National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2391b

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 and for instructional law enforcement or non-clinical research purposes. This SRM can also be used for quality assurance when assigning values to in-house control materials. It is not intended for any human or animal clinical diagnostic use. Note that SRM 2391b is slightly modified from SRM 2391, in that there is more emphasis on Short Tandem Repeats (STRs) and less emphasis on D1S80 [1,2] reflecting the growing interest and utility of STRs [3 to 14]. Additional information on each STR locus can be found at a NIST-sponsored database on the internet: http://www.cstl.nist.gov/biotech/strbase [14].

This SRM is composed of well-characterized human deoxyribonucleic acid (DNA) in two forms: genomic DNA and DNA to be extracted from cells spotted onto filter paper. A unit of the SRM is composed of 12 frozen components packaged in one box. See the section in this certificate entitled *Description of Components* for a complete listing of the components.

Certified Values: The SRM is certified for genetic loci of forensic interest that were commercially available at the time of production. Genetic types for these loci can be found in Tables 1, 2, and 3. The tables are organized as follows: Table 1 lists the genetic types for the Federal Bureau of Investigation's (FBI's) CODIS (COmbined DNA Index System) core STR loci; Table 2 lists additional STR loci of interest; and Table 3 lists the genetic types for D1S80, AmpliType® PM + HLADQA1, and Amelogenin.

Expiration of Certification: The certification of this SRM is valid until **31 December 2008**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is contaminated or otherwise modified.

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 certification, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

Storage: Store frozen at a temperature of -20 ºC. **DO NOT** use a self-defrosting freezer because periodic cycling of temperatures may cause shortened shelf life of this SRM.

The overall direction and coordination of the technical activities leading to certification were under the chairmanship of J.M. Butler of the NIST Biotechnology Division.

Analytical determination and technical measurements leading to the certification of this SRM were performed by M.C. Kline and J.W. Redman of the NIST Biotechnology Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Group by C.S. Davis.

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NOTICE AND WARNINGS TO USER

Warning: SRM 2391b IS A HUMAN SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE.

Use: Sample aliquots for analysis should be withdrawn immediately after opening the vials and should be processed without delay for the certified values to be applicable.

Source of Material: Genomic DNA components 1 through 8 were obtained from Roche Molecular Systems, Inc., Alameda, CA.[1] Cell Lines GM09947A and GM09948 were obtained from Marligen Biosciences Inc., Ijamsville, MD. [1]

Interlaboratory Analysis: The STR values for this SRM represent the pooled results from analyses performed at NIST; Pennsylvania State Police DNA Laboratory, Greensburg, PA; Oregon State Police Forensic Laboratory, Portland, OR; Promega Corp., Madison, WI; and Applied Biosystems, Foster City, CA. A detailed list of the amplification kits used at NIST to obtain the STR values and other genetic loci types are shown in Table 4.

Description of Components: Twelve components are included in each unit; all components must be stored at -20 ºC. Components #1 through #10 each contain 20 µL of genomic DNA at a concentration of approximately 1 ng/µL.

#1	Genomic DNA 1
#2	Genomic DNA 2
#3	Genomic DNA 3
#4	Genomic DNA 4
#5	Genomic DNA 5
#6	Genomic DNA 6
#7	Genomic DNA 7
#8	Genomic DNA 8
#9	Genomic GM09947A
#10	Genomic GM09948
#11	Cell GM09947A – 2 x 10^5 cells on a 7 mm Scleicher & Schull 903™ filter paper circle[1]
#12	Cell GM09948 – 2 x 10^5 cells on a 7 mm Scleicher & Schull 903™ filter paper circle[1]

NOTE: DNA concentrations given are nominal values and are not intended for use as concentration standards.

Typing results are shown in Tables 1 through 3. All results are identical with those of SRM 2391a with the following quantitative exceptions:

The relative intensities of the three alleles of GM09948 cell line at the HUMCSF1PO locus (CSF1PO) are not the same. While this cell line was determined to be karyologically stable by Fregeau et al. [3], these authors did not study the CSF1PO locus. The observed CSF1PO locus differences may be due to differences in cell generation passage numbers of the two materials. While less noticeable, these CSF1PO allele intensity differences have been present in the earlier SRM 2391 releases, see figure below Table 1.

[1]Certain commercial 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 identified are necessarily the best available for the purpose.

Table 1. Certified Values for the FBI's CODIS 13 STR Loci

Component Number	Description	CSF1PO	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D21S11	FGA	TH01	TPOX	vWA
1	Genomic 1	12,12	14,17	12,12	9,10	13,13	11,13	12,14	14,14	29,33.2	21,22	6,7	8,11	17,17
2	Genomic 2	11,12	15,16	12,12	9,10	11,16	8,11	12,12	10,14	29,30	20,22	8,9.3	8,10	14,16
3	Genomic 3	11,12	15,15	11,11	12,13	14,16	11,12	11,12	16,20	28,31.2	23,25	9.3,9.3	8,11	18,19
4	Genomic 4	11,12	15,17	11,11	8,10	14,14	12,12	9,10	18,18	28,30	18,22	7,9	8,9	17,17
5	Genomic 5	10,12	15,18	11,12	8,10	15,16	11,12	9,11	14,16	28,30	23,26	7,7	10,11	16,20
6	Genomic 6	10,13	14,17	12,12	8,11	10,16	12,13	12,13	18,18	28,29	21,26	9,9.3	8,8	16,18
7	Genomic 7	10,11	14,15	11,12	9,9	13,15	11,12	10,10	13,16	28,31.2	23,24	6,7	8,11	16,16
8	Genomic 8	10,12	15,18	12,13	9,10	12,14	9,13	9,11	15,18	30,31	24,28	7,8	8,12	15,17
9	Genomic GM09947A	10,12	14,15	11,11	10,11	13,13	11,11	11,12	15,19	30,30	23,24	8,9.3	8,8	17,18
10	Genomic GM09948	10,11, 12	15,17	11,13	11,11	12,13	11,11	11,11	15,18	29,30	24,26	6,9.3	8,9	17,17
11	GM09947A Cells	10,12	14,15	11,11	10,11	13,13	11,11	11,12	15,19	30,30	23,24	8,9.3	8,8	17,18
12	GM09948 Cells	10,11,or 10,11,12*	15,17	11,13	11,11	12,13	11,11	11,11	15,18	29,30	24,26	6,9.3	8,9	17,17

- The relative intensity of the 12 allele is less than 7 %
- of that of the dominant 10 allele. See accompanying figure.

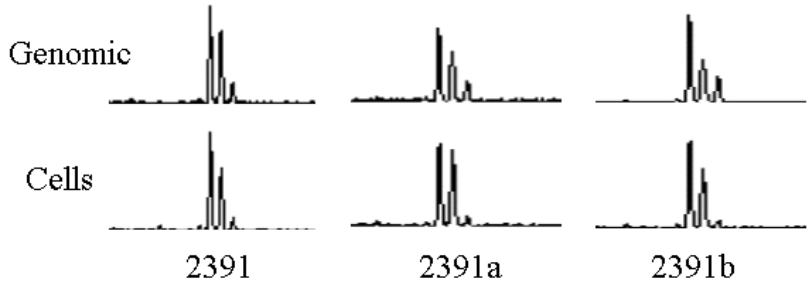
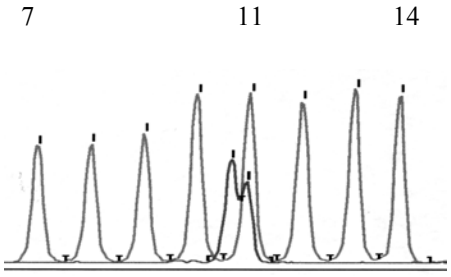


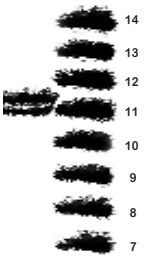
Table 2. Certified Values for Additional STR Loci

Component Number	Description	F13A01	F13B	FES/FPS	LPL	Penta D	Penta E	D2S1338	D19S433	SE 33
1	Genomic 1	6,7	10,10	12,12	10,11	10,15	7,12	17,23	13,16.2	20,30.2
2	Genomic 2	7,7	8,10	10,11	10,11	9,11	7,12	17,26	14,16	23.2,28.2
3	Genomic 3	3.2,4	9,10	11,12	11,12	11,12	13,14	20,24	12,14	14.2,26.2
4	Genomic 4	5,6	6,9	10,13	10,12	8,9	5,12	17,23	11,13	22,28.2
5	Genomic 5	5,7	8,9	11,13	10,12	10,13	7,13	17,19	12.2,14	14,30.2
6	Genomic 6	3.2,5	9,10	11,11	10,12	9,12	12,14	25,25	12,14	20,21
7	Genomic 7	5,8	6,8	11,11*	11,12	3.2,11	12,16	17,22	13,15.2	13.2,20
8	Genomic 8	3.2,5	6,8	10,11	9,11	8,9	5,10	22,22	12.2,15	16,27.2
9	Genomic GM09947A	6,16	8,10	10,12	11,12	12,12	12,13	19,23	14,15	19,29.2
10	Genomic GM09948	6,6	8,8	11,11	10,12	8,12	11,11	23,23	13,14	23.2,26.2
11	GM09947A Cells	6,16	8,10	10,12	11,12	12,12	12,13	19,23	14,15	19,29.2
12	GM09948 Cells	6,6	8,8	11,11	10,12	8,12	11,11	23,23	13,14	23.2,26.2

*Genomic 7 has a variant allele at the FES/FPS locus. Many analysis systems type this as an 11 homozygote; however, this sample displays an 11 and an 11 variant allele when separated in a Genetic Analyzer 310 Capillary Electrophoresis unit and in a gel-based electrophoretic system used at NIST as shown below. Preliminary sequence information of this 11 variant allele indicates two sequence changes, one on either side of the repeat region.



Capillary Electrophoresis of FES/FPS locus, Genomic 7 sample and ladder peaks 7 through 14 with the variant allele peaks appearing as a doublet at allele 11



Gel-based separation and image of FES/FPS ladder bands 7 through 11, with Genomic 7 band appearing as a doublet at allele 11

Table 3. Certified Values for Additional Genetic Loci

Component Number	Description	AmpliType® HLA-DQA1	AmpliType® PM	D1S80	Amelogenin
1	Genomic 1	2,4.1	AA,AB,AA,AA,CC	28,31	XY
2	Genomic 2	1.1,3	AB,BB,AB,AB,AC	18,24	XX
3	Genomic 3	1.3,4.1	BB,AA,BB,BB,AA	18,18	XY
4	Genomic 4	4.1,4.2/4.3	AB,AA,AB,AA,AB	21,24	XX
5	Genomic 5	4.1,4.1	BB,AA,BC,AA,BC	17,28	XX
6	Genomic 6	4.1,4.1	AB,AB,AB,AB,AC	24,37	XX
7	Genomic 7	1.2,4.1	BB,BB,CC,AB,BB	24,28	XY
8	Genomic 8	1.2,2	BB,BB,AC,AA,BB	17,21	XX
9	Genomic GM09947A	4.1,4.2/4.3	AA,AB,AB,AA,AC	18,31	XX
10	Genomic GM09948	1.2,3	AB,AB,BB,AB,BC	18,25	XY
11	GM09947A Cells	4.1,4.2/4.3	AA,AB,AB,AA,AC	18,31	XX
12	GM09948 Cells	1.2,3	AB,AB,BB,AB,BC	18,25	XY

Table 4. Amplification "kits" Used at NIST to Genotype STR Loci

	Applied Biosystems				Promega			
Genetic Locus	Identifiler	Profiler Plus	COfiler	SGM Plus	Power Plex 16	Power Plex ES	Power Plex 16 BIO	FFFL
Amelogenin	X	X	X	X	X	X	X	
CSF1PO	X		X		X		X	
D2S1338	X			X				
D3S1358	X		X	X	X	X	X	
D5S818	X	X			X		X	
D7S820	X	X	X		X		X	
D8S1179	X	X		X	X	X	X	
D13S317	X	X			X		X	
D16S539	X		X	X	X		X	
D18S51	X	X		X	X	X	X	
D19S433	X			X				
D21S11	X	X		X	X	X	X	
F13A01								X
F13B								X
FES/FPS								X
FGA	X	X		X	X	X	X	
LPL								X
PENTA D					X		X	
PENTA E					X		X	
SE33						X		
TH01	X		X	X	X	X	X	
TPOX	X		X		X		X	
vWA	X	X		X	X	X	X	

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Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Group at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.