National Institute of Standards and Technology

Certificate of Analysis

Standard Reference Material® 2391

PCR-based DNA Profiling Standard

(In Cooperation with the National Institute of Justice - U.S. Department of Justice)

Standard Reference Material (SRM) 2391 is intended primarily for use 1) in the standardization of forensic and paternity quality assurance procedures for Polymerase Chain Reaction (PCR)-based genetic testing and 2) for instructional law enforcement or non-clinical research purposes. It is not intended for any human or animal clinical diagnostic use.

This SRM is composed of well-characterized human DNA in three forms: genomic DNA, amplified DNA, and DNA to be extracted from cells spotted onto filter paper. It also contains a DlS80 allelic ladder for characterization of amplified DNA. Additionally, a DNA size marker is included to assure that electrophoretic separations are properly performed. A unit is composed of 20 frozen components packaged in two separate boxes. Unamplified DNA is packaged separately from amplified DNA. See the section on page 2 of this certificate entitled "Description of Components" for a complete listing of the components. The Annex to this certificate provides a detailed description and use for each of the SRM 2391 components.

Certified Values: The SRM is certified for the genetic locus D1S80 (pMCT118) [1]. The certified values are only valid if the primers specified by Kasai, et. al. [2] are used. Table 1 lists the certified DlS80 types (nominal alleles) for each of the components.

Reference Values: Reference values for additional genetic systems are included in Tables 2 and 3. All values were typed by NIST and collaborating laboratories listed in Table 4. All data from all laboratories were unanimously confirmed to be the values provided. All typing was done with commercially available reagents. For some loci, allelic calls are listed that show higher resolution than can be seen on some analytical systems, e.g. 11,11.2 of FES/FPS genomic 7-component 9. In such cases, binning to the nominal allele, e.g., 11, is valid. Additional information on each STR locus in Table 2 can be found at a NIST-sponsored database on the internet: http://ibm4.carb.nist.gov:8800/dna/home.htm.

Expiration of Certification: Stored as specified in the section below, the certified values should remain valid for at least 3 months from the date of shipment from NIST. NIST will keep samples of this material under surveillance for at least 6 months after the date of last sale. If changes occur, NIST will notify purchasers. In absence of such notification, the user should not use the material beyond 3 months after the date of shipment from NIST.

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 Box A and Box B 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.

Internal quality control checks at NIST have indicated that some of the screw-top lids were not sufficiently tightened at the time of manufacturing of "Box B " which contains the Genomic DNA Samples and Cell Lines. At -20 °C storage, the liquid contents of some of the tubes may have dried or desiccated. Please reconstitute any dry tubes with 21 µL of sterile water and proceed as usual with your test protocols. In all cases when the procedure was done at NIST, the contents of the tubes, when reconstituted as above, performed in the same manner as when they were originally certified.

The technical and support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by J.C. Colbert.

Gaithersburg, MD 20899
Certificate Issue Date: 3 June 1998*
26 May 95 (original certificate date)
*Revised to include additional genetic loci.

Thomas E. Gills, Chief
Standard Reference Materials Program

The overall direction of the technical measurement leading to certification was under the chairmanship of D.J. Reeder of the NIST Biotechnology Division.

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

NOTICE AND WARNINGS TO USER

The DNA and cells were derived from healthy human subjects. The suppliers of these materials have tested the source from which the DNA and cells were derived and found them to be nonreactive for hepatitis B surface antigen (HB_sAG) and HIV by FDA-approved testing. However, no test method can ensure that a product derived from human blood does not contain HIV, hepatitis, or other infectious agents. HANDLE AS IF CAPABLE OF TRANSMITTING 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. Certified values are not applicable to material stored in vials that have been opened, even if they are resealed.

Source of Material: The DNA, Amplified and Genomic, as well as the D1S80 Ladder were obtained from Roche Molecular Systems, Inc.[1], Alameda, CA. The Cell Lines, GM09947A and GM09948 as well as the 100bp ladder were obtained from Life Technologies, Inc., Gaithersburg, MD. Roche Molecular Systems, Inc. supplied amplified DlS80 from Cell Lines GM09947A and GM09948 (from genomic DNA provided by Life Technologies, Inc.).

Interlaboratory Analysis: The certified values for this SRM represent the pooled results from analyses performed at NIST and two collaborating laboratories. Prior to certification, three rounds of interlaboratory testing of various components were accomplished with the help of 20 laboratories. Additional laboratories participated in providing values for the STRs. Laboratories participating in these studies are listed in Table 4.

Description of Components: Twenty components are included in each unit; all components must be stored at -20 °C.

Box A: Amplified products and ladders

#1 - DlS80 Allelic Ladder (60 µL)
#2 - 100 bp Ladder (25 µL)
#13 - Amplified DNA 1 (15 µL)
#14 - Amplified DNA 2 (15 µL)
#15 - Amplified DNA 3 (15 µL)
#16 - Amplified DNA 4 (15 µL)
#17 - Amplified DNA GM09947A (15 µL)
#18 - Amplified DNA GM09948 (15 µL)

Box B: Genomic DNA samples and Cell Lines

#3 - Genomic DNA 1 (0.5 ng/µL, 20 µL)
#4 - Genomic DNA 2 (0.5 ng/µL, 20 µL)
#5 - Genomic DNA 3 (0.5 ng/µL, 20 µL)
#6 - Genomic DNA 4 (0.5 ng/µL, 20 µL)
#7 - Genomic DNA 5 (0.5 ng/µL, 20 µL)
#8 - Genomic DNA 6 (0.5 ng/µL, 20 µL)
#9 - Genomic DNA 7 (0.5 ng/µL, 20 µL)
#10 - Genomic DNA 8 (0.5 ng/µL, 20 µL)
#11 - Genomic GM09947A (0.5 ng/µL, 20 µL)
#12 - Genomic GM09948 (0.5 ng/µL, 20 µL)
#19 - Cell GM09947A - 6 x 10^4 cells on a 6 mm Scleicher & Schull 903™ filter paper circle
#20 - Cell GM09948 - 6 x 10^4 cells on a 6 mm Scleicher & Schull 903™ filter paper circle

[1]Certain commercial materials are identified on this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards & Technology, nor does it imply that the materials identified are necessarily the best available for the purpose.

Table 1. Certified Values for Genetic Locus D1S80

Component #	Description	D1S80
3	Genomic 1	28,31
4	Genomic 2	18,24
5	Genomic 3	18,18
6	Genomic 4	21,24
7	Genomic 5	17,28
8	Genomic 6	24,37
9	Genomic 7	24,28
10	Genomic 8	17,21
11	Genomic GM09947A	18,31
12	Genomic GM09948	18,25
13	Amplified 1	28,31
14	Amplified 2	18,18
15	Amplified 3	24,37
16	Amplified 4	17,21
17	Amplified GM09947A	18,31
18	Amplified GM09948	18,25
19	GM09947A Cells	18,31
20	GM09948 Cells	18,25

Table 2. Reference Values for Short Tandem Repeats (STR) Loci

Component #	Description	CSF1P0	D3S1358	D5S818	D7S820	D13S317	D16S539	F13A01	F13B	FES/ FPS	FGA	LPL	TH01	TPOX	vWA	D8S1179	D18S51	D21S11
3	Genomic 1	12,12	14,17	12,12	9,10	11,13	12,14	6,7	10,10	12,12	21,22	10,11	6,7	8,11	17,17	13,13	14,14	29,33.2
4	Genomic 2	11,12	15,16	12,12	9,10	8,11	12,12	7,7	8,10	10,11	20,22	10,11	8,9.3	8,10	14,16	11,16	10,14	29,30
5	Genomic 3	11,12	15,15	11,11	12,13	11,12	11,12	3.2,4	9,10	11,12	23,25	11,12	9.3,9.3	8,11	18,19	14,16	16,20	28,31.2
6	Genomic 4	11,12	15,17	11,11	8,10	12,12	9,10	5,6	6,9	10,13	18,22	10,12	7,9	8,9	17,17	14,14	18,18	28,30
7	Genomic 5	10,12	15,18	11,12	8,10	11,12	9,11	5,7	8,9	11,13	23,26	10,12	7,7	10,11	16,20	15,16	14,16	28,30
8	Genomic 6	10,13	14,17	12,12	8,11	12,13	12,13	3.2,5	9,10	11,11	21,26	10,12	9,9.3	8,8	16,18	10,16	18,18	28,29
9	Genomic 7	10,11	14,15	11,12	9,9	11,12	10,10	5,8	6,8	11,11.2	23,24	11,12	6,7	8,11	16,16	13,15	13,16	28,31.2
10	Genomic 8	10,12	15,18	12,13	9,10	9,13	9,11	3.2,5	6,8	10,11	24,28	9,11	7,8	8,12	15,17	12,14	15,18	30,31
11	Genomic GM09947A	10,12	14,15	11,11	10,11	11,11	11,12	6,16	8,10	10,12	23,24	11,12	8,9.3	8,8	17,18	13,13	15,19	30,30
12	Genomic GM09948	10,11,12	15,17	11,13	11,11	11,11	11,11	6,6	8,8	11,11	24,26	10,12	6,9.3	8,9	17,17	12,13	15,18	29,30
19	GM09947A Cells	10,12	14,15	11,11	10,11	11,11	11,12	6.16	8,10	10,12	23,24	11,12	8,9.3	8,8	17,18	13,13	15,19	30,30
20	GM09948 Cells	10,11,12	15,17	11,13	11,11	11,11	11,11	6,6	8,8	11,11	24,26	10,12	6,9.3	8,9	17,17	12,13	15,18	29,30

Table 3. Additional Reference Values for Commonly Used Loci

Component Number	Description	AmpliType®[2] HLADQAI	AmpliType®[2] PM	Amelogenin
3	Genomic 1	2,4.1	AA,AB,AA,AA,CC	XY
4	Genomic 2	1.1,3	AB,BB,AB,AB,AC	X
5	Genomic 3	1.3,4.1	BB,AA,BB,BB,AA	XY
6	Genomic 4	4.1,4.2/4.3	AB,AA,AB,AA,AB	X
7	Genomic 5	4.1,4.1	BB,AA,BC,AA,BC	X
8	Genomic 6	4.1,4.1	AB,AB,AB,AB,AC	X
9	Genomic 7	1.2,4.1	BB,BB,CC,AB,BB	XY
10	Genomic 8	1.2,2	BB,BB,AC,AA,BB	X
11	Genomic GM09947A	4.1,4.2/4.3	AA,AB,AB,AA,AC	X
12	Genomic GM09948	1.2,3	AB,AB,BB,AB,BC	XY
19	GM09947A Cells	4.1,4.2/4.3	AA,AB,AB,AA,AC	X
20	GM09948 Cells	1.2,3	AB,AB,BB,AB,BC	XY

[2]The use of a trademark on this certificate is for identification only and does not imply endorsement of the product by the National Institute of Standards and Technology.

Table 4. Cooperative Analyses for Value Assignment were Performed in the Following Laboratories

Alabama Department of Forensic Sciences*	Birmingham, AL
Armed Forces DNA Identification Laboratory	Rockville, MD
Broward Sheriff's Crime Laboratory	Ft. Lauderdale, FL
California Department of Justice NA Laboratory	Berkeley, CA
Chicago Police Crime Laboratory	Chicago, IL
Commonwealth of Virginia Division of Forensic Sciences*	Richmond, VA
Connecticut Department of Public Safety Forensic Science Laboratory	Meriden, CT
Directions des Expertises Judiciaries	Montreal, Quebec, Canada
Dubai Police Headquarters C.I.D. Criminal Laboratory	Dubai, United Arab Emirates
FBI Academy - Forensic Science Research and Training Center	Quantico, VA
Forensic Science Associates	Richmond, CA
Georgia Bureau of Investigation Division of Forensic Sciences*	Decatur, GA
Illinois State Police Crime Laboratory*	Springfield, IL
Kentucky State Police	Frankfort, KY
Metro-Dade Police Department	Miami, FL
Maine State Police Crime Laboratory**	Augusta, ME
Michigan State Police DNA Laboratory	East Lansing, MI
Minnesota Forensic Science Laboratory	St. Paul, MN
New York State Police Crime Laboratory**	Albany, NY
North Carolina State Bureau of Investigation**	Raleigh, NC
Office of Chief Medical Examiner	New York, NY
Orange County Sheriff-Coroner Department Forensic Science Services	Santa Ana, CA
Roche Biomedical Laboratories, Inc.	Research Triangle Park, NC
Royal Canadian Mounted Police Central Forensic Laboratory*	Ottawa, Ontario, Canada
Suffolk County Crime Laboratory**	Hauppauge, NY
University of California, Berkeley School of Public Health, Forensic Science Group	Berkeley, CA
University of North Texas Health Science Center of Fort Worth	Ft. Worth, TX

* Those laboratories who participated in all three interlaboratory tests.
** Those laboratories who contributed STR data.

REFERENCES

[1] Nakamura, Y., Carlson, M., Krapcho, V., and White, R., Isolation and Mapping of a Polymorphic DNA Sequence (pMCT118) on Chromosome lp (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).

It is the responsibility of users of this SRM to assure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: Phone (301) 975-6776 (select "Certificates"), Fax (301) 926-4751, e-mail srminfo@nist.gov, or via the intemet http://ts.nist.gov/srm.