



# 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. SRM 2391b 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 certification of D1S80 has been dropped [1,2], reflecting the growing interest and utility of STRs [3–16]. 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 (components 1 through 10) and DNA to be extracted from cells that are spotted onto filter paper (components 11 and 12). A unit of SRM 2391b is composed of twelve frozen components packaged in one box. See the section in this certificate entitled “Description of Components” and Table 1 for a complete listing of the components.

**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 [17]. The SRM is certified for genetic loci of forensic interest that were commercially available at the time of the certificate update. Genetic types for these loci can be found in Tables 2 and 3. The tables are organized as follows: Table 2 lists the genetic types for the U.S. Federal Bureau of Investigation’s (FBI’s) CODIS (Combined DNA Index System) core STR loci and Table 3 lists additional STR loci of interest. Table 4 lists the genetic types for 26 non-CODIS (NC) miniSTR loci. Certification of these NC miniSTR loci alleles is based on sequencing results in combination with genotyping results. Those allele calls, listed in Table 4 as bold font, are certified values based on the presence of allele sequence information for all alleles of the SRM components that is concordant with the genotypes obtained. Complete sequencing results, listed as repeat motifs for the SRM components, are available at <http://www.cstl.nist.gov/biotech/strbase/srm2391b.htm>.

**Reference Values:** A NIST Reference Value is a best estimate of the true value provided by NIST where all known or suspected sources of bias have not been fully investigated by NIST [17]. Table 4 also lists the genetic types for 26 non-CODIS (NC) miniSTR loci in normal font that are considered reference values. Reference values have been assigned when there is sequence information for only a few alleles of a particular locus. The alleles that do not have sequence information for the components of this SRM are considered reference values in terms of the repeat count based on base pair (bp) size differences of an unsequenced allele compared to a sequenced allele for that locus. While these are reference values there is confidence in their allele assignments.

**Expiration of Certification:** The certification of SRM 2391b is valid, within the measurement uncertainties specified, until **31 December 2013**, 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.

The overall direction and coordination of the technical activities leading to certification were under the leadership of M.C. Kline of the NIST Biochemical Science Division.

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*See Certificate Revision History on Last Page*

Analytical determinations and technical measurements leading to the certification of this SRM were performed by staff of the NIST Biochemical Science Division: M.C. Kline and J.W. Redman prepared the samples; M.C. Kline initially evaluated the components using selected PCR amplification kits to determine the certified genotypes; A.E. Decker and M.C. Kline evaluated the stability of the components in selected quantitative PCR (qPCR) assays; C.R. Hill performed a concordance evaluation of the components by comparing the initial genotypes with recently obtained genotypes using short tandem repeat (STR) PCR amplification kits; and C.R. Hill performed sequencing and genotyping of 26 miniSTR loci to determine certified and reference values.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

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

**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) will facilitate notification.

## **NOTICE AND WARNINGS TO USER**

**Storage:** Store frozen at a temperature of  $-20^{\circ}\text{C}$ . **DO NOT** use a self-defrosting freezer because periodic cycling of temperatures may cause shortened shelf life of this SRM. Let the material warm up to lab ambient temperature for at least 2 hours before use. Studies indicate that thawed material stored at  $4^{\circ}\text{C}$  for several weeks resulted in an increased recovery of genomic DNA that had been adsorbed to the walls of the storage tubes. For more stability information, see <http://www.cstl.nist.gov/biotech/strbase/srm2391b.htm>.

**Warning:** SRM 2391b IS A HUMAN SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE PRODUCT AS BIOSAFETY LEVEL 1 MATERIALS CAPABLE OF TRANSMITTING INFECTIOUS DISEASE [18]. SRM 2391b components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

## **INSTRUCTIONS FOR USE**

After opening the vials, sample aliquots for analysis should be withdrawn immediately and processed without delay for the certified values to be applicable.

## **SOURCE AND ANALYSIS<sup>1</sup>**

**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 Marligen Biosciences, Inc. (Ijamsville, MD).

**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 5.

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<sup>1</sup> 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.

**Description of Components:** Twelve components are included in each unit. Components 1 through 10 each contain 20 µL of genomic DNA. The DNA concentration ([DNA]) ranges are provided in Table 1 for information purposes indicating the range found at initial thawing through one-month storage at 4 °C. Component A of SRM 2372 Human DNA Quantitation Standard was used to calibrate the qPCR runs establishing the [DNA] ranges.

Table 1. Description of Components in SRM 2391b

Component	Description	DNA Concentration Range <sup>(a)</sup> (ng/µL)	No. of cells (on 7 mm Schleicher & Schull 903 Filter Paper Circle)
1	Genomic DNA 1	0.08 – 0.61	
2	Genomic DNA 2	0.17 – 0.89	
3	Genomic DNA 3	0.17 – 0.41	
4	Genomic DNA 4	0.21 – 0.49	
5	Genomic DNA 5	0.34 – 0.70	
6	Genomic DNA 6	0.24 – 0.72	
7	Genomic DNA 7	0.16 – 0.56	
8	Genomic DNA 8	0.42 – 0.87	
9	Genomic GM09947A	0.31 – 0.88	
10	Genomic GM09948	0.33 – 0.87	
11	Cell GM09947A		$2 \times 10^5$ cells
12	Cell GM09948		$2 \times 10^5$ cells

<sup>(a)</sup> DNA concentrations given are nominal values and are not intended for use as concentration standards.

Typing results are shown in Tables 2 through 4. All results are identical to 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 1 below).

Table 2. 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 <sup>(a)</sup>	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

<sup>(a)</sup> The relative intensity of the 12 allele is less than 10 % of the dominant 10 allele. See accompanying Figure 1 below.

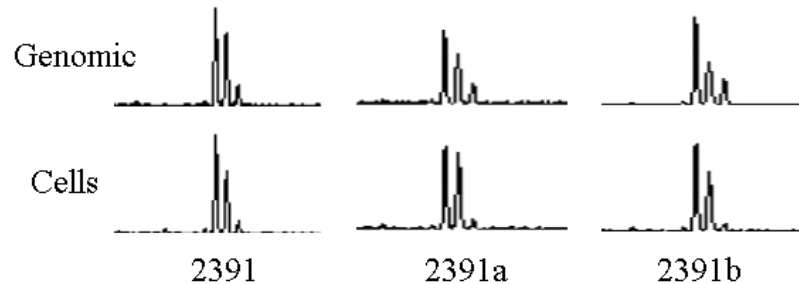


Figure 1. Relative intensities of the three CSF1PO alleles of GM09948 cell line in different lots of 2391.

Table 3. Certified Values for Additional STR Loci Available in Manufactured Kit Form.

Component Number	Description	Amelogenin	F13A01	F13B	FES/FPS	LPL	Penta D	Penta E	D2S1338	D19S433	SE33
1	Genomic 1	X,Y	6,7	10,10	12,12	10,11	10,15	7,12	17,23	13,16.2	20,30.2
2	Genomic 2	X,X	7,7	8,10	10,11	10,11	9,11	7,12	17,26	14,16	23.2,28.2
3	Genomic 3	X,Y	3.2,4	9,10	11,12	11,12	11,12	13,14	20,24	12,14	14.2,26.2
4	Genomic 4	X,X	5,6	6,9	10,13	10,12	8,9	5,12	17,23	11,13	22,28.2
5	Genomic 5	X,X	5,7	8,9	11,13	10,12	10,13	7,13	17,19	12.2,14	14,30.2
6	Genomic 6	X,X	3.2,5	9,10	11,11	10,12	9,12	12,14	25,25	12,14	20,21
7	Genomic 7	X,Y	5,8	6,8	11,11 <sup>(a)</sup>	11,12	3.2,11	12,16	17,22	13,15.2	13.2,20
8	Genomic 8	X,X	3.2,5	6,8	10,11	9,11	8,9	5,10	22,22	12.2,15	16,27.2
9	Genomic GM09947 A	X,X	6,16	8,10	10,12	11,12	12,12	12,13	19,23	14,15	19,29.2
10	Genomic GM09948	X,Y	6,6	8,8	11,11	10,12	8,12	11,11	23,23	13,14	23.2,26.2
11	GM09947 A Cells	X,X	6,16	8,10	10,12	11,12	12,12	12,13	19,23	14,15	19,29.2
12	GM09948 Cells	X,Y	6,6	8,8	11,11	10,12	8,12	11,11	23,23	13,14	23.2,26.2

<sup>(a)</sup> 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 a variant allele, 10.3, when separated in a Genetic Analyzer 3130xl Capillary Electrophoresis unit used at NIST as shown in Figure 2 below. Preliminary sequence information of this variant allele indicates two sequence changes: one on either side of the repeat region resulting in an electrophoretic mobility shift.

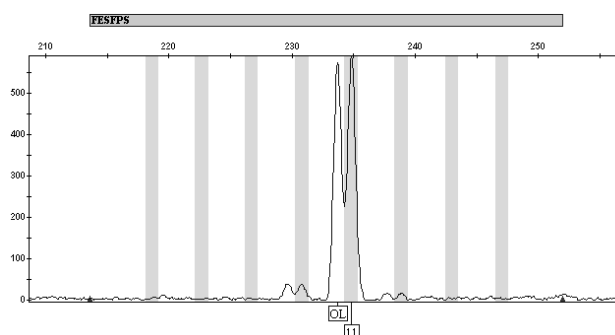


Figure 2. Genetic Analyzer 3130 xl Capillary Electrophoresis of FES/FPS locus, Genomic 7 sample.

Table 4. Certified Values and Reference Values for Additional Genetic Loci Alleles Not Commercially Available in Kit Format<sup>(a)</sup>.

Locus	Genomic 1	Genomic 2	Genomic 3	Genomic 4	Genomic 5	Genomic 6	Genomic 7	Genomic 8	Genomic 9 - 9947A	Genomic 10 - 9948	Cells 11 - 9947A	Cells12 - - 9948
D1GATA113	11,11	12,13	11,11	13,13	11,12	11,12	10,12	10,12	11,12	7,12	11,12	7,12
D1S1627	10,14	13,14	13,14	11,12	14,15	11,13	11,14	13,14	13,14	11,13	13,14	11,13
D1S1677	<b>12,13</b>	<b>14,16</b>	<b>14,17</b>	<b>14,15</b>	<b>13,14</b>	<b>13,14</b>	<b>12,13</b>	<b>14,16</b>	<b>13,14</b>	<b>13,14</b>	13,14	13,14
D2S441	<b>11,14</b>	<b>11,14</b>	<b>10,14</b>	<b>12,14</b>	<b>11,14</b>	<b>10,11</b>	<b>11,14</b>	<b>11,11.3</b>	<b>10,14</b>	<b>11,12</b>	10,14	11,12
D2S1776	11,12	11,11	8,10	11,12	12,13	11,12	11,12	11,12	10,10	10,12	10,10	10,12
D3S3053	9,12	10,11	9,11	11,11	11,11	9,9	11,11	9,9	9,11	9,12	9,11	9,12
D3S4529	14,15	13,16	14,16	15,16	13,15	15,17	14,16	14,14	13,13	12,12	13,13	12,12
D4S2364	<b>9,9</b>	<b>9,10</b>	<b>9,10</b>	<b>9,9</b>	<b>9,10</b>	<b>8,9</b>	<b>9,9</b>	<b>9,9</b>	<b>9,10</b>	<b>9,10</b>	9,10	9,10
D4S2408	10,10	9,9	8,9	9,10	10,11	9,9	8,11	11,11	9,10	10,10	9,10	10,10
D5S2500	17,18	17,24	17,18	17,18	14,15	14,18	14,20	14,18	14,23	14,17	14,23	14,17
D6S474	15,17	14,17	14,15	14,16	15,18	14,17	15,17	17,17	14,18	17,17	14,18	17,17
D6S1017	10,10	10,12	10,12	7,10	8,9	10,10	7,12	10,12	9,10	8,8	9,10	8,8
D8S1115	16,16	16,16	16,17	9,17	9,15	9,16	9,18	15,16	9,18	15,17	9,18	15,17
D9S1122	11,12	12,13	12,12	12,12	11,13	11,12	11,12	13,13	12,13	12,15	12,13	12,15
D9S2157	8,13	9,11	11,13	11,11	7,14	11,13	12,15	11,11	7,13	7,11	7,13	7,11
D10S1248	<b>14,16</b>	<b>13,15</b>	<b>13,16</b>	<b>12,12</b>	<b>14,15</b>	<b>14,15</b>	<b>13,14</b>	<b>11,15</b>	<b>13,15</b>	<b>12,15</b>	13,15	12,15
D10S1435	13,13	11,14	13,14	12,12	11,12	12,12	12,12	11,13	10,11	12,13	10,11	12,13
D11S4463	14,14	13,14	14,15	11,12	14,16	16,17	14,15	14,17	12,13	12,14	12,13	12,14
D12ATA63	14,17	13,17	12,15	16,18	13,15	14,18	16,17	14,15	13,13	13,18	13,13	13,18
D14S1434	<b>13,14</b>	<b>11,13</b>	<b>14,15</b>	<b>10,11</b>	<b>13,14</b>	<b>13,14</b>	<b>10,14</b>	<b>13,13</b>	<b>11,13</b>	<b>13,14</b>	11,13	13,14
D17S974	9,11	9,10	9,9	7,9	11,12	9,9	11,11	8,9	7,10	10,11	7,10	10,11
D17S1301	11,11	11,12	11,12	12,13	11,11	11,11	11,12	12,12	12,12	11,12	12,12	11,12
D18S853	11,14	11,11	11,11	11,13	10,15	11,14	14,14	12,13	11,14	11,11	11,14	11,11
D20S482	14,14	14,16	15,15	14,15	14,15	14,14	14,14	15,16	14,15	13,14	14,15	13,14
D20S1082	11,15	14,15	11,11	14,15	11,14	11,15	14,15	11,15	11,14	11,15	11,14	11,15
D22S1045	<b>14,15</b>	<b>11,16</b>	<b>15,16</b>	<b>17,18</b>	<b>11,14</b>	<b>11,15</b>	<b>11,15</b>	<b>16,17</b>	<b>11,14</b>	<b>16,18</b>	11,14	16,18

<sup>(a)</sup> All allele calls listed in bold print are certified values. All allele calls that are in normal print are reference values.

Table 5. Commercially Available Amplification “Kits” Used at NIST to Genotype STR Loci.

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

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<b>Certificate Revision History:</b> 03 September 2008 (Extension of certificate period and editorial revisions); 06 December 2002 (Original certificate issue date).
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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 Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*