# NIST Standard Reference Materials (SRMs) for the Human Identity Testing Community:
## Past, Present, and Future Directions You Can Assist in Making

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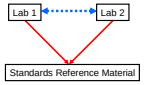
The National Institute of Standards and Technology (NIST) supports accurate and compatible measurements by certifying and providing over 1300 Standard Reference Materials® with well-characterized composition or properties, or both. These materials are used to perform instrument calibrations in units as part of overall quality assurance programs, to verify the accuracy of specific measurements and to support the development of new measurement methods. The Human Identity Project at NIST as part of the Chemical Science and Technology Laboratory, Biochemical Science Division, Applied Genetics Group has been producing DNA based Standard Reference Materials (SRMs) for the Forensic Human DNA identity community since the 1992 release of SRM 2390 DNA Profiling Standard for Restriction Fragment Length Polymorphism (RFLP). Other SRMs of interest to this community include: SRM 2391(a,b) PCR-Based DNA Profiling Standard, SRM 2395 Human Y-Chromosome DNA Profiling Standard, SRM 2392 Mitochondrial DNA Sequencing (Human), SRM 2392-I Mitochondrial DNA Sequencing,(Human HL-60 DNA) and SRM 2372 Human DNA Quantitation Standard. Over the years the Certificates of Analysis for these SRMs have been updated with new information in order to keep the materials current to the areas of interest. When materials start getting low we actively pursue replacement of the material. Such is the case with SRM 2391 that is in its third generation as SRM 2391b. Presentation of not only the history of the SRM certificate updating but also a questionnaire is available for input into the design for the next generation SRM 2391c.

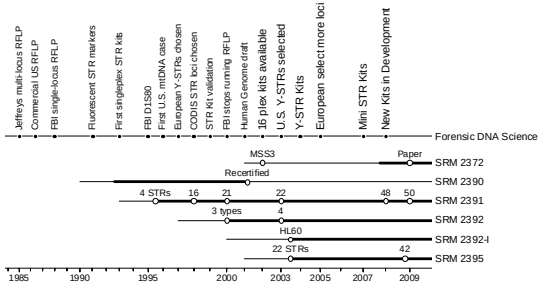
### Why do forensic DNA labs care about the use of reference materials?


**QAS Standard 9.5.5.** The laboratory shall check its DNA procedures annually or whenever substantial changes are made to a procedure against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

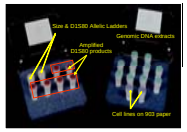



**Calibration with SRMs enables confidence in comparisons of results between laboratories**

### History of SRM Work and Certificates




### PCR-based DNA Profiling Standard

| SRM 2391 (1995) | SRM 2391a (2000) | SRM 2391b (2003, r2008) | SRM 2391c (planned 2010) |
|---|---|---|---|
|  |  |  | **Would like your input into the desired number of components & loci certified** |

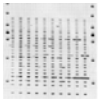


***coverage for all commercially available kit STR loci at the time of release**

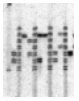
**1995** – released SRM 2391 with certified values for D1S80, DQA1+PM, and 4 monoplex STR loci*
**November 1997** – FBI selection of 13 core U.S. loci
**1998** – updated SRM 2391 certificate with 17 STRs (13 core loci + FFFL)*
**October 1998** – DAB Quality Assurance Standard 9.5 requires use of NIST SRM or NIST-traceable material
**2000** – renewal (SRM 2391a) due to Quality Assurance Standard requirement; now includes 21 STRs (Penta D, Penta E, D19S433, D2S1338 added)*
**2003** – renewal (SRM 2391b) due to Quality Assurance Standard required use; includes 22 STRs (SE33 added)*
**2007** – MiniFiler kit released *(with different primer sets* and D16 dropout seen in Component 8*)*, new miniSTR assays developed at NIST, and new commercial kits with new loci on the horizon
**2008** – SRM 2391b certificate revised with additional 26 miniSTR loci including D2S441, D10S1248, and D22S1045
**2009** – sequence analysis performed on new kit loci*: D12S391, D1S1656, and SE33
**2010** – due to limited supply of current sample components, **new DNA sources will be needed for SRM 2391c**

**Certified values for the NIST reference materials have evolved as the technology for DNA testing has improved…**

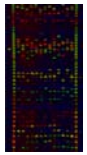
**PM + DQA1 reverse dot blot**



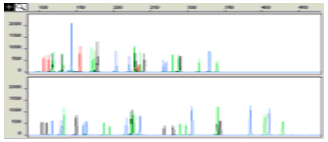
**D1S80 silver stain gels**



**STR silver stain gels**



**Fluorescent STR FMBIO gel image**



**Fluorescent STR megaplexes CE electropherogram**

**Genomic materials in SRM 2391 were originally selected to "light-up" all the types on a PM+DQA1 (PolyMarker and DQα) reverse dot blot strip to confirm that all probes were working properly**. Cell lines 9947A and 9948 were added due to work by Ron Fourney's RCMP lab. Components for the analysis of the D1S80 locus as well as cells to extract from paper. A laboratory could check all steps of their typing procedure.


### Effort that goes into SRM production

**Homogeneity**: single lot in a single container aliquoted to individual tubes packaged as components in each SRM unit

**Purity** (absence of significant impurities): single source DNA samples used; while it is not certified to be "mixture-free", foreign, contaminating alleles should not be seen; thus, the solutions can be considered >~90% pure (mixture detection limit ~10%)

**Stability: g**enerally certified for 5-6 years but likely stable much longer under appropriate storage conditions (refrigerated or frozen, out of sunlight)

**Concentration:** for genotyping reference materials, amount of DNA is not certified; some variability in amount of DNA present can be expected; samples generally supplied at near "ready-to-use" concentrations (~1-2 ng/µL)

There are three levels of values: certified, reference, and information. To be a "certified value", the measurement must be done at NIST using a primary method with confirmation by other methods or using two independent critically-evaluated methods.

**For more information on NIST SRMs, see:**
**http://www.nist.gov/srm**
http://www.cstl.nist.gov/biotech/strbase/srm2391b.htm
http://www.cstl.nist.gov/biotech/strbase/srm2395.htm
http://www.cstl.nist.gov/biotech/strbase/srm2372.htm

### Some STR Typing Measurement Issues

STR genotypes are generated using PCR amplification and electrophoretic sizing that involves an internal size standard with each sample.

The forensic DNA community almost exclusively uses STR typing kits to obtain results (there are different kits available that examine the same common markers).

PCR amplification is expected to generate consistent genotypes as long as primer positions are not changed between kits. Primer changes can result in allele dropout due to primer site mutations.

Occasionally new commercial kits are created with additional loci.

General STR repeat nomenclature rules have been established but do have some subjectivity in them permitting possible differences in how STR alleles are named.

### Make Your Own (MYO) Traceable Material

Prepare a "lot" of DNA samples: stain, swab, cell pellet, extract, etc.

Assure that the MYO samples are:
- Homogenous
- Stable
- Reproducible

Analyze the **appropriate SRM** and MYO "in parallel"

Confirm that your results for the SRM are correct (agree with certificate) and your results for the MYO are consistent (agree with your prior results).

Maintain the records of the now **traceable** MYO and the SRM analysis. You may use the MYO as frequently as you desire in your Laboratory System **instead of** the SRM. Keep a record of the use of the MYO and results.

**IF AT ANY TIME THERE IS A DISCREPANCY WITH THE RESULTS OBTAINED FOR THE MYO, A NEW LOT MUST BE MADE!!!!!**

Remember:
There must always be a direct comparison to the SRM.
The "Lot" is Traceable ***not*** the source of the material.

Example:
- Obtain human use approval for 10 mL whole blood.
- Obtain blood and appropriate stain cards / stain-media
- Following protocol, prepare 500 stains (20 µL/stain)
- Dry and store for at least a week
- Analyze at least 5 randomly selected samples
- Evaluate results: are they all qualitatively identical?
- **Now** analyze at least two samples in parallel with SRM
- Maintain records that the SRM data obtained was correct as well as the data from your stain.
- Package and store stains appropriately (**dry** and **cold**!)
- Use MYO as traceable to NIST material.

**References**

[1] **NIST Special Publication 260-136** "Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements"

[2] Reeder, D.J., Kline, M.C., Richie, K.L (1995) An overview of reference materials prepared for standardization of DNA typing procedures. *Fresenius J Anal Chem*, 352: 246-249.

[3] Levin, B.C., Reeder, D. J. (2004) Reference Materials for DNA Analysis. In: Evolutionary Methods in Biotechnology (Susanne Brakmann, Andreas Schwienhorst., ed.) Wiley-VCH Publishers: Germany.

[4] Levin, B.C., Richie, K.L., Kline, M.C., Redman, J.W., Hancock, D.K. (2006) Human DNA standard reference materials developed by the National Institute of Standards and Technology. Trends in Genome Research (Williams, C.R., ed.) Nova Science Publishers: New York, pp. 173-205.

[5] Kline MC, Duewer DL, Travis JC, Smith MV, Redman JW, Vallone PM, Decker AE, Butler JM. (2009) Production and certification of NIST Standard Reference Material 2372 Human Quantitation Standard. *Anal Bioanal Chem*, 394: 1183-1192.

| SRM | Name | FY06 | FY07 | FY08 | FY09 | Avg | Remaining | Current $* |
|---|---|---|---|---|---|---|---|---|
| 2372 | Human DNA Quantitation Std | 0 | 0 | 160 | 147 | 153.5 | 1,078 | $372 |
| 2390 | DNA Profiling | 2 | 0 | 1 | 0 | 0.8 | 3 | $833 |
| 2391B | PCR-Based DNA Profiling | 86 | 81 | 125 | 140 | 108 | 107 | $811 |
| 2392 | Mitochondrial DNA Sequencing | 8 | 6 | 0 | 12 | 6.8 | 165 | $883 |
| 2392-I | Mitochondrial DNA Sequencing (Human HL-60 DNA) | 6 | 32 | 20 | 19 | 19.3 | 176 | $365 |
| 2395 | Human Y-Chromosome DNA Profiling | 34 | 39 | 72 | 88 | 58.3 | 136 | $383 |

*As of Oct 7, 2009

Poster available for download from STRBase: http://www.cstl.nist.gov/biotech/strbase/pub_pres/Promega2009poster_SRM2391c.pdf