



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 2391a

PCR-based DNA Profiling Standard

Standard Reference Material (SRM) 2391a 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 2391a is slightly modified from SRM 2391, in that there is more emphasis on Short Tandem Repeats (STRs) and less emphasis on D1S80, reflecting the growing interest and utility of STRs. Additional information on each STR locus can be found at a NIST-sponsored database on the internet: <http://www.cstl.nist.gov/biotech/strbase>.

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 2003**, 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 °C. 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 D.J. Reeder 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 Program by J.C. Colbert.

Gary L. Gilliland, Chief
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Gaithersburg, MD 20899
Certificate Issue Date: 17 March 2000

Thomas E. Gills, Director
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NOTICE AND WARNINGS TO USER

Warning: SRM 2391a 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. Cell Lines GM09947A and GM09948 were obtained from Life Technologies, Inc., Gaithersburg, MD.

Interlaboratory Analysis: The STR values for this SRM represent the pooled results from analyses performed at NIST, DNA Identity Laboratory, Institute of Legal Medicine, University of Berne, Switzerland, Promega Corp., Madison, WI, and PE-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 detailed in Table 4.

Description of Components: Twelve components are included in each unit; all components must be stored at -20 °C. Components #1 through #10 each contain 20 µL of genomic DNA at a concentration of 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 – 5 x 10 ⁴ cells on a 6 mm Schleicher & Schull 903™ filter paper circle ¹ ,
#12	Cell GM09948 – 5 x 10 ⁴ cells on a 6 mm Schleicher & Schull 903™ filter paper circle ¹

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

¹Certain 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.

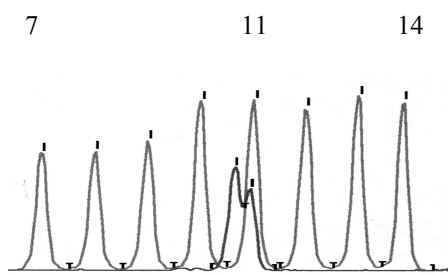
Table 1. Certified Values for the FBI's CODIS 13 STR Loci

Component Number	Description	CSFIPO	D3S1358	D5S818	D7S820	D8S1179	D13S317	DI6S539	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	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
12	Cells 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

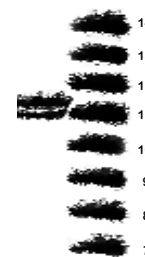
Table 2. Certified Values for Additional STR Loci

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

*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® HLADQA1	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	X
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	X
5	Genomic 5	4.1,4.1	BB,AA,BC,AA,BC	17,28	X
6	Genomic 6	4.1,4.1	AB,AB,AB,AB,AC	24,37	X
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	X
9	Genomic GM09947A	4.1,4.2/4.3	AA,AB,AB,AA,AC	18,31	X
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	X
12	GM09948 Cells	1.2,3	AB,AB,BB,AB,BC	18,25	XY

Table 4. Amplification “Kits” Used at NIST to Validate STRs Loci

Genetic Locus	PE-Biosystems				Promega Corp.	
	Profiler	Profiler Plus	COfiler	SGM Plus	Power Plex 2.1	FFFL
Amelogenin	X	X	X	X		
CSF1PO	X		X			
D2S1338				X		
D3S1358	X		X	X	X	
D5S818	X	X				
D7S820	X	X	X			
D8S1179		X		X	X	
D13S317	X	X				
D16S539			X	X		
D18S51		X		X	X	
D19S433				X		
D21S11		X		X	X	
F13A01						X
F13B						X
FES/FPS						X
FGA	X	X		X	X	
LPL						X
PENTA E					X	
TH01	X		X	X	X	
TPOX	X		X		X	
vWA	X	X		X	X	

REFERENCES

- [1] Nakamura, Y., Carlson, M., Krapcho, V., and White, R., "Isolation and Mapping of a Polymorphic DNA Sequence (pMCT118) on Chromosome 1p (D1S80)," *Nucleic Acids Res.* **16**, p. 9364, (1988).
- [2] Kasai, K., Nakamura, Y., and White, R., "Amplification of a Variable Number of Tandem Repeats (VNTR) Locus (pMCT118) by the Polymerase Chain Reaction (PCR) and its Application to Forensic Science," *Journal of Forensic Sciences* **35**, pp. 1196-1200, (1990).

Certificate Revision History: 17 March 2000 (This revision corrects the measurement units on page 2); 28 December 1999 (original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: Telephone (301) 975-6776 (select "Certificates"), Fax (301) 926-4751, e-mail srminfo@nist.gov, or via the Internet <http://ts.nist.gov/srm>.