



Proper Use of Reference Materials

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Disclaimers

Funding: Interagency Agreement 2008-DN-R-121 between the **National Institute of Justice** and NIST Office of Law Enforcement Standards.

Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

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Why Use Reference Materials?

Reference materials are important tools in realizing a number of aspects of measurement quality and can be/are used for:

- method validation
- calibration
- estimation of measurement uncertainty
- training
- internal QC
- external QA (proficiency testing) purposes.

Why do you care about the use of reference materials?



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.

Definitions

- **reference material RM**
material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in **measurement** or in examination of **nominal properties** (JCGM 200: 2008)

Reference material (certified or standard) is a material for which values are certified by a technically valid procedure and accompanied by, or traceable to, a certificate or other documentation which is issued by a **certifying body**.

Forensic Science Communications October 2008—Volume 10—Number 4 Standards and Guidelines
Quality Assurance Standards for Forensic DNA Testing Laboratories effective date July 1, 2009

Definitions

- **certified reference material CRM reference material**, accompanied by documentation issued by an **authoritative body** and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures. (JCGM 200: 2008)
- **Standard Reference Material® (SRM®):** A CRM issued by NIST that also meets additional NIST certification criteria. (NIST SP 260-136: 2000)

NIST and other National Metrology Institutes (NMIs) worldwide:

- provide and maintain primary standards
- provide linkages for traceability to the international system of measurement
- disseminate these realizations in a manner and of a quality that is consistent with the needs of the measurement community

The typical role of an NMI is to establish and maintain:

Scientifically-Sound, Metrologically-Based Competencies and Measurement Capabilities that are Internationally Vetted and Recognized

↓

To provide calibration and measurement services disseminated to Customers via mechanisms such as:

- Validated Reference Methods
- Certified Reference Materials
- Reference Data
- Value-assignment of customer-provided samples or materials
- Value-assignment of Proficiency Testing samples
- Measurement Services for other Government Agencies
- Etc.

Although physical quantities such as length, mass, temperature, time, etc. are the first to come to mind when one thinks about metrology and measurement standards, chemical measurement research and standards have been a major activity at NIST since its inception in 1901.

NIST (NBS) established in 1901

“It is therefore the unanimous opinion of your committee that no more essential aid could be given to

- manufacturing
- commerce
- the makers of scientific apparatus
- the scientific work of Government
- schools, colleges, and universities

than by the establishment of the institution proposed in this bill.”



House Committee on Coinage, Weights and Measures ... May 3, 1900 on the establishment of the National Bureau of Standards (now NIST)

National Institute of Standards and Technology

NIST is a non-regulatory agency in the Technology Administration of the US Department of Commerce.



Mission ...

to promote U.S. innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve our quality of life

The NIST Laboratories – serve as the ultimate reference point for measurements, standards, and technology research for the U.S. to support industry, science, health, safety, and national defense



NIST traditionally has focused its research and measurement service activities on the physical science and engineering disciplines

Bioscience has now been identified as an area for significant emphasis and growth

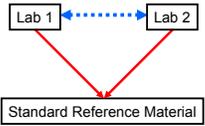
Standard Reference Materials (SRMs)

<http://www.nist.gov/srm>

Traceable standards to ensure accurate and comparable measurements between laboratories



SRM 2391b – autosomal STRs
 SRM 2392 &-I – mtDNA sequencing
 SRM 2395 – Y-STRs
 SRM 2372 – DNA quantitation



Calibration with SRMs enables confidence in comparisons of results between laboratories

Helps meet ISO 17025 needs for traceability to a national metrology institute

The Tools of DNA Typing and SRM Needs

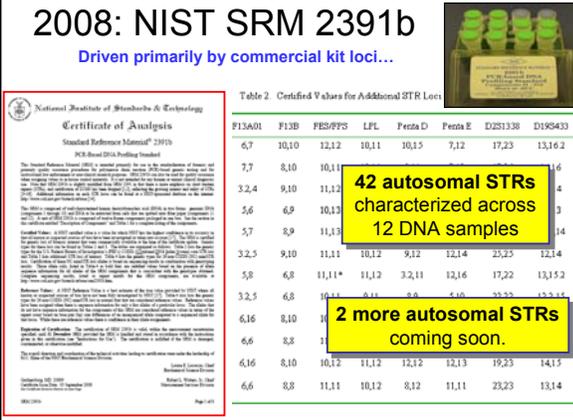
- RFLP Testing (Late 1980's) SRM 2390
 - Radioactive Based
 - Chemiluminescent Based

Technology no longer used
- PCR-Based Testing (Mid 1990's)
 - ~~Dot Blot~~
 - ~~VNTR~~
 - STR (Fluorescent markers used today)

SRM 2391..a..b
- DNA Sequencing (Late 1990's) SRM 2392, 2392-I
 - Mitochondrial DNA
- Y-Chromosome Testing (early 2000's) SRM 2395
- DNA Quantification (Oct 2007) SRM 2372

2008: NIST SRM 2391b

Driven primarily by commercial kit loci...



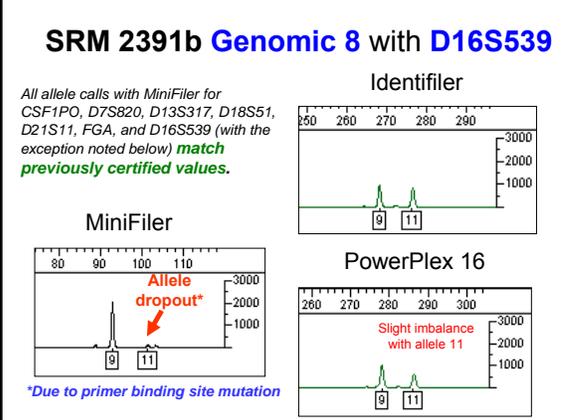
42 autosomal STRs characterized across 12 DNA samples

2 more autosomal STRs coming soon.

FLXAD1	F13D	FES/PPS	LPL	Penta D	Penta E	DEB1338	D19S433
6,7	10,10	12,12	10,11	10,15	7,12	17,23	13,16,2
7,7	8,10	10,11					
3,2,4	9,10	11,12					
5,6	6,9	10,13					
5,7	8,9	11,13					
3,2,5	9,10	11,11	10,12	9,12	12,14	25,25	12,14
5,8	6,8	11,11*	11,12	3,2,11	12,16	17,22	13,15,2
3,2,5	6,8	10,11	8,11	8,9	6,10	33,33	13,5,14
6,16	8,10						
6,6	8,8						
6,16	8,10	10,12	11,12	12,12	12,13	19,23	14,13
6,6	8,8	11,11	10,12	8,12	11,11	23,23	13,14

SRM 2391b Genomic 8 with D16S539

All allele calls with MiniFiler for CSF1PO, D7S820, D13S317, D18S51, D21S11, FGA, and D16S539 (with the exception noted below) match previously certified values.



Identifiler

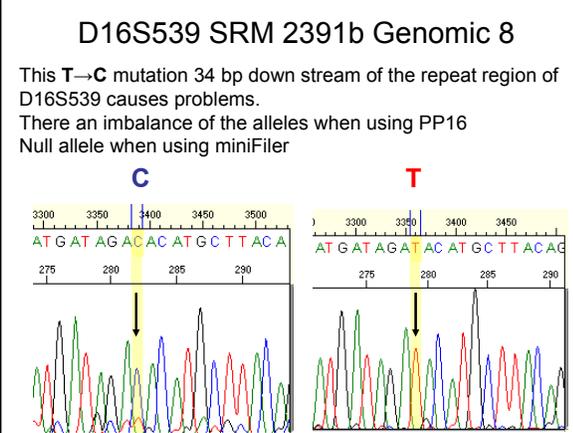
MiniFiler
Allele dropout*

PowerPlex 16
Slight imbalance with allele 11

*Due to primer binding site mutation

D16S539 SRM 2391b Genomic 8

This T→C mutation 34 bp down stream of the repeat region of D16S539 causes problems. There is an imbalance of the alleles when using PP16. Null allele when using miniFiler.



C **T**

3300 3350 3400 3450 3500 3300 3350 3400 3450

ATGATAGACACATGCTTACAA ATGATAGATACATGCTTACAG

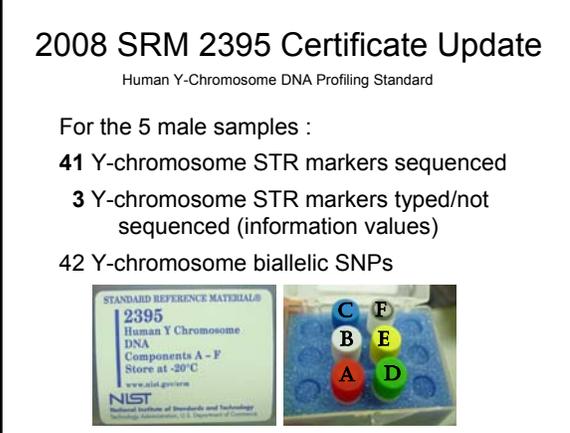
275 280 285 290 275 280 285 290

2008 SRM 2395 Certificate Update

Human Y-Chromosome DNA Profiling Standard

For the 5 male samples :

- 41 Y-chromosome STR markers sequenced
- 3 Y-chromosome STR markers typed/not sequenced (information values)
- 42 Y-chromosome biallelic SNPs



SRM 2372: Human DNA Quantitation Standard

released in October 2007



Manuscript describing SRM 2372 production has been accepted for publication in:

“Analytical and Bioanalytical Chemistry”.

[Anal. Bioanal. Chem.](http://dx.doi.org/10.1002/anie.200900011) 2009 Apr 18. [Epub ahead of print]

[SpringerLink](http://www.springerlink.com)

Production and certification of NIST Standard Reference Material 2372 Human DNA Quantitation Standard.

Kline MC, Dunover DL, Truitt JC, Smith MY, Redman JW, Yallone PM, Decker AJ, Butler JM

Establishing Traceability to NIST SRMs

Traceability requires the establishment of an unbroken chain of comparisons to stated references (see <http://ts.nist.gov/traceability/>). In the case of DNA testing with autosomal STR markers, the reference materials would be SRM 2391b.

Just as *chain of custody* is used to convey integrity of connections between the final result obtained on a DNA sample and the evidence collected, **materials deemed traceable to NIST SRMs must have associated records.**

SRM Usage

- Method validation
- Instrument validation
- Instrument calibration
- Production of a daily use NIST Traceable material

Definitions

Validation is a process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis and includes the following:

1. Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic samples.
2. Internal validation is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

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Definitions

Internal validation is the accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

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Definitions

- **traceability:** The property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.
(ISO VIM: 1993, 6.10)

Traceability is the property of a result of a measurement whereby it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons.

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Definitions

- **reproducibility:** Closeness of the agreement between the results of measurements of the same measurand carried out under changed conditions of measurement.
(ISO VIM: 1993, 3.7)

Definitions

- **Homogeneous**
Uniform throughout in structure or make-up
- **Stable**
Resisting sudden change in position or condition.
Unchanging and permanent : enduring.
Not easily decomposed or otherwise modified chemically.
(Webster's II New Riverside University Dictionary)

Traceable to NIST?



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.**

Making a NIST Traceable Material for SRM 2391b or SRM 2395

Prepare a “lot” of samples:
stain, swab, cell pellet, extract, etc.

Are the samples?

Homogeneous

- all aliquots contain similar material, appropriate for use.
 - calibrate dispensing equipment prior to use
 - continuous stirring while the material is aliquoted

Stable

- the material will exhibit the same properties throughout it’s proposed shelf life at the proposed storage conditions.
 - prepared stains are all dried to the same extent
 - prepared extracts are in appropriately sealed tubes

Making a NIST Traceable Material for SRM 2391b or SRM 2395

Are the samples?

Reproducible

- the material produces the same results on analysis.
 - select random samples of the “lot” and analyze
 - assure that all results are the same every time
 - assure that there are no signs of degradation

Making a NIST Traceable Material for SRM 2391b or SRM 2395

The traceability step: unbroken chain of comparisons

- Analyze the **appropriate SRM** and the prepared samples “in parallel”.
- Confirm that your results for the SRM are correct: they must agree with the current certificate available at: <http://ts.nist.gov/MeasurementServices/ReferenceMaterials/index.cfm>
- Confirm that your results for the samples are consistent with your previous analyses.
- Maintain the records of the now **traceable** material and the SRM analysis.

Making a NIST Traceable Material for SRM 2391b or SRM 2395

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

Remember:

There must always be a *direct* comparison to the appropriate SRM.
The “Lot” is traceable; the donor/source is ***not!***

Definitions

Calibration is the set of operations which establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material, and the corresponding known values of a measurement.

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Making a NIST Traceable Material for SRM 2372
AKA assigning a DNA concentration to qPCR kit stds/or DNA extract (Calibration)

Prepare/Purchase a “lot” of DNA extract for use.
 Are the samples?

Homogeneous

- all aliquots contain similar material, appropriate for use.
 - calibrate dispensing equipment prior to use
 - continuous stirring while the material is aliquoted

Stable

- the material will exhibit the same properties throughout it’s proposed shelf life at the proposed storage conditions.
 - prepared extracts are in appropriately sealed tubes

Making a NIST Traceable Material for SRM 2372
AKA assigning qPCR kit stds/or DNA extract a DNA concentration (Calibration)

Are the samples?

Reproducible (*ie.* the material always yields the same results)

- select random samples of the “lot” and analyze
- assure that all results are the same every time
- assure that there are no signs of degradation

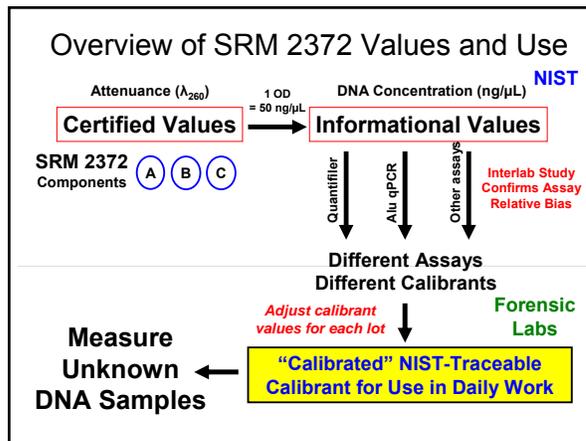
SRM 2372
Human DNA Quantitation Standard

Components

A: Male/single donor/RNased/NIST
 B: Female/multiple donors/NIST
 C: Mixture/male & female/commercial

Quantities supplied:
 110 µL of Human Genomic DNA = 50ng/µL

Certification
 Decadic Attenuance (**Absorbance**) by a US National Reference Spectrophotometer
 Homogeneity by a Cary 100 Bio Spectrophotometer
Validation of conventional [DNA] by Interlaboratory Study and NIST qPCR studies.



Nominal DNA Concentrations
 Using 1 OD = 50 ng/µL double stranded DNA.

Informational Values

Component	Nominal [DNA], ng/µL
A	52.5
B	53.6
C	54.3

- So how do you use this SRM?**
- You calibrate your materials and make them NIST Traceable by using [SRM 2372 to create the standard Curve](#).
 - How?
 - By analyzing **your materials** with **your DNA Quantification Methods** and assigning a [DNA] based on the values obtained **using SRM 2372 materials to generate your standard curve.**

Examples of Value Assignment

- Take the DNA you plan to use as the Calibration Standard in your qPCR assay.
- Make serial dilutions of this material to run in your qPCR value assignment assay:
 $S1_{1:10} \rightarrow S2_{1:5} \rightarrow S3_{1:2} \rightarrow S4_{1:2}$
 (or whatever you normally use)
- The SRM 2372 components are used as the calibration standards** (Serial 1:2 dilutions).
- All samples and standards are analyzed in duplicate.

qPCR plate setup

	1	2	3	4	5	6	7	8
A	A_52.5	A_52.5	B_53.6	B_53.6	C_54.3	C_54.3	S1 unknown	S1 unknown
B	A_26.3	A_26.3	B_26.8	B_26.8	C_27.2	C_27.2	S2 unknown	S2 unknown
C	A_13.1	A_13.1	B_13.4	B_13.4	C_13.6	C_13.6	S3 unknown	S3 unknown
D	A_6.6	A_6.6	B_6.7	B_6.7	C_6.8	C_6.8	S4 unknown	S4 unknown
E	A_3.3	A_3.3	B_3.4	B_3.4	C_3.4	C_3.4	NTC	NTC
F	A_1.6	A_1.6	B_1.7	B_1.7	C_1.7	C_1.7		
G	A_0.8	A_0.8	B_0.8	B_0.8	C_0.8	C_0.8		
H	A_0.4	A_0.4	B_0.4	B_0.4	C_0.4	C_0.4		

Quantifiler Human results: value assignment

Dilution code	Serial Dilutions	qPCR Results	SD	Multiple by	[DNA]	SD
S1	1:10	12.6	0.58	10	126	5.8
S2	1:5	2.9	0.02	50	145	0.8
S3	1:2	1.4	0.01	100	138	0.5
S4	1:2	0.7	0.02	200	137	3.9

New assigned value is:
Average of [DNA] column

= 136 ng/μL

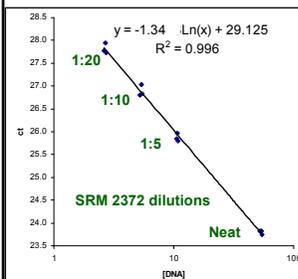
Definitions

Performance check is a quality assurance measure to assess the functionality of laboratory instruments and equipment that affect the accuracy and/or validity of forensic sample analysis.

Reproducibility is the ability to obtain the same result when the test or experiment is repeated.

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Reproducibility



Everything is under control!

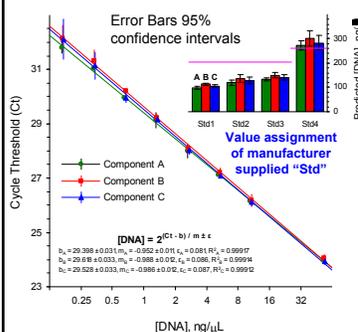
1:10 dilutions of these materials were made prior to analysis

Quantifiler Human Kit was used in conjunction with a 7900 instrument.

Results from two runs were combined to produce the following results.

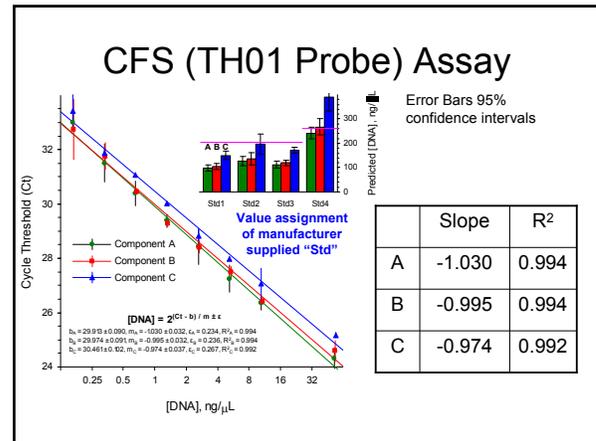
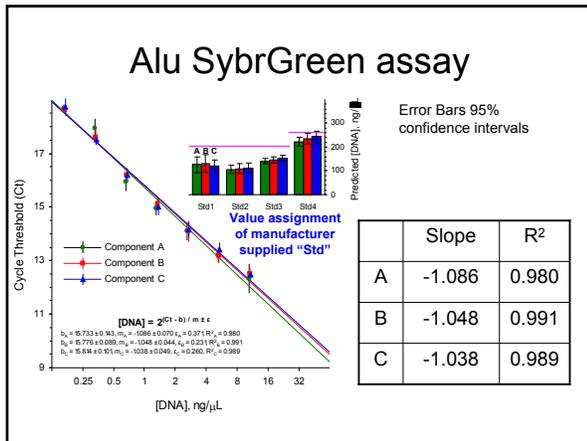
n = 34	Ct	sd	%
A_boxes	26.95	0.09	0.3
B_boxes	27.02	0.10	0.4
C_boxes	26.90	0.10	0.4

Qfiler Assay



With qPCR, 100% reaction efficiency results in a doubling of the DNA target each cycle. Using \log_2 the "perfect" fit regression slope would be -1.0 Ct per \log_2 .

	Slope	R ²
A	-0.952	0.999
B	-0.988	0.999
C	-0.986	0.999



STRBase: a Community Resource for Forensic DNA Applications of STRs...

Short Tandem Repeat DNA
Internet DataBase

NIST Standard Reference Database SRD 130

For more information on STRBase

Lab Resources and Tools

- o [Addresses for scientists working with STRs](#)
- o [Training Materials](#)
- o [STR Allele Sequencing](#)
- o [Population data](#)
- o [Data from NIST U.S. Population Samples](#)
- o [NIST-Developed Software including AutoDimer, mixSTR, and Multiplex_OA](#)
- o [NIST Standard Reference Material for PCR-Based Testing](#)
- o [New STR Markers under Development at NIST](#)
- o [Chromosomal Locations](#)
- o [DNA Advisory Board Quality Assurance Standards](#)
- o [Interlaboratory Studies](#)
- o [NIST Mixture 2005 Interlab Study MIX05 Data](#)
- o [Validation information](#)
- o [DNA Quantitation - SRM 2372 \(available as of October 5, 2007\)](#)
- o [Technology for resolving STR alleles](#)

← Two buttons

Find our talks on STRBase

General Information

- o [Purpose of STRBase/NAR 2001 Paper describing STRBase/Overview Presentation](#)
- o [Publications and Presentations from NIST Human Identity Project Team](#)
- o [NIJ-Funded Projects](#)
- o [Training Materials](#)
- o [Links to other web sites](#)
- o [Glossary of commonly used terms](#)

Publications and Presentations from the NIST Forensics Human Identity Project Team

[Publications] [Presentations] [Training Workshops]

Thank You for Your Attention...

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