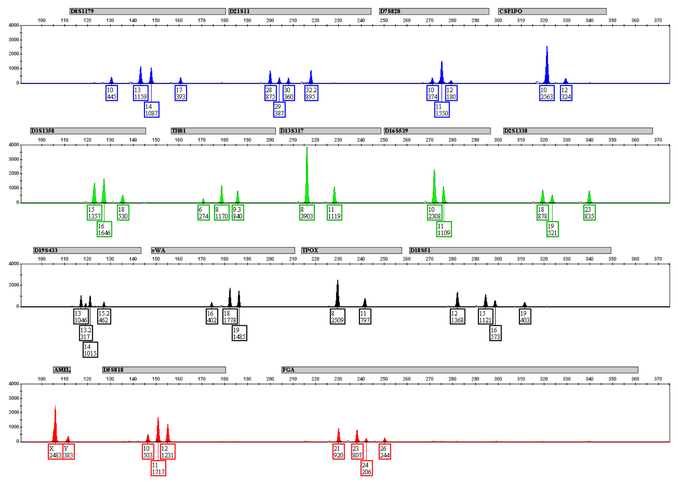

Figure 1. Illustrative electropherogram of Component D amplified with Identifiler

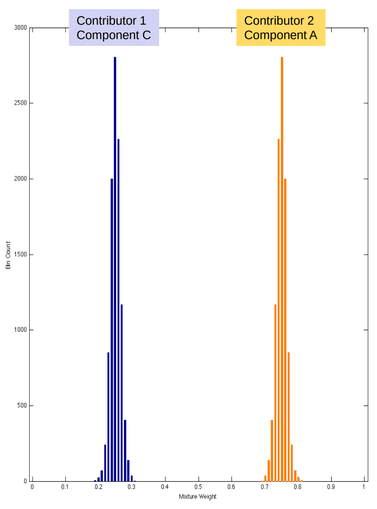

Figure 2: Illustrative TrueAllele deconvolution of Component D contributors.

The analysis used the .fsa file from a single amplification of Component D with the Identifiler multiplex on the ABI 3130xl. The values below the graph are the relative contributions of two contributors. The genotype of "Contrib 1" corresponds to Component C, that of "Contrib 2" to Component A.

Mixture Variance: NIST SRM2391c

Template	Contrib	Weight	Stdev	95% Interval
SRM2391c	1	0.252	0.015	[0.223, 0.281]
SRM2391c	2	0.748	0.015	[0.719, 0.777]

Table 5. Results for TrueAllele Deconvolution of Component D Profiles

		TrueAllele		Weight / (1-Weight)		
Multiplex	Input ng	Weight	Stdev	Ratio	u	U_{95}
COfiler	1	0.775	0.036	3.44	0.79	1.53
Identifiler	1	0.748	0.015	2.97	0.24	0.47
PP16 HS	1	0.743	0.025	2.89	0.39	0.76
PP16 HS	2	0.751	0.029	3.02	0.49	0.96
Profiler Plus	1	0.766	0.019	3.27	0.36	0.69
SGM Plus	1	0.734	0.024	2.76	0.35	0.68

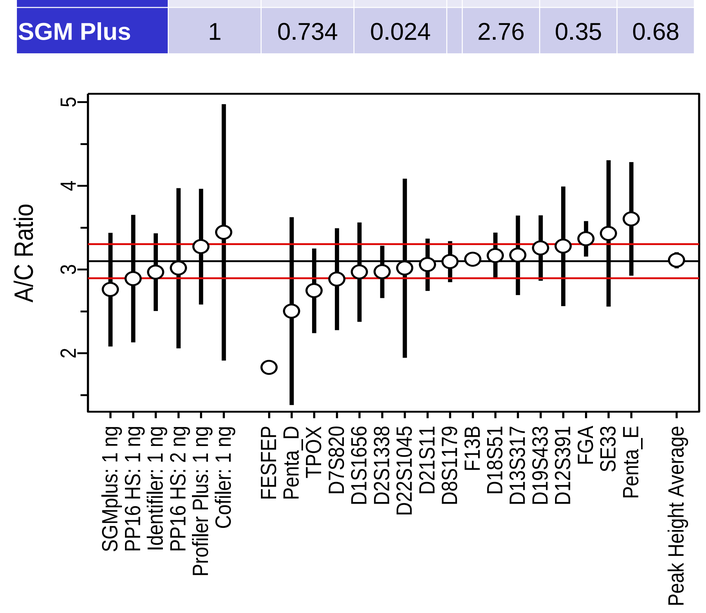

Figure 3: Summary of mass ratio evidence for Component D

The dots represent the mean estimate for each of the lines of evidence, with bars spanning the full 95 % level of confidence interval on the mean. Bars are not shown for the two loci characterized with only one multiplex. The "Peak Height Average" is the mean of the **140** discrete peak height ratios provided by the Life Technologies and Promega STR kits listed in Table 2 for 17 loci with unshared alleles for Components A and C. The red horizontal lines bound the 3.1 ± 0.1 95 % level of confidence interval about the preparative value.