Biotech SRMs Designed for the Rapidly Evolving Forensic DNA and Human Identity Testing Communities

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Abstract

As advances in biotechnology have been applied to human identification, the National Institute of Standards and Technology (NIST) has provided Standard Reference Materials® (SRMs®) to aid the forensic community to assure the quality of their measurements. SRM 2390 DNA Profiling Standard for Restriction Fragment Length Polymorphism (RFLP) genetic loci was issued in 1992. SRM 2391 PCR-Based DNA Profiling Standard for Variable Number of Tandem Repeat (VNTR), Short Tandem Repeat (STR) and Single-Nucleotide Polymorphism (SNP) loci was released in 1995, with renewals issued in 1998 and 2003. SRM 2392 Mitochondrial DNA Sequencing Standard (Human) was released in late 1999; it is now being updated. SRM 2395 Human Y-Chromosome DNA Profiling Standard will be issued in mid-2003. SRM 2372 Human DNA Quantitation Standard is in development.

1. Introduction.

Advances in recombinant DNA technologies in the 1970s and early 1980s led to the development of several powerful systems for establishing human identity. The first human DNA profiling system, based on the use of Jeffreys' multi-locus RFLP probes [1], was introduced in 1985 by the U.K. Forensic Science Service (FSS). By 1990, single-locus RFLP methods were well established in Europe and in North America [2, 3]. The first commercial PCR-based short tandem repeat (STR) "singleplex" systems became available in 1993, followed shortly by "multiplex" systems of ever greater power and complexity [4]. Developments in genomic sequencing enabled forensic exploitation of subtle differences in mitochondrial DNA starting in 1991 [5]. Information from the Human Genome Project facilitated the development and commercial release of Y-chromosome STR- and SNP-based systems in 2003 [6].

Nearly from the first use of DNA profiling in the U.S., NIST has been actively engaged with the North American forensic community: helping harmonize protocols, evaluating equipment and materials, sponsoring interlaboratory challenge exercises, and developing quality assurance standards. NIST became even more integrally involved in 1998, when the Director of the U.S. Federal Bureau of Investigation (FBI) issued Standard 9.5 [7] that includes the requirement "The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST Standard Reference Material or

standard traceable to a NIST standard." Figure 1 provides an approximate timeline of developments in DNA profiling technologies and of NIST's involvement.

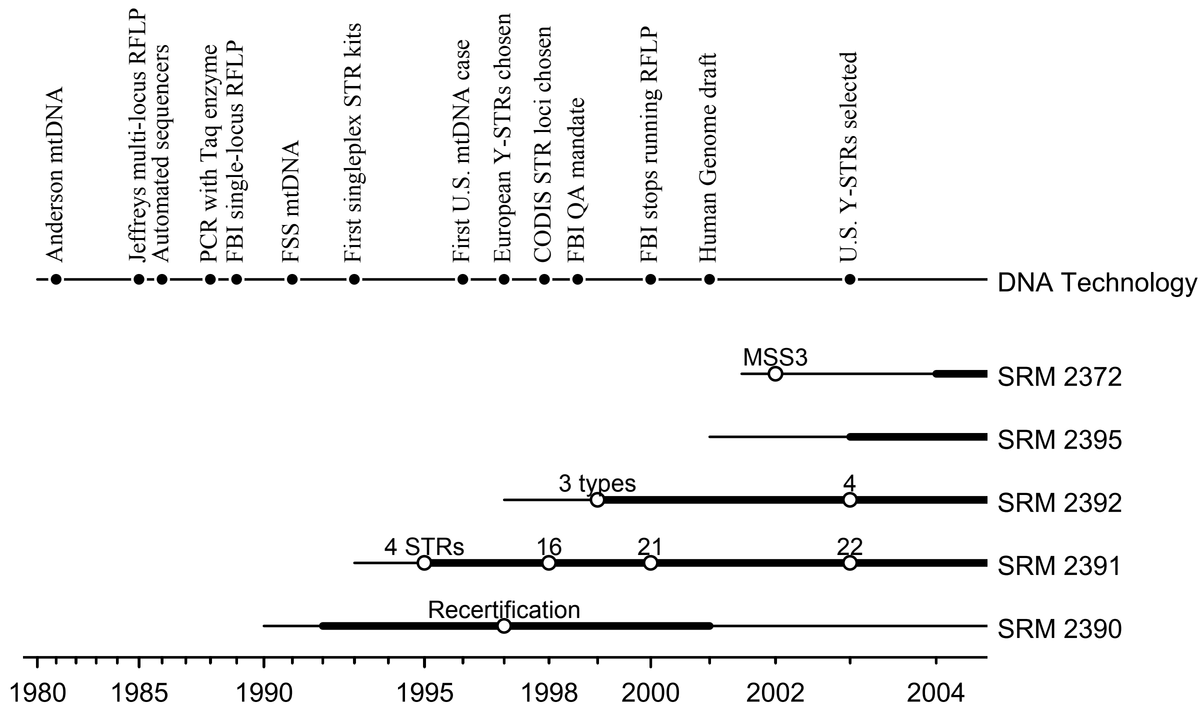

Figure 1. Forensic DNA timeline.

2. SRM 2390 DNA Profiling Standard for RFLP analysis.

The FBI promulgated its single-locus RFLP measurement system in 1990; however, by this time a number of North American forensic laboratories had developed their own protocols. Worldwide sharing of RFLP data was not possible because most European laboratories used the restriction enzyme *Hin*fI while most North American laboratories used *Hae*III [8]. Laboratories using the same general method often employed slight variations that resulted in minor but reproducible measurement differences. Standard Reference Material 2390 DNA Profiling Standard was released in 1992. This SRM was designed to help assure that (1) each step in a *Hae*III-based RFLP protocol is performing correctly and (2) RFLP measurement results remain stable among laboratories and over time. Table 1 lists the SRM 2390 components and their intended uses.

While the RFLP technique proved to be robust [10], it required 100 ng to 1 μg of relatively intact DNA – 1000 to 20,000 base pairs (bp) in size. There is often too little DNA in evidential samples, and what is present is often too degraded, for successful RFLP analysis. The original ^{32}P-based autoradiographic detection systems required 5 to 7 days to probe one locus. In general, 3 to 7 RFLP probes were required to establish genetic identity.

Table 1. RFLP procedures and relevant SRM 2390 quality assurance materials

RFLP Steps	Materials Supplied in SRM 2390 (1992)
Sample identification	Cell pellet (2 materials)
DNA extraction	Genomic DNA (2 materials)
DNA quantification	Genomic DNA yield gel standards (6 materials)
*Hae*III restriction	"Pre-cut" (*Hae*III digested) DNA (2 materials)
Electrophoresis	DNA Analysis Marker System, argarose, buffer (7 materials)
Sizing	Adenovirus sizing ladder
Final analysis	Certified values for 6 probes, information values for 5 others

3. SRM 2391 PCR-Based DNA Profiling Standard

The second generation of forensically useful genetic markers was developed using the polymerase chain reaction (PCR) with the thermostable *taq* polymerase [11]. This technique "amplifies" specific genetic loci thereby reducing the amount of genomic DNA required for analysis. SRM 2391 PCR-Based DNA Profiling Standard was issued in 1995. It was initially designed for use with the Variable Number of Tandem Repeats (VNTR) locus D1S80 [12, 13], HLA DQα1 and PM SNP systems [14], and four then "new" short tandem repeat (STR) genetic loci [4].

D1S80 VNTR range in size from 300 bp to 1000 bp; STRs are typically in the 100 bp to 500 bp range. The reduction in the size of intact DNA required for analysis made it possible to process relatively degraded samples. The quantity of DNA required to obtain a genetic "profile" was reduced to 1 ng to 2 ng per PCR amplification. However, more STR loci are required to obtain the power of discrimination equal to that provided by RFLP analysis [15]. The FBI adopted 13 "core" STR loci in November 1997 [16]. The SRM 2391 certificate was updated in mid-1998 to provide certified values for these loci plus three others of interest. SRM 2391a, a renewal with certified values for 21 STR loci, was released in 2000. SRM 2391b, a second renewal certifying values for 22 STR loci (adding a marker used by European forensic laboratories), was released in January 2003. Table 2 lists the SRM 2391 components and their intended uses.

Table 2. PCR procedures and relevant SRM 2391 quality assurance materials

	Materials Supplied in	
PCR Steps	SRM 2391 (1995)	SRM 2391b (2003)
Sample identification	Cells on 903 paper (2 materials)	Cells on 903 paper (2 materials)
DNA extraction	Genomic DNA (10 materials)	Genomic DNA (10 materials)
PCR amplification	D1S80 PCR products (6 materials)	
Electrophoresis	100 bp sizing ladder	
Typing	Consensus D1S80 ladder	
Final analysis	Certified values for D1S80, 6 SNPs, 4 STRs	Certified values for D1S80, 6 SNPs, 22 STRs

Current commercial STR multiplexing technology allows 16 genetic loci to be amplified simultaneously, enabling identification of a complete profile from 1 ng or less of genomic DNA [17].

4. SRM 2392 Mitochondrial DNA Sequencing Standard

Forensically useful human identity information can be obtained from even highly degraded samples (such as old skeletal material) using mitochondrial DNA (mtDNA). Each human mitochondria has its own circular, double-stranded mtDNA. There are thousands of mitochondria in each cell; there are often sufficient mtDNA copies embedded in hair shafts to enable successful analysis. Since mtDNA is maternally inherited, comparison samples can be obtained from relatives of the same maternal lineage [18].

Anderson *et al*. completely sequenced the 16,569 bp of human mtDNA in 1981 [19]. The FSS first used mtDNA for casework in 1991, using the non-coding mtDNA control region (CR, also termed the D-loop) [20]. The CR is approximately 1125 bp in length. Portions of the CR are highly variable among individuals [18]. The FBI began mtDNA casework in 1996, mainly for the analysis of hair.

SRM 2392 Mitochondrial DNA Sequencing Standard, designed for use by both the forensic and clinical communities, was released in 1999. It certifies the entire mtDNA sequence information for the apparently normal lymphoblastoid cell lines CHR, GM09947a and GM09948. The SRM provides DNA extracts of CHR and GM09947a along with cloned DNA from the CHR HV1 region that contains a poly-C stretch that can be difficult to sequence [21]. It will soon include sequence information for and an extract of cell line HL-60, used for quality assurance by the FBI.

5. SRM 2395 Human Y-Chromosome DNA Profiling Standard

Genetic markers unique to the Y-chromosome are particularly useful for male-female mixtures such as those common in sexual assault cases, where they enable detection of small amounts of male DNA in a high background of female DNA. While mtDNA follows the maternal lineage, Y markers follow the paternal.

The first polymorphic Y-chromosome marker was described in 1992 [22]. Only 30 such markers were available at the beginning of 2002. The availability of DNA sequence information from the Human Genome Project greatly facilitated the discovery of new Y-chromosome STRs (Y-STRs) [23]; over 200 markers were deposited in the Genome Database (http://www.gdb.org) by the beginning of 2003.

The European forensic community selected the core set of Y-STRs known as the "minimal haplotype" in 1997 [24]. The U.S. Scientific Working Group on DNA Analysis Methods (SWGDAM) selected a core set in early 2003.

The soon-to-be-released SRM 2395 consists of six DNA extracts: a female component supplied as a negative control and five male components. DNA sequencing has been used to characterize

all of the alleles in all of the male samples for 20 Y chromosome STR markers. The genetic types for 50 different Y-chromosome biallelic SNPs determined by several different technologies are also provided. Table 3 details the Y-STRs certified in SRM 2395 and their current commercial availability.

Table 3. Y-STR Loci Certified in SRM 2395

SRM 2395	**Core Loci**		**Commercially Available Amplification Kits**		
Loci	**Europe**	**U.S.**	**Y-PLEX™ 6** [a]	**Y-PLEX™ 5** [a]	**PowerPlex® Y** [b]
DYS19	X	X	X		X
DYS385a	X	X	X		X
DYS385b	X	X	X		X
DYS388					
DYS389I	X	X		X	X
DYS389II	X	X	X	X	X
DYS390	X	X	X		X
DYS391	X	X	X		X
DYS392	X	X		X	X
DYS393	X	X	X		X
DYS426					
DYS435					
DYS436					
DYS437					X
DYS438		X		X	X
DYS439		X		X	X
DYS447					
DYS448					
DYS460					
DYS461					
DYS462					
H4					

a) ReliaGene Technologies, Inc., New Orleans, LA, USA.
b) Promega Corporation, Madison, WI, USA.

6. SRM 2372 Human DNA Quantitation Standard

Multiplex STR assays are now the dominant forensic DNA human identification technology. Our group at NIST has coordinated a series of interlaboratory examinations of the performance of commercial STR multiplexes [17, 25, 26]. These studies have sought to discover latent analytical difficulties by challenging analysts and assay systems with designedly difficult samples, presented in atypical contexts, and described with minimal instructions. While multi-step and chemically complex, the STR systems have proved to provide results that are robust to typical among-laboratory differences in sample preparation, PCR equipment and protocols, and separation and visualization systems.

However, linkages between certain STR measurement anomalies and inaccurate DNA quantitation were observed in the 1999 Mixed Stain Study #2 (MSS2) [25]. The 2001 Mixed Stain Study #3 (MSS3) was designed to further explore the performance of high-plexity STR systems and to resolve the DNA quantitation issues raised in MSS2. The among-participant consensus DNA concentrations in MSS3 agreed very well with the design values, but with more than a 20-fold range in reported values [26].

The forensic community has requested a "human DNA standard for quantitation" for several years. The MSS3 results indicate that a consensus quantitation standard can be produced. SRM 2372 is currently being developed. However, this material should be more than a consensus standard. The current challenge is to clearly define what is included in a "nanogram of genomic DNA", accounting for the associated mtDNA as well as counter-ions and the minimum amount of water needed to prevent denaturation.

Disclaimer: Certain commercial equipment, instruments, or materials are identified in this report to specify adequately experimental conditions or reported results. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the equipment, instruments, or materials identified are necessarily the best available for the purpose.

References:

1. P. Gill, A.J. Jeffreys, and D.J. Werrett, Forensic applications of DNA 'fingerprints', *Nature*, 318, pp. 577-579, 1985.

2. B. Budowle and F.S. Baechtel, Modifications to improve the effectiveness of restriction fragment length polymorphism typing, *Appl. Theor. Electrophoresis,* 1, pp. 181-187, 1990.

3. I.W. Evett and P. Gill, A discussion of the robustness of methods for assessing the evidential value of DNA single locus profiles in crime investigations, *Electrophoresis*, 12, pp. 226-230, 1991

4. J.M. Butler, *Forensic DNA Typing: Biology and Technology Behind STR Markers*, Academic Press, London, 2001, pp. 61-75.

5. K.M. Sullivan, R. Hopgood, B. Lang and P. Gill, Automated amplification and sequencing of human mitochondrial DNA. *Electrophoresis*, 12, pp. 17-21, 1991.

6. J.M. Butler, Recent developments in Y-STR and Y-SNP analysis, *Forensic Sci. Rev.*, in press.

7. Standards for Forensic DNA Testing Laboratories, 1998, http://www.fbi.gov/hq/lab/codis/qualassur.htm

8. D.J. Reeder, M.C. Kline, and K.L Richie, An overview of reference materials prepared for standardization of DNA typing procedures, *Fresenius J. Anal. Chem.*, 352, pp. 246-249, 1995.

9. D.L. Duewer, K.T. Gary, and D.J. Reeder. RFLP band size standards: Cell line K562 values from 1991 – 1997 proficiency studies. *J. Forensic Sci.*, 45, pp. 1106-1118, 2000.

10. D.L. Duewer, K.L Richie, and D.J. Reeder. RFLP band size standards: NIST Standard Reference Material® 2390. *J. Forensic Sci.*, 45, pp. 1093-1105, 2000.

11. R.K. Saiki, D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis and H.A. Erlich, Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239, pp. 487-491, 1988.

12. K. Kasai, Y. Nakamura, and R. White, Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application to forensic science, *J. Forensic Sci.* 35, pp. 1196-1200, 1990.

13. M.C. Kline, J.W. Redman, D.J. Reeder, and D.L. Duewer, Intercomparison of DNA sizing ladders in electrophoretic separation matrices and their potential for accurate typing of the D1S80 locus, *Appl. Theor. Electrophoresis,* 6, pp. 33-41, 1996.

14. R.K. Saiki, P.S. Walsh, C.H. Levenson, and H.A. Erlich, Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes, *Proc. Natl. Acad. Sci.*, 86, pp. 6230-6234, 1989.

15. R. Chakraborty, D.N. Stivers, B. Su, Y.X. Zhong, and B. Budowle, The utility of short tandem repeat loci beyond human identification: Implications for development of new DNA typing systems, *Electrophoresis*, 20, pp. 1682-1696, 1999.

16. B. Budowle, T.R. Moretti, S.J. Niezgod, and B.L. Brown, CODIS and PCR-based short tandem repeat loci: Law enforcement tools. In *Second European Symposium on Human Identification*, Promega Corporation, Madison, Wisconsin, 1998 pp. 73-88.

17. D.L. Duewer, M.C. Kline, J.W. Redman, P.J. Newall, and D.J. Reeder, NIST mixed stain studies #1 and #2: Interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples, *J. Forensic Sci.*, 46, pp. 1199-1210, 2001.

18. M.M. Holland and T.J Parsons, Mitochondrial DNA sequence analysis-validation and use for forensic casework, *Forensic Sci. Rev.*, 11(21), pp. 22-48, 1999.

19. S. Anderson, A.T. Bankier, B.G. Barrell, M.H.L. deBrujin, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Roe, F. Sanger, P.H. Schreier, A.J.H. Smith, R. Staden, and I.G. Young, Sequence and organization of the human mitochondrial genome, *Nature*, 290, pp. 457-465, 1981.

20. K.M. Sullivan, R. Hopgood, and P. Gill, Identification of human remains by amplification and automated sequencing of mitochondrial DNA, *Int. J. Leg. Med.*, 105, pp. 83-86, 1992.

21. B.C. Levin, H. Cheng, M.C. Kline, J.W. Redman, and K.L. Richie, A review of DNA Standard Reference Materials developed by the National Institute of Standards and Technology, *Fresenius J. Anal. Chem.*, 370, pp. 213-219, 2001.

22. L. Roewer and J.T. Epplen, Rapid and sensitive typing of forensic stains by PCR amplification of polymorphic simple repeat sequences in case work, *Forensic Sci. Int.* 53, pp. 163, 1992.

23. Q. Ayub, A. Mohyuddin, R. Qamar, K. Mazhar, T. Zerjal, S.Q. Mehdi, and C. Tyler-Smith, Identification and characterization of novel human Y-chromosome microsatellites from sequence database information, *Nucleic Acids Res.* 28, p. e8, 2000.

24. M. Kayser, A. Caglia, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. PerezLezaun, A. Piccinini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. TeifelGreding, G. Weichold, P. deKnijff, and L. Roewer, Evaluation of Y-chromosomal STRs: A multicenter study, *Int. J. Legal Med.*, 110, pp. 125, 1997.

25. M.C. Kline, D.L. Duewer, P.J. Newall, J.W. Redman, D.J. Reeder, and M. Richard. Interlaboratory evaluation of STR triplex CTT, *J. Forensic Sci.*, 42, pp. 897-906, 1997.

26. M.C. Kline, D.L. Duewer, J.W. Redman, and J.M. Butler, NIST mixed stain study #3: DNA quantification practice and its influence on short tandem repeat multiplex performance, *Anal. Chem.*, in press, 2003.