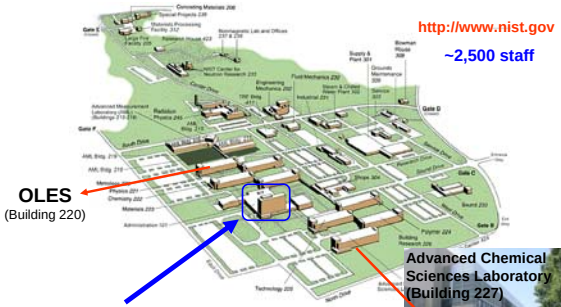
Forensics @ NIST
December 6, 2010 – Gaithersburg, MD

NIST DNA Efforts and SRM Update

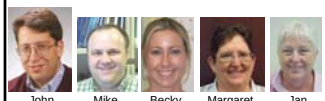
John M. Butler
Margaret C. Kline

NIST Gaithersburg Campus



NIST Human Identity Project Teams
within the Applied Genetics Group

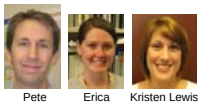
Forensic DNA Team



John Butler, Mike Coble, Becky Hill, Margaret Kline, Jan Redman

Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

DNA Biometrics Team



Pete Vallone, Erica Butts, Kristen Lewis O'Connor

Funding from the **FBI S&T Branch** through NIST Information Access Division

Data Analysis Support

Dave Duewer

Our Team Mission Statement
(Scope of Our Work)

The NIST Human Identity Project Team is trying **to lead the way in forensic DNA**… through research that helps bring **traceability and technology to the scales of justice**.

Support to the Community

…Bringing traceability and technology to the scales of justice…

- Conduct interlaboratory studies
- Perform beta-testing of new human identity testing products
- **We collaborate with other NIJ grantees**
- We provide input to (or have aided):
  - Scientific Working Group on DNA Analysis Methods (**SWGDAM**)
  - Department of Defense Quality Assurance Oversight Committee for DNA Analysis
  - Virginia DFS Science Advisory Committee
  - American Prosecutor's Research Institute (**APRI**) DNA Forensics Program "Course-in-a-Box" for training lawyers
  - WTC Kinship and Data Analysis Panel (**KADAP**) and Hurricane Katrina efforts
  - NIJ Expert System Testbed (**NEST**) Project

Current Activities at NIST
Enabled by Our NIJ Partnership

- **Standard Reference Materials** — **STRBase website**
  - SRM 2372 (DNA quant) released Oct 2007 (>640 units in use)
  - SRM 2391b (STRs), 2395 (Y-STRs), 2392 (mtDNA), *SRM 2391c (FY11)*
- **Technology Evaluation and Development**
  - Rapid multiplex PCR protocols* (PCR: 3 hr to <20 min; ***now FBI-funded***)
  - Low-level DNA studies and kinship analysis
  - Mixture interpretation – research and training materials
  - Variant STR allele characterization
  - New STR loci & assays (STR 26plex, SNP testing) and kit concordance
  - U.S. population data (23 autosomal STR loci)
  - Y-chromosome characterization (mutation rates, deletions, nomenclature)
- **Training Materials**
  - AAFS, ISHI, ISFG & lab workshops on mixture interpretation, CE, etc.
  - Third edition of *Forensic DNA Typing* textbook

Our Team's Consistent Funding and Productivity

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Year	Funding		Staff	Publications	Presentations	Workshops
Pre-2000	$100-200k		5	~3	~5	0
FY2000	$750k		5	3	11	0
FY2001	$500k	All NIJ DNA	6	8	24	0
FY2002	$950k		6	6	33	0
FY2003	$900k		6	10	23	0
FY2004	$1.1M	$7.0M	7	15	24	1
FY2005	$1.2M	$7.8M	7	16	38	2
FY2006	$1.2M	??	6	14	42	7
FY2007	$1.1M	$5.1M	6	14	44	9
FY2008	$1.0M	$10.2M	7	11	29	6
FY2009	$1.1M+	$7.0M	7	7	50	11
FY2010	$1.2M+	$8.6M	9	14	51	2
TOTALS	**$11M**	***12.3%*** of R&D budget		**118**	**369**	**38**
Average	*~$1.0M*			*10.7*	*33.5*	*3.4*

+FBI-funding (DNA biometrics)

Summary of NIJ-Funded Research

One-third of all NIJ DNA publications come from our NIST DNA Team

http://www.dna.gov/research/

Publications from the NIST DNA Program

 # Publications as listed on DNA.gov

- Alternative Genetic Markers (2/16)
- Compromised DNA Evidence (9/20)
- **General Tools and Information (17/33)**
- Human DNA Quantitation (2/13)
- Miniaturization and Automation (5/26)
- Mitochondrial DNA (7/25)
- Non-Human DNA (1/17)
- Sperm Detection and Separation (0/3)
- **Y-Chromosome (17/34)**

60/187 (32%) of all NIJ DNA publications are from NIST

As of February 17, 2010

How We Built Our Program

- Our customer is the entire forensic DNA community (we are involved at multiple levels for maximum impact)
- Consistent and substantial funding provided by the National Institute of Justice (**$11M since 2000**)
- **Consistently deliver on EVERYTHING we agree to do**
- Hard work and maintaining focus on customer needs
- Regular participation in every major forensic DNA meeting and communicate information to team members
- Visits to individual labs to understand their needs and provide useful training (and listening to their feedback)

Seminars and Training Workshops to Individual Forensic DNA Laboratories

MYRIAD — Feb 3, 2005

May 19, 2005
Oct 24, 2007

June 8, 2005

June 13-14, 2005

Apr 27-28, 2006

June 6, 2006

Aug 7, 2006

Nov 15, 2006

Dec 5-6, 2006

March 7, 2007

March 14, 2007

Apr 3-4, 2007

Apr 5, 2007

June 5, 2007

Presentations from Our Group

Forensics @ NIST (Dec 6-8, 2010)

1. John Butler, Margaret Kline — NIST DNA Efforts and SRM Update
2. Mike Coble, John Butler — Mixture Interpretation
3. Dave Duewer, Margaret Kline — Inter-Laboratory Studies
4. John Butler — STRBase and Information Resources
5. Becky Hill, Kristen O'Connor — Low-Copy Number and Kinship Analysis
6. John Butler, Becky Hill — ABI 3500 Studies
7. Margaret Kline — DNA Stability Studies: FTA & Whatman

Please Ask Questions…

Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

See also http://www.dna.gov/research/nist
http://www.cstl.nist.gov/biotech/strbase
john.butler@nist.gov

Congress Passed **the DNA Identification Act of 1994** (Public Law 103 322)

Formalized the FBI's authority to establish a national DNA index for law enforcement purposes.

FBI's DNA Advisory Board

Quality Assurance Standards for Forensic DNA Testing Laboratories

(October 1, 1998)

STANDARD 9.5

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.

"Quality" In Measurements

Method Validation

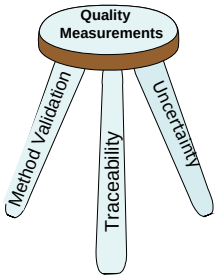
am I measuring what I set out to measure?

Uncertainty

how well do I know the result of what I've measured?

Traceability of Result (Reference Materials)

can I compare this result with other results?

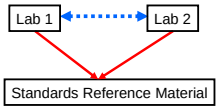


Standard Reference Materials (SRMs)

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

Requirements for a NIST SRM

Material must be fit for purpose:

- **Homogeneity**
  - All tubes are the same — Test random samples
- **Stability**
  - Will withstand shipping and normal storage and is periodically tested over the life time of the SRM
- **Recoverability** — Appropriate storage containers
  - What went in the tubes comes out
- **Traceability**
  - Values assigned are traceable to the designated certification method
- **Commutability** — Inter-laboratory study
  - Is the SRM what the intended user needs?

Steps Involved in SRM Production

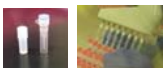
Attend conferences, read the literature, talk to potential customers

Receive input on priorities for projects and potential SRMs

Sequence & Copy Number

Research potential properties and samples to be characterized and measurement method to be used

Obtain candidate components/make measurements



Decide on number of SRM units to produce (impacts price/unit), sample packaging, concentration, etc.

Bottle components and conduct homogeneity and stability studies; finalize uncertainty

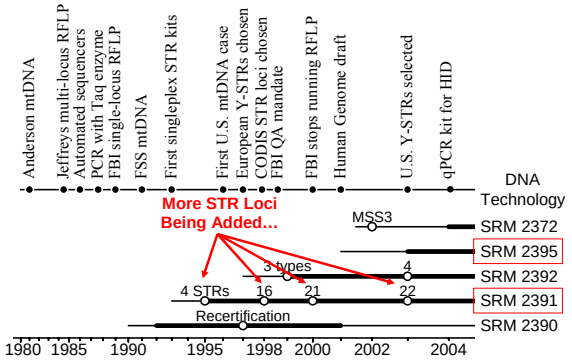
Write Report of Analysis and Certificate of Analysis

Certificate Reviewed and Approved by NIST Measurement Services Division

SRM Made Available for Purchase
http://www.nist.gov/srm

Forensic DNA Timeline

Slide not updated since its creation in 2004…

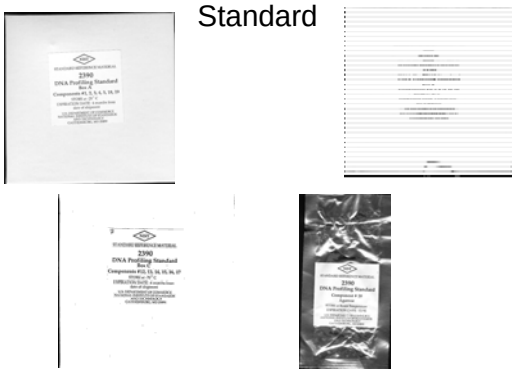


The Tools of DNA Typing and SRM Needs

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

  Technology no longer used
- PCR-Based Testing (Mid 1990's) SRM 2391..a..b
  - ~~Dot Blot~~
  - ~~VNTR~~
  - STR (Fluorescent markers used today)
- 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

1992: SRM 2390 DNA Profiling Standard



1992: SRM 2390 DNA Profiling Standard

Box A
Molecular Weight Marker DNA
Molecular Weight Marker Dilution
Molecular Weight Marker Probe
DNA Klenow Fragment
(For labeling Marker Probe)
Stop Solution
Adenovirus Visible Ladder
10X Buffer

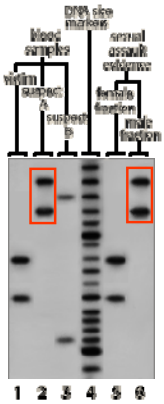
Box B
250 ng DNA standard
100 ng DNA standard
50 ng DNA standard
25 ng DNA standard
12.5 ng DNA standard
6 ng DNA standard
For evaluating extracted DNA on a Yield Gel

Box C
K562 Cell Pellet
K562 Undigested DNA
K562 DNA *Hae*III Digested
TAW Male Cell Pellet
TAW Male Undigested DNA
TAW Male DNA, *Hae*III Digested

Agarose
low electroendosmosis

RFLP Analysis

7-10 days for these results. The membrane would be stripped and reprocessed 4 to 6 more times.



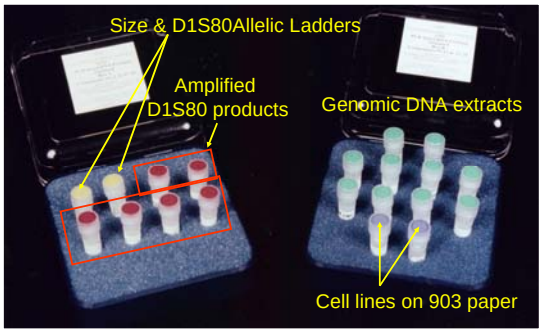
Which Suspect, A or B, cannot be excluded from potential perpetrators of this assault?

RFLP Drawbacks:

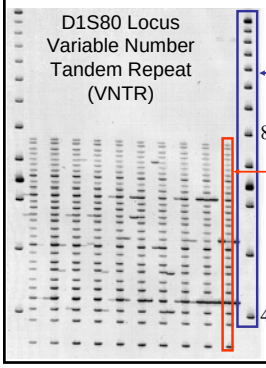
- Requires 100 ng to 1 µg of DNA (stain the size of a dime)
- The DNA must be relatively intact 1000-20,000 bp in size (not always possible to obtain)
- ^{32}P visualization requires 3 – 7 days @ –80 ºC
- 5 – 7 probes required for matching
- Time required weeks to months

Technology moves forward

1995: SRM 2391 PCR-based DNA Profiling Standard

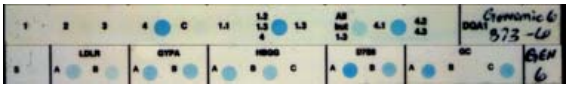


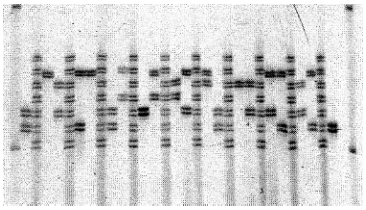
1995: SRM 2391 PCR-based DNA Profiling Standard



6 - Pre-amplified DNA's
10 - Genome DNA Extracts
2 - Cell Line cells spotted on 903 paper for extraction

DQ alpha & Polymarker Reverse Dot Blot Hybridization





Silver stained gels for STR monoplexes

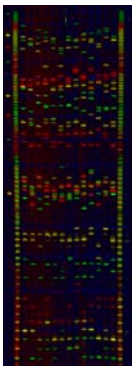
2000: SRM 2391a

NIST SRM 2391a Certificate of Analysis

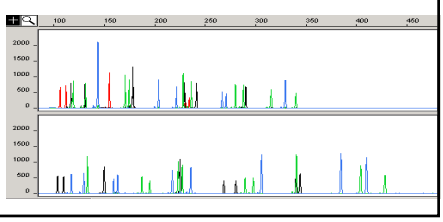
National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2391a
PCR-based DNA Profiling Standard

- When SRM 2391 was renewed, STRs loci are now the primary focus in forensic DNA analysis
- 21 STR loci were included in the certificate
- D1S80 amplified products were no longer supplied (only a single box with 12 components)
- Fluorescent labeling of the PCR products enabled new analysis technologies and multiplexing

2003: SRM 2391b



- 22 STR Loci,
- D1S80, and DQa1/PM
- Many labs using 16-plexes
- 0.5 – 1 ng DNA



NIST SRM 2391b

Certificate update September 2008

National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2391b
PCR-Based DNA Profiling Standard

Table 2. Certified Values for Additional STR Loci

F13A01	F13B	FES/FPS	LPL	Penta D	Penta E	D2S1338	D19S433
6,7	10,10	12,12	10,11	10,15	7,12	17,23	13,16.2
7,7	8,10	10,11					16
3.2,4	9,10	11,12					4
5,6	6,9	10,13					3
5,7	8,9	11,13					14
3.2,5	9,10	11,11					4
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	9,11	8,9	5,10	22,22	12.2,15
6,16	8,10	10,12	11,12	12,12	12,13	19,23	14,15
6,6	8,8	11,11	10,12	8,12	11,11	23,23	13,14
6,16	8,10	10,12	11,12	12,12	12,13	19,23	14,15
6,6	8,8	11,11	10,12	8,12	11,11	23,23	13,14

48 autosomal STRs
characterized across
12 DNA samples
As of Sept. 2008

NIST Standard Reference Material (SRM) for Forensic DNA Testing

SRM 2391b (2003-2011)

- **48 autosomal STR loci** with certified values
- **10 liquid genomic DNA** components + **2 punches** (cells on 903 paper)
- All single source samples
- 4 males + 6 females
- 9947A & 9948 included

SRM 2391c (2011-future)

- **23 autosomal STR loci and 17 Y-STRs** certified
- **4 liquid genomic DNA** components + **2 punches** (cells on **FTA** & 903 paper)
- 5 single source + 1 mixture
- 3 males + 2 females (unique)
- All new samples
  - no 9947A or 9948

SRM 2391c to replace SRM 2391b and SRM 2395 (price reduction)

SRM Production Process for Preparing Cells on FTA or 903 Paper

Required >200 million cells (43 mL of media) to spot 2688 paper punches

Paper is punched and placed into a sterile 96 well tray

Cell suspension is stirred to keep homogeneous

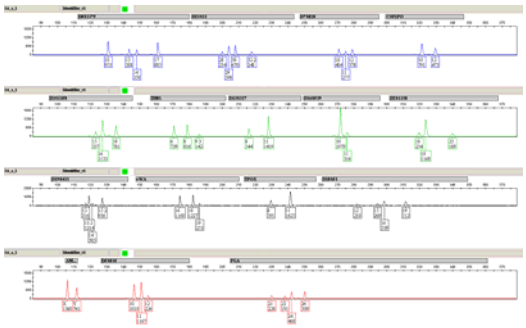
8-channel pipette is used to load cell plates and spot paper punch

Punches are first air-dried and then stored in a desiccator

Each punch, containing hopefully a similar amount of cells, is then placed into a tube and packaged with the other SRM 2391c components

Making a Mixture for SRM 2391c

Carefully considering allele combinations & mixture ratios



Additional Information on SRM 2391c

- Liquid genomic DNA components
  - Considering **50 µL volume with ~2 ng/µL** concentration (will not be certified for DNA quantity)
  - Mixture will be 3 parts male, 1 part female (total ~2 ng/µL)
  - For production purposes, we will **need 140 µg** of each DNA sample
  - PFA (Teflon) tubes to reduce DNA binding to walls
- Paper punches (6 mm diameter)
  - Enables multiple punches from a single spot
  - Theoretically **400 ng of DNA per 6 mm punch** (recovery will depend on extraction efficiency)
- Will have sequence information or multiple STR kit confirmation results for every certified allele call
- **Will verify performance on every commercially available STR typing kit**

Commercially Available STR Kits

Applied Biosystems (17)
- ~~AmpFlSTR Blue (1996)~~
- ~~AmpFlSTR Green I (1997)~~
- Profiler (1997)
- Profiler Plus (1997)
- COfiler (1998)
- SGM Plus (1999)
- **Identifiler** (2001)
- Profiler Plus ID (2001)
- ~~SEfiler (2002)~~
- Yfiler (2004)
- MiniFiler (2007)
- SEfiler Plus (2007)
- Sinofiler (2008) – China only
- **Identifiler Direct** (2009)
- NGM (2009)
- **Identifiler Plus** (2010)
- NGM SElect (2010)

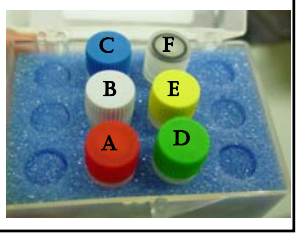
Promega Corporation (13)
- PowerPlex 1.1 (1997)
- PowerPlex 1.2 (1998)
- PowerPlex 2.1 (1999)
- **PowerPlex 16** (2000)
- PowerPlex ES (2002)
- PowerPlex Y (2003)
- PowerPlex S5 (2007)
- **PowerPlex 16 HS** (2009)
- PowerPlex ESX 16 (2009)
- PowerPlex ESX 17 (2009)
- PowerPlex ESI 16 (2009)
- PowerPlex ESI 17 (2009)
- PowerPlex 18D (2010)

Qiagen (2010)
Primarily selling kits in Europe
Due to patent restrictions cannot sell in U.S.
- ESSplex
- ESSplex SE
- Decaplex SE
- IDplex
- Nonaplex ESS
- Hexaplex ESS
- HD (Chimera)
- Argus X-12
- Argus Y-12
- DIPlex (30 indels)

~1/3 of all STR kits were released in the last year

2003: SRM 2395 Human Y-Chromosome DNA Profiling Standard

Extracted genomic DNAs: 5 Male, 1 Female (neg Control)

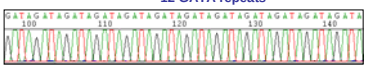


2003 SRM 2395 Certificate Information

For the 5 male samples :
22 Y-chromosome STR markers sequenced
5 Y-chromosome STR markers typed/not sequenced
42 Y-chromosome biallelic SNPs

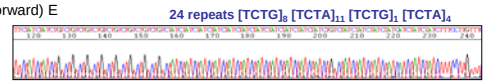
DYS439 (forward) A

12 GATA repeats



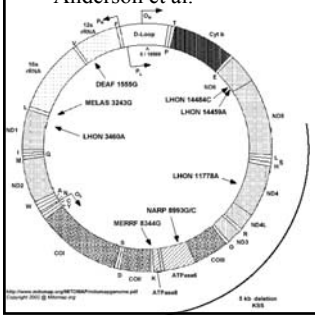
DYS390 (forward) E

24 repeats [TCTG]$_8$ [TCTA]$_{11}$ [TCTG]$_1$ [TCTA]$_4$

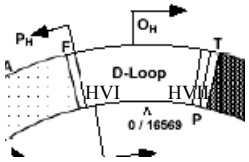


Human Mitochondrial Genome

1981: sequenced by Anderson et al.



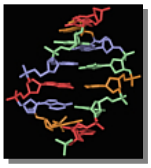
1991: FSS uses the non-coding region, control region for casework
1996: FBI began mtDNA casework



Hypervariable Region I & II

Mitochondrial DNA Standard

Human Mitochondrial DNA Standard Reference Material (SRM 2392) for Quality Control in Sequencing, Forensic Identification, Medical Diagnostics and Mutation Detection



SRM 2392 includes extracted DNA and all information for performing

- **PCR amplification process**
- **cycle sequencing steps**
- **gel separation**
- **data analysis to determine DNA sequence**
- **materials to assess accuracy of results**

Sequences of 58 sets of unique primers are also included to allow any area or all mtDNA to be amplified and sequenced

SRM provides necessary *quality control* for DNA sequence data

1999: SRM 2392 Mitochondrial DNA Sequencing Standard

- SRM 2392 certifies the entire mtDNA sequence information for apparently normal cell lines : CHR, GM09947a, and GM09948.
- Included with SRM 2392:
  DNA extracts of CHR and GM09947a
  Cloned DNA from CHR HVI region
- **2003: SRM 2392-I, Cell line HL-60 extract and sequence information.**

General qPCR Comments from the Forensic Community

- "I have feeling that the calibrant may exhibit a two-fold difference from the "true" value"
- "In practice we have found that utilizing a target range of 1-2 ng based on a method X result oftentimes yields STR data below our rfu threshold"
- "There appears to be an obvious difference between the two lots of a calibrant"
- "We have not had any problems with the lot_X calibrant and our results have been relatively stable"

SRM 2372: Human DNA Quantitation Standard

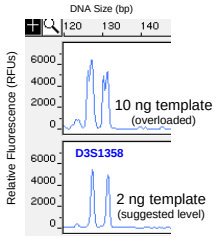
Challenge:
What is a nanogram of genomic DNA ?

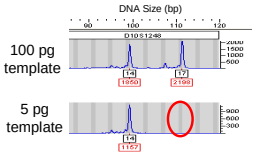
From interlaboratory studies we know there is a factor of 1.6 in the measurement systems currently in use. But the range is 20 fold.

Impact of DNA Amount into PCR

Reason that DNA Quantitation is Important Prior to Multiplex Amplification

- Too much DNA
  - Off-scale peaks
  - Split peaks (+/-A)
  - Locus-to-locus imbalance
- Too little DNA
  - Heterozygote peak imbalance
  - Allele drop-out
  - Locus-to-locus imbalance





Stochastic effect when amplifying low levels of DNA produces allele dropout

SRM 2372
Human DNA Quantitation Standard

Released Oct 2007

Component A: Male
Component B: Female
Component C: Mixture

Amounts: Each component 50 µL of Human Genomic DNA with a concentration targeted @ 50 ng/µL. The [DNA] for each component is list in the materials Certificate of Analysis.

SRM 2372
Human DNA Quantitation Standard

Component A: Male (blood)
Component B: Female (blood)
Component C: Mixture (placenta)

- Genomic DNA isolated by Salt out procedure
- Treated with RNAse and re-precipitated
- UV spectroscopy 340-220 nm on a NIST calibrated spectrophotometer
- Assume $A^{260} = OD^{260} = 1$ for a 50 µg/mL solution

Forensic SRM sales (Oct 2007-Nov 2010)

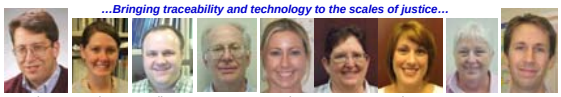
SRM :	2372 Quant	2391b STR	2395 Y-STR	2392 mtDNA	2392-I mtDNA HL60
FY 08	160	125	72	0*	20
FY 09	147	140	88	12	19
FY 10	196	139	55	4	15
FY 11	35	20	14	1	1

* Sales restricted while a certificate revision was accomplished

The NIST Human Identity Project Team
(Forensic DNA & DNA Biometrics)

Funding from the **National Institute of Justice (NIJ)** through the NIST Office of Law Enforcement Standards and the **FBI S&T Branch** through the NIST Information Access Division

...Bringing traceability and technology to the scales of justice...



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Workshops & Textbooks | Mixtures, mtDNA & Y | Concordance & LT-DNA | Kinship Analysis | Rapid PCR & Biometrics | Direct PCR & DNA Extraction | Software Tools & Data Analysis | Variant alleles & Cell Line ID | STRBase Support

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