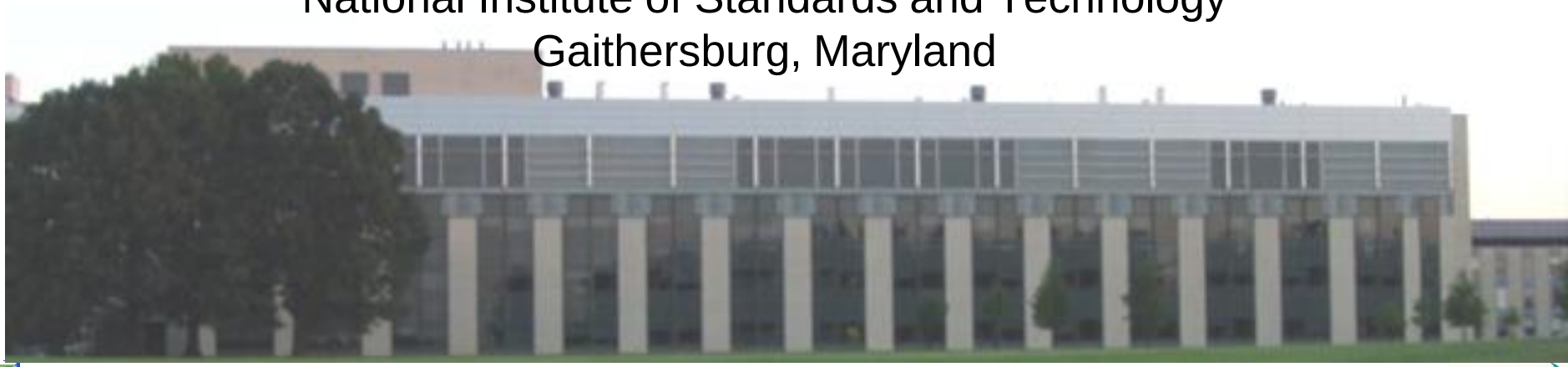
## Association for Molecular Pathology
### November 17, 2010 – San Jose, CA

# Dialogue with NIST:
## Standard Reference Materials for Molecular Diagnostics

**John M. Butler, Ph.D.**

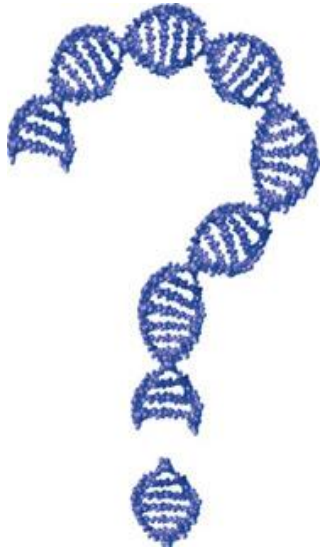
NIST Applied Genetics Group
National Institute of Standards and Technology
Gaithersburg, Maryland



NIST
Applied Genetics

# Presentation Outline

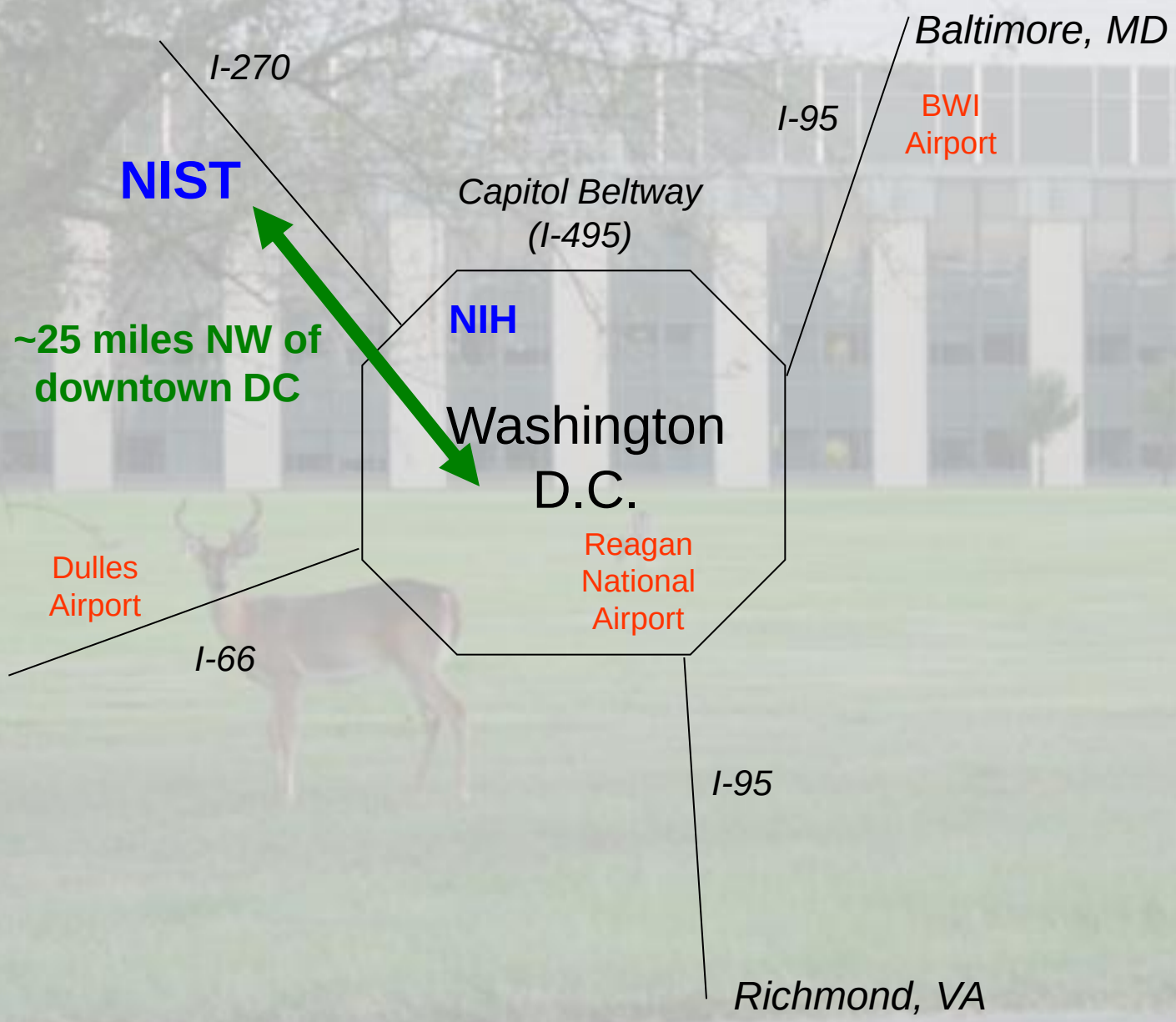
**Please ask questions**



- **NIST**
  - location, role, organizational structure, funding
- **Applied Genetics Group**
  - members, expertise, equipment, funding
  - programs, projects, priorities
- **Standard Reference Materials (SRMs)**
  - Purpose, principles, production process, pricing
  - Differences from WHO standards (certification vs consensus)
- **Clinical DNA SRMs**
  - Pipeline, plans, and path forward
  - Partnerships: needs/priorities from the community

# NIST Background

# Location of NIST



# National Institute of Standards & Technology (NIST)

- **Non-regulatory agency** established in 1901 in the US Department of Commerce.
- Mission to promote US innovation and industrial competitiveness by advancing measurement science, standards & technology.
- NIST is at the top of the US standards pyramid for a wide variety of physical standards, test methods, and calibrations.

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# Organic Act of 1901; Updated in 2008

"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."


THE EVENING STAR, MONDAY, MARCH 11, 1901

CORRECT MEASURES

Passing of the New Bureau of Standards

LABORATORY TO BE ERECTED

Prof. Stratton, the Director, Details Need of Establishment

A HANDICAP REMOVED

## Functions and Activities include:

- Custody and dissemination of national standards
  - Calibrations, Certified Reference Materials, Reference Data, Reference Methods
- Determination of physical constants and properties of materials
- Comparison of US National standards with those of the world
- Solution of standards and measurement problems for industry and other agencies

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# Early Driver for U.S. Standards

1904

- Out-of-town fire companies arriving at a Baltimore fire cannot couple their hoses to the hydrants. 1526 buildings razed.

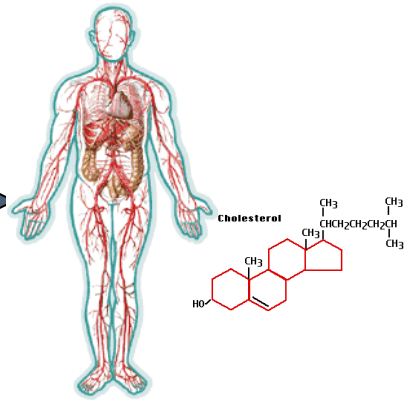
1905

- National Fire Protection Association adopted NBS-developed national hose coupling standard.

# NIST

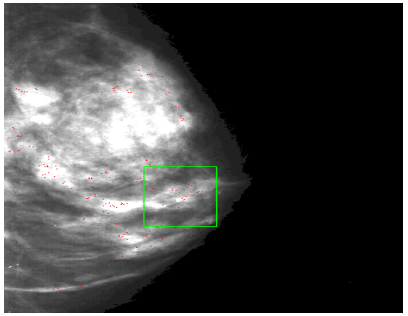
**Since its inception NBS/NIST has focused its research and measurement services activities on contemporary societal needs.**

1901 2010




*Standards for train tracks, couplings, steel manufacturing …*

*Standards for clinical analytes, medical imaging, cybersecurity …*



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# NIST Today

## Major Assets

- ~ 2,900 employees
- ~ 2600 associates and facilities users
- ~ 400 NIST staff on about 1,000 national and international standards committees
- 3 Nobel Prizes in past 15 years

## Major Programs

- **NIST Laboratories**
- Baldridge National Quality Program
- Hollings Manufacturing Extension Partnership
- Technology Innovation Program

**Joint NIST/University Institutes:**

- JILA
- Joint Quantum Institute
- Institute for Bioscience & Biotechnology Research
- Hollings Marine Laboratory

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# Role of NIST


NIST is a non-regulatory federal agency within the Technology Administration of the U.S. Department of Commerce. NIST's primary mission is to **promote economic growth by working with industry to develop and apply technology, measurements, and standards**.

**Measurement and Standards Program**
planned and conducted in cooperation with industry and focused on infrastructural technologies

**Baldridge National Quality Program**
an outreach program recognizing organizational performance excellence

**Hollings Manufacturing Extension Partnership Program**
a nationwide network of extension centers that provides hands-on technical assistance to smaller manufacturers

**Technology Innovation Program**
Planned to provide cost-shared awards to industry, universities and consortia for research on potentially revolutionary technologies that address critical national and societal needs.

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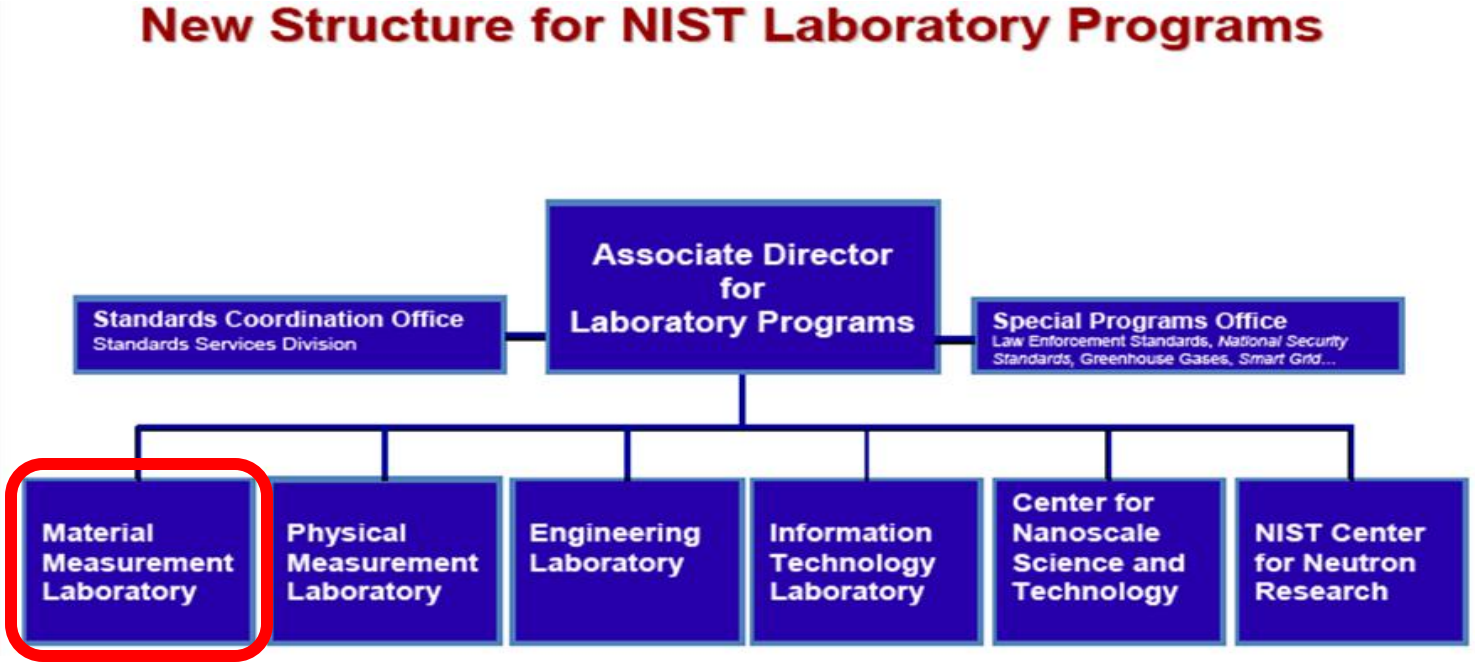
# The NIST Laboratories




**Traditionally focused research and measurement service activities on physical science and engineering disciplines**

**Bioscience and Health identified as a new area for significant emphasis for NIST labs**

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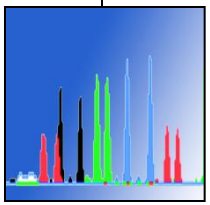
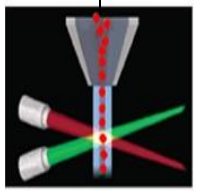
# NIST Biochemical Science Division

**Laurie Locascio**
**Division Chief**

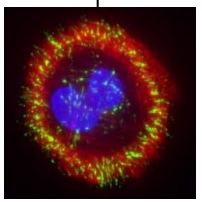
Process Sensing
Dean Ripple
Group Leader



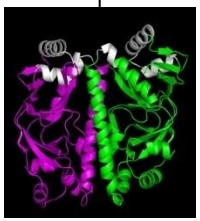
**Applied Genetics**
John Butler
Group Leader



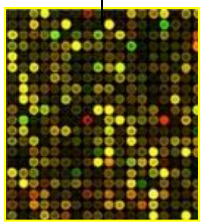
Bioassay Methods
Steve Choquette
Group Leader



Cell Systems Science
Anne Plant
Group Leader



Macromolecular Structure & Function
John Marino
Group Leader



Multiplexed Biomolecular Science
Marc Salit
Group Leader

# NIST Applied Genetic Group

# Applied Genetics Group Mission Statement

***Advancing technology and traceability*** through quality genetic measurements to aid work in

- **forensic DNA testing**
- **clinical diagnostics**
- **cell line authentication**
- **agricultural biotechnology**
- **DNA biometrics**

# APPLIED GENETICS Group

## *Major Programs Currently Underway*

- **Forensic DNA**
  - New loci and assays (26plex)
  - STR kit testing
  - Ancestry SNP assays
  - Low-template DNA studies
  - Mixture interpretation
  - STR nomenclature
  - Variant allele cataloging and sequencing
  - Expert systems review
  - Training workshops to forensic DNA laboratories
  - Validation information and software tools
  - Textbook – 3rd ed. (2 vol.)

- **Clinical Genetics**
  - **Huntington's Disease SRM**
  - **CMV SRM**
  - **Exploring future needs**

- **Ag Biotech**
  - "universal" GMO detection/ quantitation (35S promoter)

- **DNA Biometrics**
  - Rapid PCR methods
  - Efforts to standardize testing of future portable DNA systems
  - Kinship analysis

- **Cell Line Authentication**

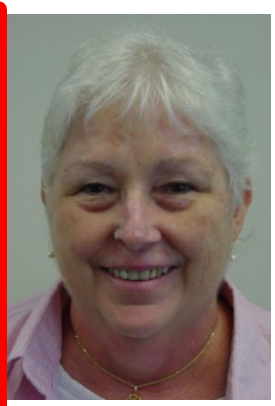
# NIST Applied Genetics Group

*Group Leader*

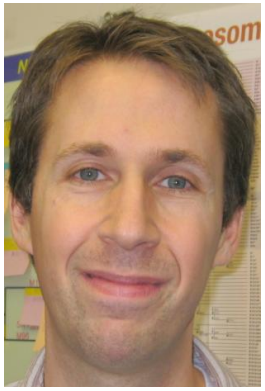
**John Butler**

**Marcia Holden**

**Margaret Kline**



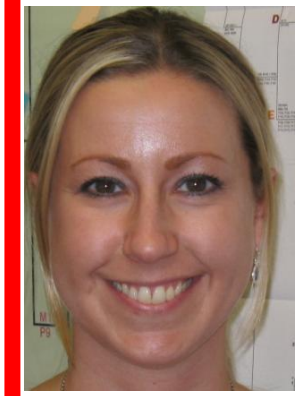
**Jan Redman**



**Pete Vallone**

**Dave Duewer***

**Ross Haynes**



**Becky Hill**

**Erica Butts**

**Kristen Lewis**

**Mike Coble**

# Group Expertise and Funding Sources

## Group Expertise

- Reference Material Characterization
- Standard Information Resource Development
- Rapid Multiplex PCR Assay Construction
- Short Tandem Repeat (STR) Genotyping
- Single Nucleotide Polymorphism (SNP) Genotyping
- DNA Sequencing
- Training Materials and Workshops (validation info)

## Current Funding Sources

- **National Institute of Justice** (Forensic DNA)
- **FBI Science & Technology Branch** (DNA Biometrics)
- **NIST SRM Program** (SRM development and production)
- **Base funding from Congress (clinical DNA)**

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# Applied Genetics Group Instrumentation

- **ABI 3130xl** for Sanger sequencing, SNP analysis, and STR genotyping
- **ABI 7500, Qiagen Roto-Gene Q, Roche LightCycler,** for qPCR (DNA quantitation)
- **ABI 9700** and Veriti thermal cyclers for PCR
- **Fluidigm BioMark** for digital PCR (copy number determination)

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# NIST Digital PCR Instrument

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# NIST Standard Reference Materials (SRMs)

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# SRM Information

- Purpose & use
- Decision to create & design of components
- Pricing: how determined?
- Characterization
  - Stability
  - Homogeneity
  - Value assignment
- Certified value: how determined?
- Difference in approach from WHO (NIBSC)

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# What are Standards and Metrology?

- Standards and Metrology are the things needed to supply good data that can be used to confidently support decision making.
  - in a good world, good decisions are informed with good data which are the results of excellent measurements!
- Metrology and standards comprise the ‘formal’ system that tells us how well we trust those data
- Establishing confidence in data

# Examples of Standards

1. **Documentary Standard**: **Guideline documentation** that reflects agreements in practices by governmental bodies or nationally or internationally recognized industrial, professional, trade associations

2. **Certified or Standard Reference Material**: **A physical entity** to serve as a reference in measuring quantities or qualities, establishing practices or procedures, or evaluating results

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1643d

Trace Elements in Water

This Standard Reference Material (SRM) is intended primarily for use in evaluating methods used in the determination of trace elements in fresh water. SRM 1643d consists of approximately 250 mL of filtered and acidified water in a polyethylene bottle, which is sealed in an aluminized plastic bag to maintain stability. SRM 1643d simulates the elemental composition of fresh water. Nitric acid is present at a concentration of 0.5 mol/L to stabilize the trace elements.

The certified values for 26 elements in SRM 1643d are listed in Table 1. The information values for an additional four elements are provided in Table 2. The analytical methods used for the characterization of this SRM are given in Table 3. All values are reported as mass concentrations [1].

NOTICE AND WARNINGS TO USERS

**Expiration of Certification:** This certification of SRM 1643d is valid, within the measurement uncertainty(ies) specified, until **31 July 2003**, provided the SRM is handled in accordance with instructions given in this certificate. This certification is nullified if the SRM is damaged, contaminated, or modified.

**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 certification, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

**Precautions:** The SRM should be shaken before use because of possible water condensation. To prevent possible contamination of the SRM, do not insert pipets into the bottle. Samples should be decanted at a room temperature of 22° C ± 5° C. After use, the bottle should be recapped tightly and returned to the aluminized plastic bag, which should be folded and sealed with sealing tape. This safeguard will protect the SRM from possible environmental contamination and long-term evaporation.

The accuracy of trace element determinations, especially at the µg/L level, is limited by contamination. Apparatus should be scrupulously cleaned and only high purity reagents employed. Sampling and manipulations, such as evaporations, should be done in a clean environment, such as a Class-100 clean hood.

Coordination of the NIST technical measurements was under the direction of J.R. Moody of the NIST Analytical Chemistry Division.

The technical and support aspects involved in the certification and issuance of this SRM were coordinated through the Standard Reference Materials Program by J.S. Kane and B.S. MacDonald.

Willie E. May, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Revised Certificate Issue Date:
*See Certificate Revision History on Las*

Thomas E. Gills, Chief

Statistical analysis of the experi

SRM 1643d

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# Importance of Reference Materials

Dr. Karen Mann, President of the Association of Molecular Pathology, in her testimony before Congress (Feb 24, 2010):

"Reference materials are important to ensure the necessary sensitivity, specificity and level of reproducibility of intra- and inter-laboratory test results. **The best approach to achieve consistent and comparable quantitative data amongst laboratories is by the use of internationally established reference reagents**."

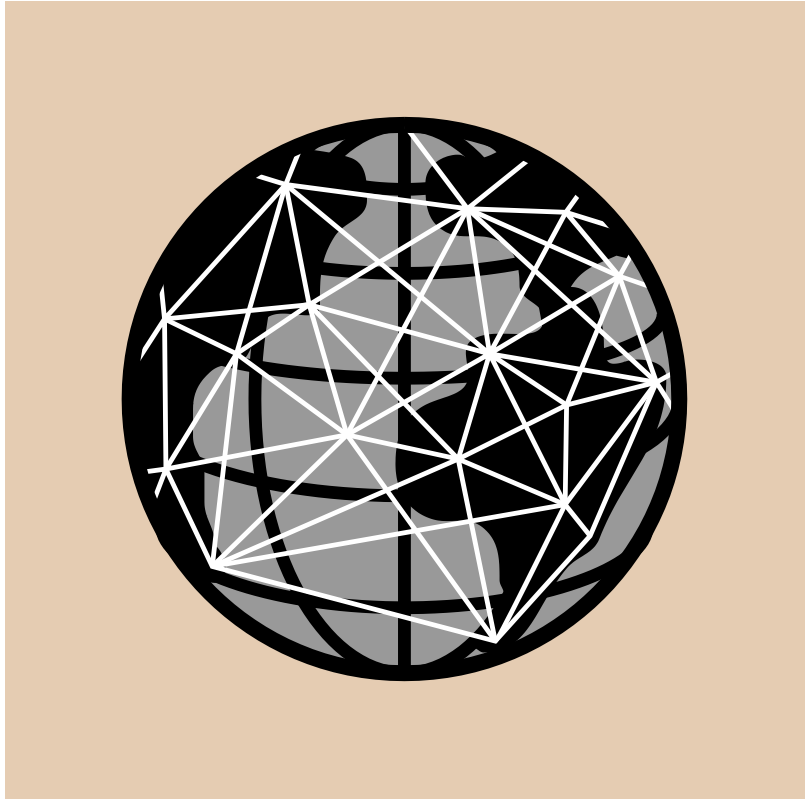
Source: http://www.amp.org

# Comparing Results

- **Results are only useful when compared**
  - to other results
    - e.g., to observe a trend
  - to limits
    - e.g., a threshold for action
  - different results in different places or measured at different times…
    - “comparability over space-and-time”

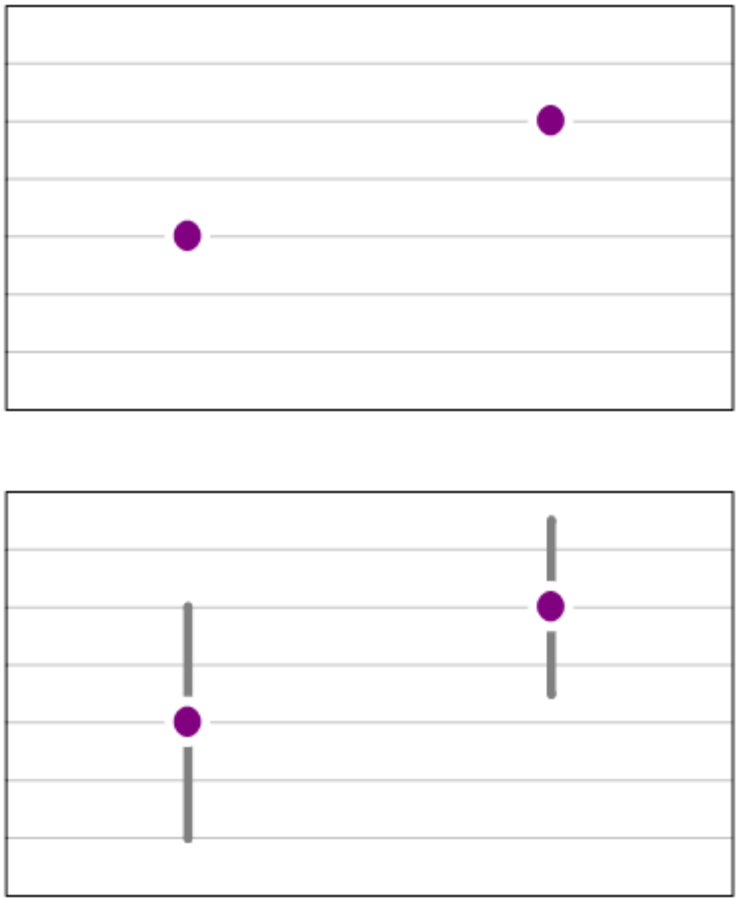
# Comparability of results

- Results linked to a common reference can be compared
- Scope of reference defines scope of comparability
  - global network
    - SI



# Measurement Uncertainty

- Are these results the same?
- How well do you know the result?
  - essential part of being able to compare!
- Are these results good enough?
  - fit-for-purpose



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# "Quality" In Measurements

## Method Validation

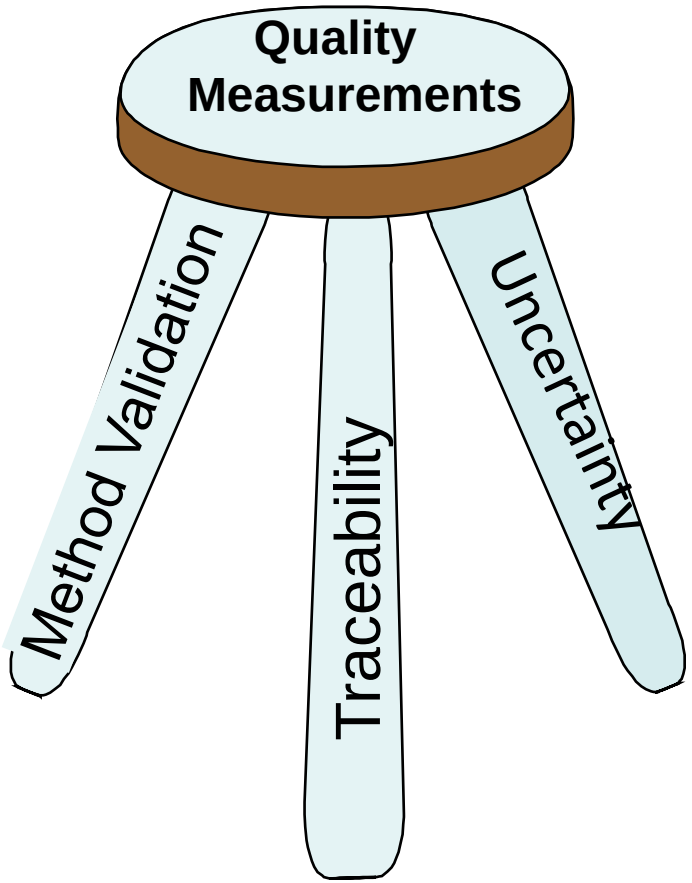
***am I measuring what I set out to measure?***

## Uncertainty

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

## Traceability of Result (Reference Materials)

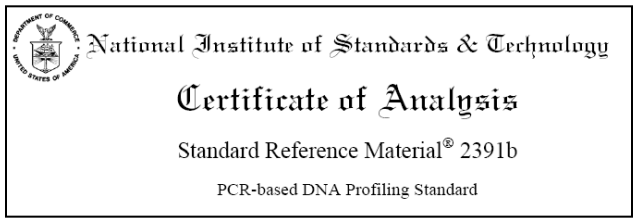
***can I compare this result with other results?***



# Standard Reference Materials (SRMs)

**http://www.nist.gov/srm**

*Traceable standards to ensure accurate and comparable measurements between laboratories*

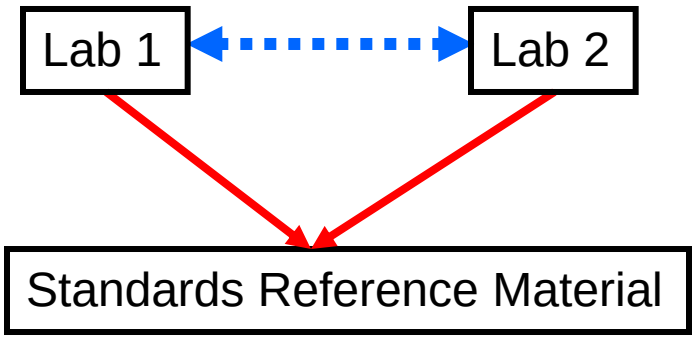

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2391b

PCR-based DNA Profiling Standard


SRM 2391b – autosomal STRs
SRM 2392 &-I – mtDNA sequencing
SRM 2395 – Y-STRs
SRM 2372 – DNA quantitation
SRM 2394 – mtDNA heteroplasmy
SRM 2399 – Fragile X




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

**Helps meet ISO 17025 needs**

**for traceability to a national metrology institute**

# How Do We Know an SRM is Needed?

- May be mandated or recommended by a scientific society or even Congress
  - SRMs are useful when there is a need for between laboratory comparability of measurements; especially if the labs use different measure equipment and/or methods
- Other agencies may come to NIST requesting an SRM
  - it is valuable to document who is requesting the reference material and why
  - The National Institute of Justice (NIJ) requested we develop SRMs for the Forensic DNA Testing Community when DNA technology started entering the courtroom
  - For NIST in general other agencies include: CDC, EPA, NIH…
  - The FDA when inspecting looks at the calibration of critical instruments and may stipulate the use of a higher order standard when available

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# 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.

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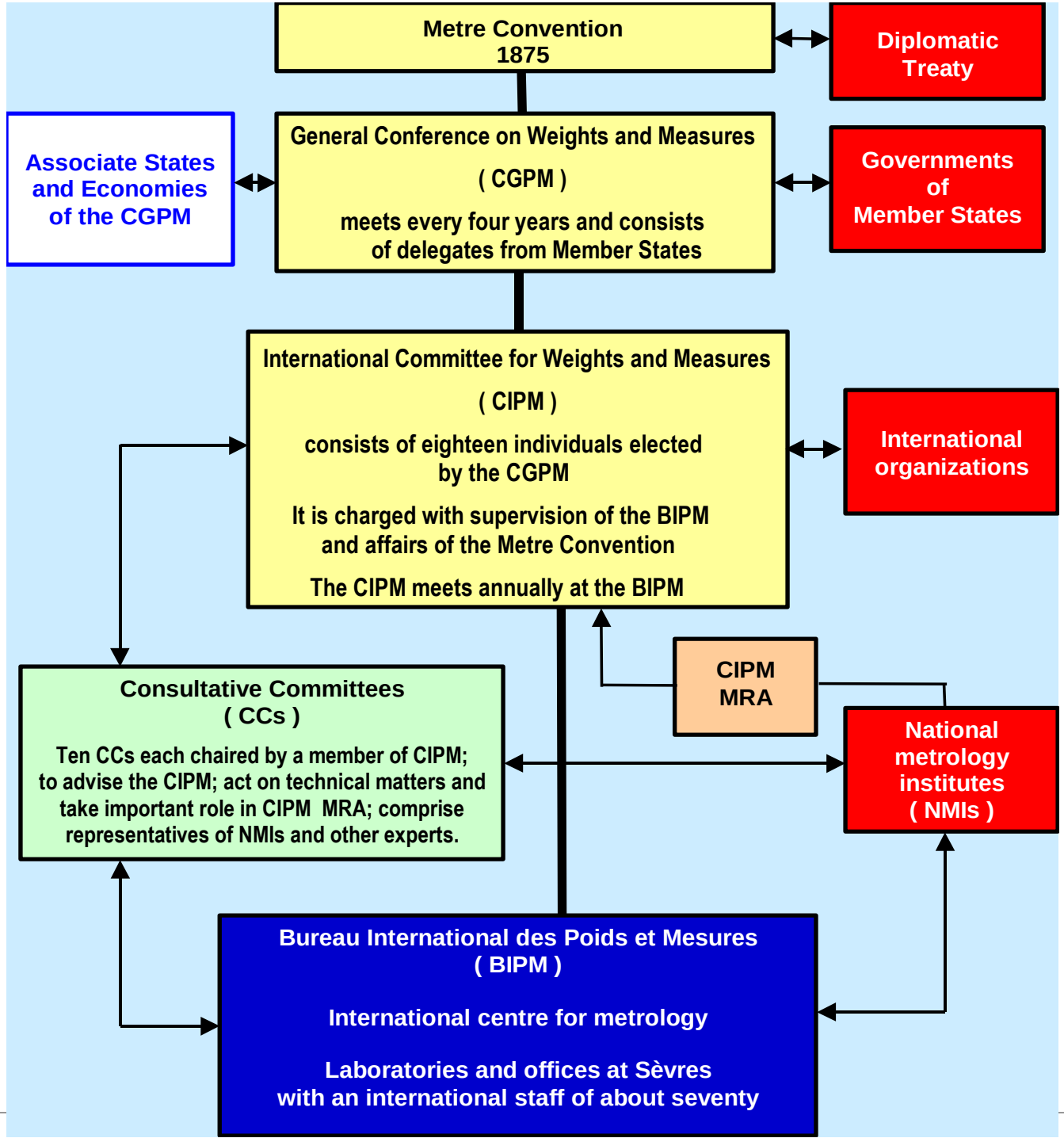
Metre Convention
1875
Diplomatic
Treaty
Associate States
and Economies
of the CGPM
General Conference on Weights and Measures
( CGPM )
meets every four years and consists
of delegates from Member States
Governments
of
Member States
International Committee for Weights and Measures
( CIPM )
consists of eighteen individuals elected
by the CGPM
It is charged with supervision of the BIPM
and affairs of the Metre Convention
The CIPM meets annually at the BIPM
International
organizations
CIPM
MRA
Consultative Committees
( CCs )
Ten CCs each chaired by a member of CIPM;
to advise the CIPM; act on technical matters and
take important role in CIPM MRA; comprise
representatives of NMIs and other experts.
National
metrology
institutes
( NMIs )
Bureau International des Poids et Mesures
( BIPM )
International centre for metrology
Laboratories and offices at Sèvres
with an international staff of about seventy

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# CIPM Mutual Recognition Arrangement

***Objectives:***

- **Establish the degree of equivalence of national measurement standards maintained by NMIs**
- **Provide for the mutual recognition of both calibration and CRM certificates issued by NMIs**
- **Provide a secure technical foundation for wider agreements related to international trade, commerce and regulatory affairs**

***The CIPM Mutual Recognition Arrangement (MRA)*** **was signed in October, 1999 by the directors of the NMIs of 47 member states of the *Metre Convention,* and representatives of two international organizations.**

# CIPM Mutual Recognition Arrangement


## *National Metrology Laboratories Must:*

- **Declare measurement capabilities that underpin services delivered to customers**
- **Participate in relevant International Key comparisons to validate claims**
- **Provide evidence of competence and Quality systems that underpin delivery of measurement services**

## *Outcome:*

- **Statements of the measurement capabilities of each NMI in a database publicly available on the Web**

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# 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?

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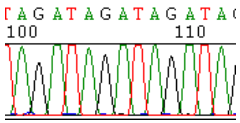
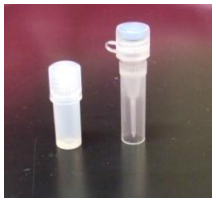
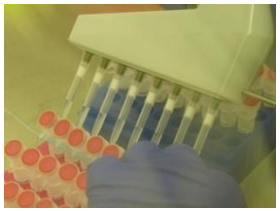
# Steps Involved in SRM Production

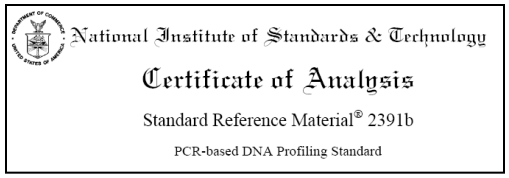
Attend conferences, read the literature, talk to potential customers

***Sequence & Copy Number***






National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2391b

PCR-based DNA Profiling Standard


**Receive input on priorities for projects and potential SRMs**

**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**

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# Pipeline for SRM Production

**Your help needed**

1. Request for SRM and internal decision to go forward
2. Understanding needs and scope of SRM (research)
3. SRM design

   what formats and amounts are best?
4. Gather materials for SRM components
5. Characterization of components

   Measure amounts with uncertainties, homogeneity, stability
6. Complete paperwork

   Report of Analysis (ROA) and Certificate of Analysis
7. Paperwork reviewed and SRM approved
8. SRM released (http://www.nist.gov/srm)
9. Customers use SRM and provide feedback

**SD** (standards development) | **WCF** (working capital funds) | SRM office

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# How is NIST SRM Cost Determined?

- SRM price is based on cost recovery of working capital funds requested in order to produce the SRM components

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# Estimated Cost of an SRM

$50K - **500 units** produced

## Calculate SRM Unit Price

| | |
|---|---|
| Laboratory | CSTL |
| SRM Category | 105 |
| Production Costs | $50,000.00 |
| Units | 500 |
| NIST Unit Production Cost | $100.00 |
| MSD SRM Operating Cost (Surcharge) | $187.00 |
| Cold Storage Fee | |
| AD VALOREM Surcharge | $50.00 |
| 49% Service Development: $49.00 | |
| 1% Obsolescence: $1.00 | |
| Dept. of Commerce surcharge | $4.32 |
| Lab Operations, if applicable | $48.00 |
| Total Price | $389.32 |
| Rounded to Nearest Dollar * | $389.00 |

$50K - **100 units** produced

## Calculate SRM Unit Price

| | |
|---|---|
| Laboratory | CSTL |
| SRM Category | 105 |
| Production Costs | $50,000.00 |
| Units | 100 |
| NIST Unit Production Cost | $500.00 |
| MSD SRM Operating Cost (Surcharge) | $187.00 |
| Cold Storage Fee | |
| AD VALOREM Surcharge | $250.00 |
| 49% Service Development: $245.00 | |
| 1% Obsolescence: $5.00 | |
| Dept. of Commerce surcharge | $4.32 |
| Lab Operations, if applicable | $48.00 |
| Total Price | $989.32 |
| Rounded to Nearest Dollar * | $989.00 |

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# How does NIST Determine the Number of Units to Produce?

We (the staff producing the SRM) are responsible for determining the appropriate number of units to produce considering items such as:

- the perceived demand for each SRM based on historical sales rates of the SRM or of similar SRMs,
- the technical difficulty of future renewals,
- the marginal cost of producing additional units,
- the cost of storage space,
- material stability

The sales price is set to collect all production costs (no profit) and repay the WCF in **no more than five years**.

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# If we build it, will they come?

Will clinical DNA labs or commercial calibrant suppliers consider the cost of the NIST material too expensive or not important to their work?

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# Lessons Learned from Forensic DNA Experience

- **Mandate use of SRMs**
  - This mandates NIST participation & SRM production
- Consistent, significant funding
  - Enables research as well as SRM production
  - National Institute of Justice partnership
- Experienced staff and appropriate equipment
- Standard techniques and targets
  - Short tandem repeat (STR) typing and core loci
- Information sharing
  - Creation of STRBase website to help community
- Inter-laboratory studies
  - Lessons learned can help with SRM needs and design

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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

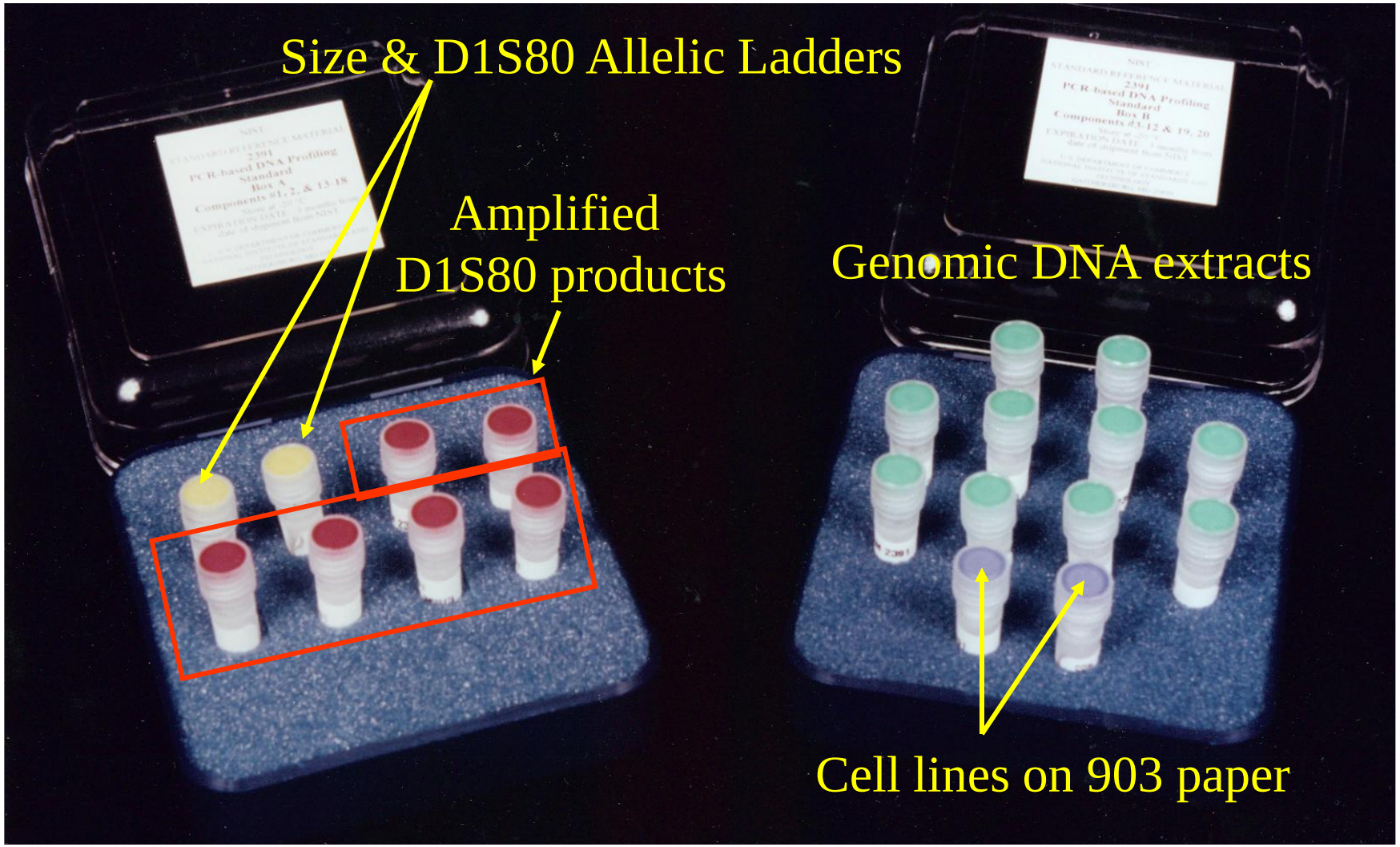
## Quality Assurance Standards for Forensic DNA Testing Laboratories

(October 1, 1998)

### STANDARD 9.5

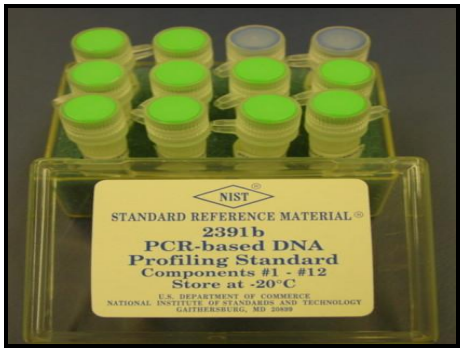
**The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.**

# 1995: SRM 2391 PCR-based DNA Profiling Standard



# 2003: NIST SRM 2391b

## Driven primarily by commercial kit loci…




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. This SRM 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 less emphasis on D1S80 [1,2] reflecting the growing interest and utility of STRs [3 to 14]. 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 and DNA to be extracted from cells spotted onto filter paper. A unit of the SRM is composed of 12 frozen components packaged in one box. See the section in this certificate entitled *Description of Components* for a complete listing of the components.

**Certified Values:** The SRM is certified for genetic loci of forensic interest that were commercially available at the time of production. Genetic types for these loci can be found in Tables 1, 2, and 3. The tables are organized as follows: Table 1 lists the genetic types for the Federal Bureau of Investigation's (FBI's) CODIS (COmbined DNA Index System) core STR loci; Table 2 lists additional STR loci of interest; and Table 3 lists the genetic types for D1S80, AmpliType® PM + HLADQA1, and Amelogenin.

**Expiration of Certification:** The certification of this SRM is valid until **31 December 2008**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is contaminated or otherwise modified.

**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 certification, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

**Storage:** Store frozen at a temperature of -20 °C. **DO NOT** use a self-defrosting freezer because periodic cycling of temperatures may cause shortened shelf life of this SRM.

The overall direction and coordination of the technical activities leading to certification were under the chairmanship of J.M. Butler of the NIST Biotechnology Division.

Analytical determination and technical measurements leading to the certification of this SRM were performed by M.C. Kline and J.W. Redman of the NIST Biotechnology Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Group by C.S. Davis.

Vincent Vilker, Acting Chief
Biotechnology Division

Gaithersburg, MD 20899
Certificate Issue Date: 06 December 2002

John Rumble, Jr., Chief
Measurement Services Division

SRM 2391b — Page 1 of 7

2. Certified Values for Additional STR Loci

| | F13B | FES/FPS | LPL | Penta D | Penta E | D2S1338 | D19S433 |
|---|---|---|---|---|---|---|---|
| | 10,10 | 12,12 | 10,11 | 10,15 | 7,12 | 17,23 | 13,16.2 |
| | 8,10 | 10,11 | | | | | 16 |
| | 9,10 | 11,12 | | | | | 4 |
| | 6,9 | 10,13 | | | | | 3 |
| | 8,9 | 11,13 | | | | | 14 |
| | 9,10 | 11,11 | 10,12 | 9,12 | 12,14 | 25,25 | 12,14 |
| | 6,8 | 11,11* | 11,12 | 3.2,11 | 12,16 | 17,22 | 13,15.2 |
| | 6,8 | 10,11 | 9,11 | 8,9 | 5,10 | 22,22 | 12.2,15 |
| | 8,10 | 10,12 | 11,12 | 12,12 | 12,13 | 19,23 | 14,15 |
| | 8,8 | 11,11 | 10,12 | 8,12 | 11,11 | 23,23 | 13,14 |
| | 8,10 | 10,12 | 11,12 | 12,12 | 12,13 | 19,23 | 14,15 |
| | 8,8 | 11,11 | 10,12 | 8,12 | 11,11 | 23,23 | 13,14 |

**22 autosomal STRs**
characterized across
12 DNA samples

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# 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

# How to use an SRM…

- The SRM material has been assigned a value through vigorous analysis methods.
- The appropriate SRM is used to value assign/validate materials and/or methods or calibrate.
- Specific SRM usage instructions are included in the Certificate of  Analysis supplied with each SRM material
- The format of the SRM determines the analysis step for it's use.  (carried through the complete analysis, or only at a later stage of analysis)

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# How to Create NIST Traceable Materials?

- Make a “lot” of material that you want to use as your control material.
- Test your control material for homogeneity and stability
- Analyze your control material in parallel with the SRM material, using the SRM material to assign the value to the control material.
- This “lot” of control material is now traceable to the NIST SRM.
- When this “lot” of control material is consumed or yields a different result it can no longer be used
- New “lots” of control materials must be analyzed in parallel with the SRM; not to one another

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# What is Documented When Creating a NIST SRM?

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# What is Documented When Creating a NIST SRM?

- **Homogeneity, Stability, Fit for Purpose, Purity**

- Well characterized material
  - 3 levels of certification: certified, reference, or informational values

- Well documented:
    - An established paper trail that would allow a future NIST scientist to reproduce the material if needed
  - **ROA: Report of Analysis**
    - An internal NIST document that is very detailed
  - **COA: Certificate of Analysis**
    - What is released with the reference material that describes the contents of the SRM along with the certified values

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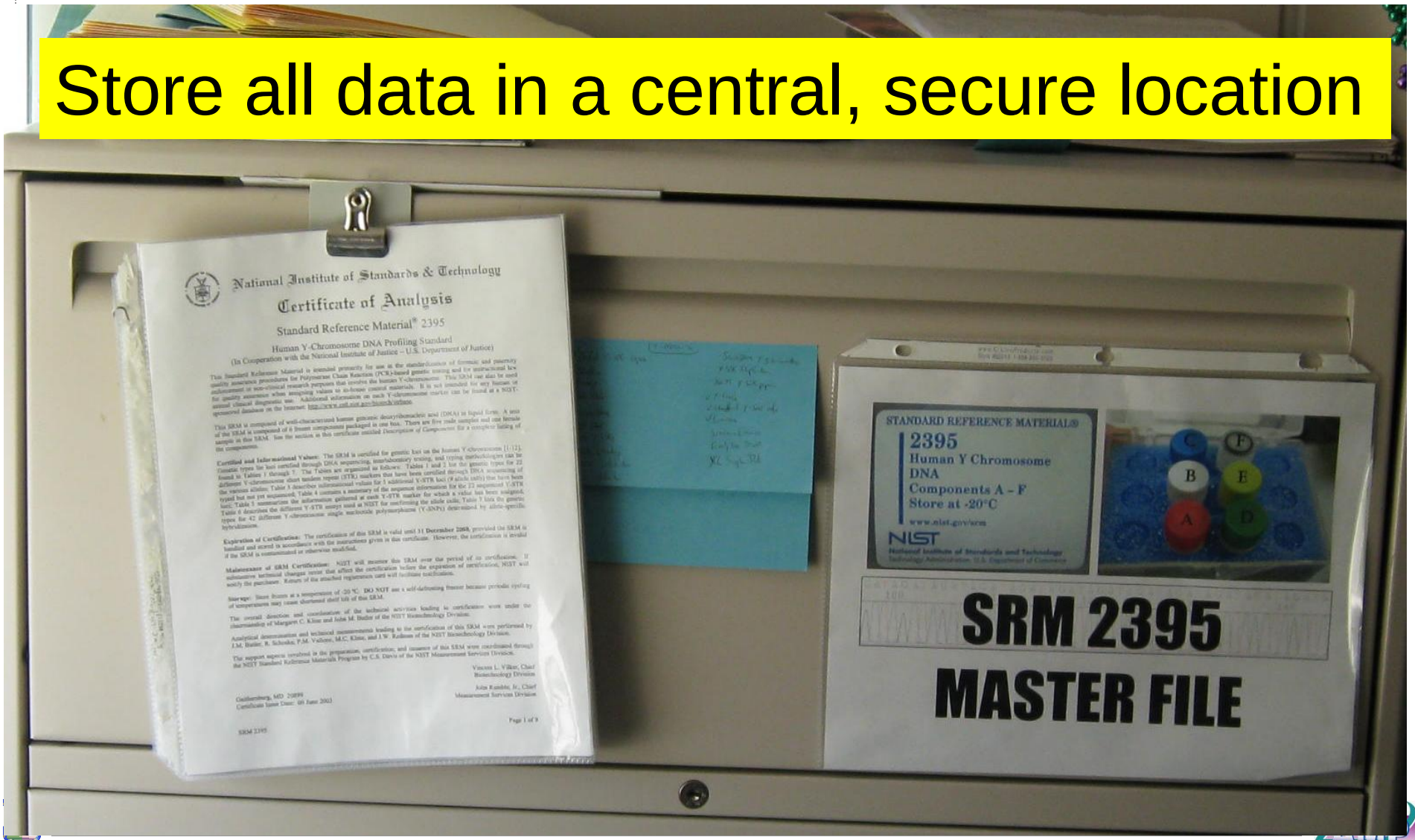
# SRM 2395 Documentation



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# SRM 2395 file drawer contents

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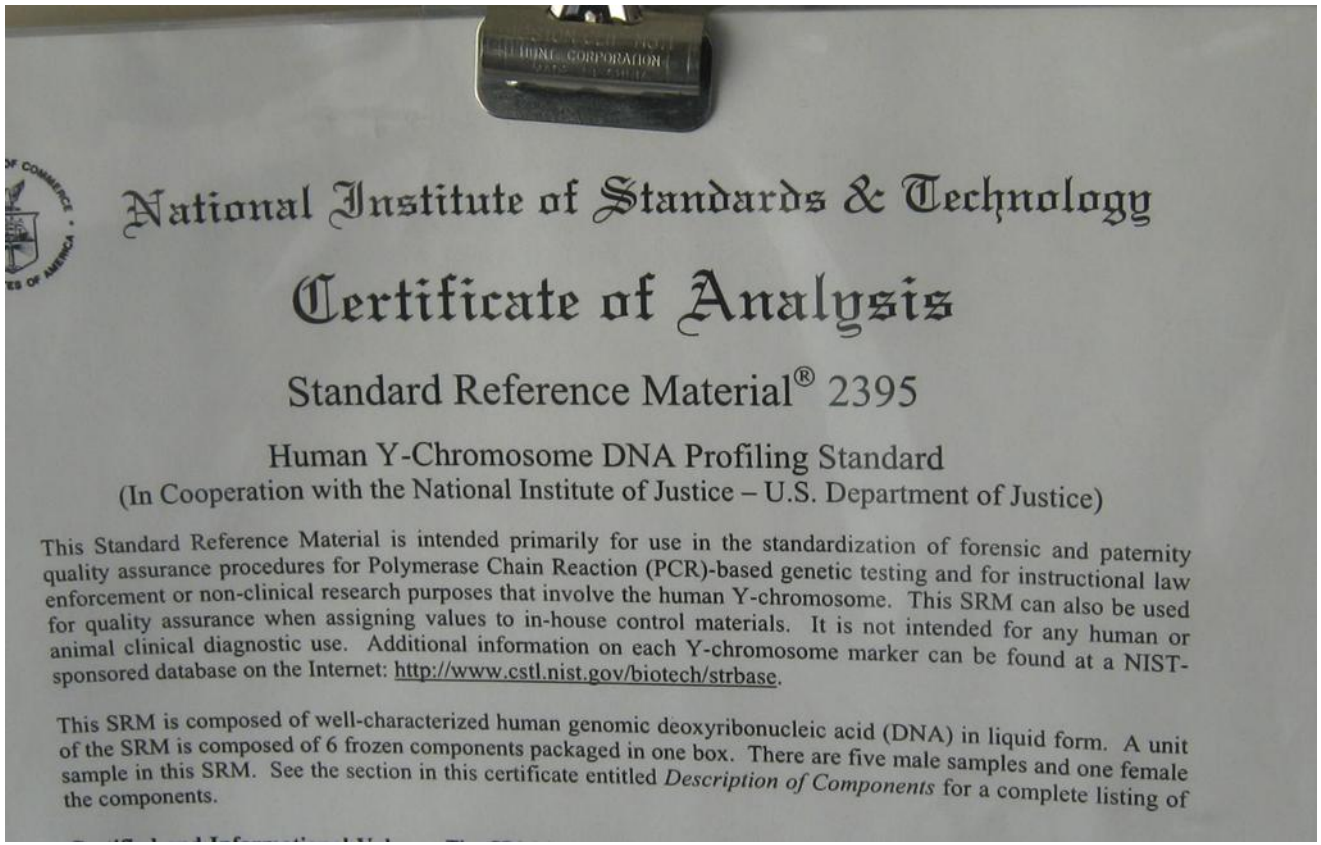
# Paper trail leads to a Certificate



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2395

Human Y-Chromosome DNA Profiling Standard

(In Cooperation with the National Institute of Justice – U.S. Department of Justice)

This Standard Reference Material 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 that involve the human Y-chromosome. This SRM 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. Additional information on each Y-chromosome marker can be found at a NIST-sponsored database on the Internet: http://www.cstl.nist.gov/biotech/strbase.

This SRM is composed of well-characterized human genomic deoxyribonucleic acid (DNA) in liquid form. A unit of the SRM is composed of 6 frozen components packaged in one box. There are five male samples and one female sample in this SRM. See the section in this certificate entitled *Description of Components* for a complete listing of the components.

**This certificate will be provided with the reference material and describes what is certified**

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# Report of Analysis (ROA)

U.S. DEPARTMENT OF COMMERCE
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
CHEMICAL SCIENCE AND TECHNOLOGY LABORATORY
BIOCHEMICAL SCIENCE DIVISION
GAITHERSBURG, MARYLAND 20899

## REPORT OF ANALYSIS

25-June-2007

This ROA is 54 pages

Submitted to: Laurie E. Locascio, Chief
Biochemical Science Division

Authors: Margaret C. Kline
Amy E. Decker
David L. Duewer
Peter M. Vallone

Title: Preparation and Homogeneity Testing of Materials for SRM 2372 Human DNA Quantitation Standard

Constituents: Human Genomic DNA

**The ROA is kept on file at the SRM Office (and the Division) and describes in great detail experimental data supporting the specified certified values**

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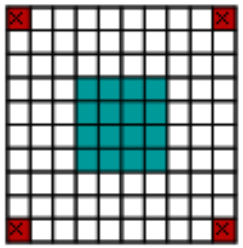
# Some of the details of SRM 2372

**Material Qualification**

Preliminary absorption spectra of the bulk materials over the wavelength range 220 nm to 345 nm were obtained using the BioCary 100 spectrophotometer located in 227/B261.

The molecular mass of the DNA in the bulk materials was assessed with a FlashGel (Cambrex Bio Science Rockland ,Inc., Rockland, ME). The gel was loaded with 1 uL of each component and the proprietary FlashGel DNA Marker. The gel was electrophoresed for 7 minute at 275 Volts. Results of the analysis were imaged with the system's proprietary gel-stain and recorded as a digital photograph on the FlashGel Dock with viewing light.

**Selection of Vials for Analysis**



Vials of each component were pulled for four different sets of analysis: UV/vis certification measurements, UV/vis homogeneity assessment, qPCR homogeneity assessment, and interlaboratory validation. The certification measurements required the total volume of 51 vials; three vials were taken from three of the corner cells (coded red in the diagram to the right) of each of the 17 boxes. The UV/vis homogeneity assessment required one vial from each box; this was taken from the "near center" cells (coded teal green in the diagram). The qPCR homogeneity assessment also required one vial from each box; this was taken from one of the edge cells (along one edge between the red cells). The interlaboratory validation study consumed 34 vials of the A, $B_{eq}$ and C materials; these were selected from the "near center" and the remaining corner.

**UV/vis Verification and Homogeneity**

Absorbance homogeneity for all components was assessed using the BioCary 100 spectrophotometer located in 227/B261. Seventeen vials, one from each of the storage boxes, were assayed for each component using 80 µL cuvettes. The temperature of the cuvettes was 22 ℃ ± 1 ℃, the same as used in the HAS II certification measurements.

The detailed UV/Vis homogeneity protocol is given in Appendix B.

## qPCR Commutability Studies.

While the interlaboratory study supplied some information about systematic differences in response to the A, $B_{old}$ and C materials among the available qPCR methods, studies were conducted at NIST to provide greater detail for specific assays [6,7,8] and to evaluate Component $B_{new}$. Four commercially available DNA quantitation standards were used for these commutability studies. These studies are documented in MCK Notebook QPCR 05 pages 38 – 43, SRM2372\2372Certification\qPCR\qPCR_Usage.

Note book details

One 96-well plate was used for each of the three qPCR methods evaluated. The design of the studies is shown schematically in Figure 4.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | A_1 | B_1 | C_1 | SP_1A | SP_1A | SP_1C | SP_1C | A_1 | B_1 | C_1 | Blank |
| B | | A_2 | B_2 | C_2 | SP_2A | SP_2A | SP_2C | SP_2C | A_2 | B_2 | C_2 | |
| C | | A_3 | B_3 | C_3 | SP_3A | SP_3A | SP_3C | SP_3C | A_3 | B_3 | C_3 | |
| D | | A_4 | B_4 | C_4 | SP_4A | SP_4A | SP_4C | SP_4C | A_4 | B_4 | C_4 | |
| E | | A_5 | B_5 | C_5 | SP_1B | SP_1B | SP_1D | SP_1D | A_5 | B_5 | C_5 | |
| F | | A_6 | B_6 | C_6 | SP_2B | SP_2B | SP_2D | SP_2D | A_6 | B_6 | C_6 | |
| G | | A_7 | B_7 | C_7 | SP_3B | SP_3B | SP_3D | SP_3D | A_7 | B_7 | C_7 | |
| H | | A_8 | B_8 | C_8 | SP_4B | SP_4B | SP_4D | SP_4D | A_8 | B_8 | C_8 | |

Figure 4. qPCR Commutability Design

Note: "X_1" (where "X" represents the component A, B or C) is the 1:10 diluted material, X_2 is a 1:5 dilution of X_1, X_3 is a 1:2 dilution of X_2, X_5 is a 1:2 dilution of X_4, X_6 is a 1:2 dilution of X_5, X_7 is a 1:2 dilution of X_6, and X_8 is a 1:2 dilution of X_7. "SP_1A" to "SP_1D" are 1:10 dilutions of four commercially available DNA calibration materials, "SP_2x" (where "x" represents one of the four commercial materials) is a 1:5 dilution of SP_1x, SP_3x is a 1:2 dilution of SP_2x, and SP_4x is a 1:2 dilution of SP_3x. Blank" denotes a buffer-only negative control. SP_A Applied Biosystems (Foster City, CA) Quantifiler Human DNA Standard Part # 4343893 Lot #0412010, SP_B Applied Biosystems Quantifiler Human DNA Standard Part # 4343893 Lot #0602018, SP_C Applied Biosystems Quantifiler Human DNA Standard Part # 4343893 Lot #0604020, SP_D Promega Corp (Madison, WI) Human Genomic DNA Male, Part# G147A Lot #13636102. Applied Biosystems Quantifiler Human DNA Standards reported [DNA] 200 ng/µL. Promega Human Genomic DNA Male reported [DNA] 262 ng/ µL.

The SRM must be useful to the community.
2372 was checked against the most frequently used qPCR methods for the forensic Human Id community. Additional commercial stds were also tested.

# Pipettes are Checked

Table 1. 2 µL Pipette Calibration

| | Electronic Pipet | | E0000798E | | 2 uL | | | |
|---|---|---|---|---|---|---|---|---|
| | Reagent Lot Code 55091 | | | | | | Grand Mean | SD CV |
| | Mal 1 | Vol | Mal 2 | Vol | Mal 3 | Vol | 1.959 | 0.039 2.0 % |
| Tips Box1 | 1 | 1.935 | 1 | 1.964 | 1 | 1.971 | | |
| | 2 | 1.95 | 2 | 1.982 | 2 | 1.955 | | |
| | 3 | 1.924 | 3 | 1.996 | 3 | 1.921 | | |
| | 4 | 1.963 | 4 | 1.949 | 4 | 1.946 | | |
| | 5 | 1.913 | 5 | 1.96 | 5 | 1.942 | | |
| | 6 | 1.925 | 6 | 1.963 | 6 | 1.959 | | |
| | 7 | 1.922 | 7 | 1.951 | 7 | 1.95 | | |
| | 8 | 1.997 | 8 | 2.008 | 8 | 1.953 | | |
| | 9 | 1.998 | 9 | 1.952 | 9 | 1.958 | | |
| | 10 | 1.99 | 10 | 1.955 | 10 | 1.932 | | |
| | 11 | 1.954 | 11 | 1.948 | 11 | 1.969 | | |
| | 12 | 1.992 | 12 | 1.943 | 12 | 1.955 | | |
| | 13 | 1.939 | 13 | 1.942 | 13 | 1.971 | | |
| | 14 | 1.917 | 14 | 1.944 | 14 | 1.973 | | |
| | 15 | 1.962 | 15 | 1.935 | 15 | 1.969 | | |
| | 16 | 1.933 | 16 | 1.993 | 16 | 1.992 | | |
| | 17 | 1.938 | 17 | 2.016 | 17 | 1.947 | | |
| | 18 | 1.982 | 18 | 1.932 | 18 | 1.933 | | |
| | 19 | 1.974 | 19 | 1.97 | 19 | 1.881 | | |
| | 20 | 1.94 | 20 | 1.931 | 20 | 1.961 | | |
| Tips Box2 | 21 | 1.948 | 21 | 1.95 | 21 | 1.972 | | |
| | 22 | 2.196 | 22 | 1.932 | 22 | 1.963 | | |
| | Avg | 1.963 | | 1.960 | | 1.953 | Setpoint | |
| | SD | 0.059 | | 0.025 | | 0.023 | -1.8 % | |

When equipment is used for CRITICAL Measurements
The calibration of the equipment must be checked!

In this case with qPCR the pipettes used to aliquot the samples where verified to be functioning properly

The 2 µL is within -1.8 % of the setpoint

Table 2. 10 µL Pipette Calibration

| Electronic Pipet | | G010134E | 10 uL |
|---|---|---|---|
| | Vol | | |
| 1 | 9.98 | | |
| 2 | 9.946 | | |
| 3 | 9.986 | | |
| 4 | 9.94 | | |
| 5 | 9.99 | | |
| 6 | 10.019 | | |
| 7 | 9.985 | | |
| 8 | 9.95 | | |
| 9 | 9.975 | | |
| Avg | 9.975 | Setpoint | |
| SD | 0.025 | -0.25 % | |
| CV | 0.253 % | | |

The 10 µL is within -0.25 % of the setpoint

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# Definition of RM vs CRM (SRM at NIST)

**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)

**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)

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# Definition of SRM Materials

Certified Reference Materials from NIST that are:

- Well-characterized
- Using state-of-the-art measurement methods and/or technologies
- For the determination of chemical composition and/or physical properties

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

# Three Possible Data Quality Descriptors

- **NIST Certified Value**
  - Highest confidence in its accuracy
  - All known sources of bias have been investigated
- NIST Reference Value
  - Best estimate of the true value
  - All known sources of bias have not been fully investigated
- NIST Information Value
  - Value will be of interest and use
  - Insufficient information is available to assess uncertainty

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

NIST Applied Genetics

# Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements **to Obtain a NIST Certified Value**

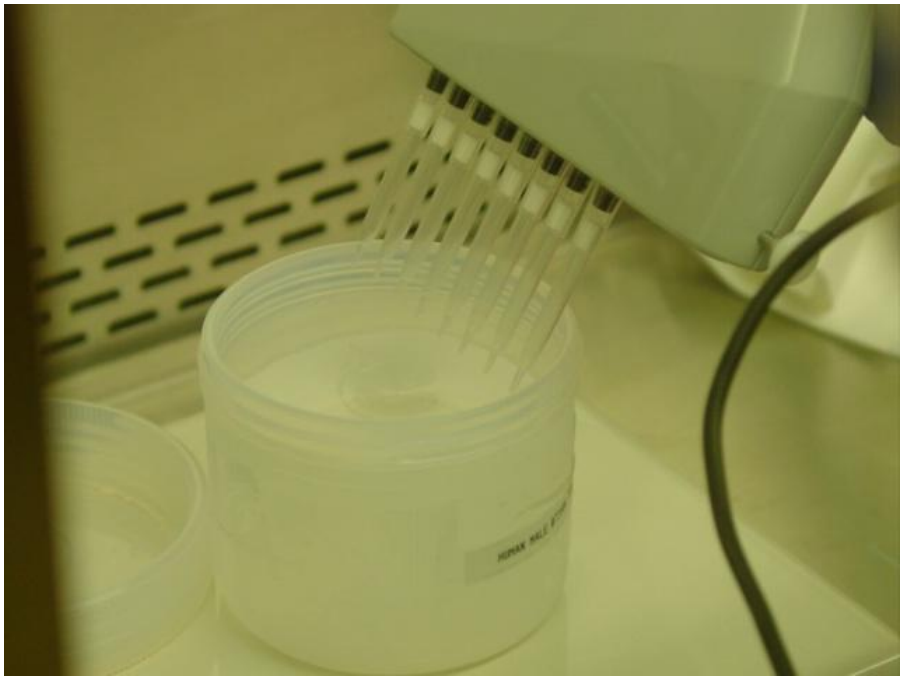
- Certification at NIST Using a Single Primary Method with Confirmation by Other Method(s)
- Certification at NIST Using Two Independent Critically-Evaluated Methods
- Certification/Value-Assignment Using One Method at NIST and Different Methods by Outside Collaborating Laboratories

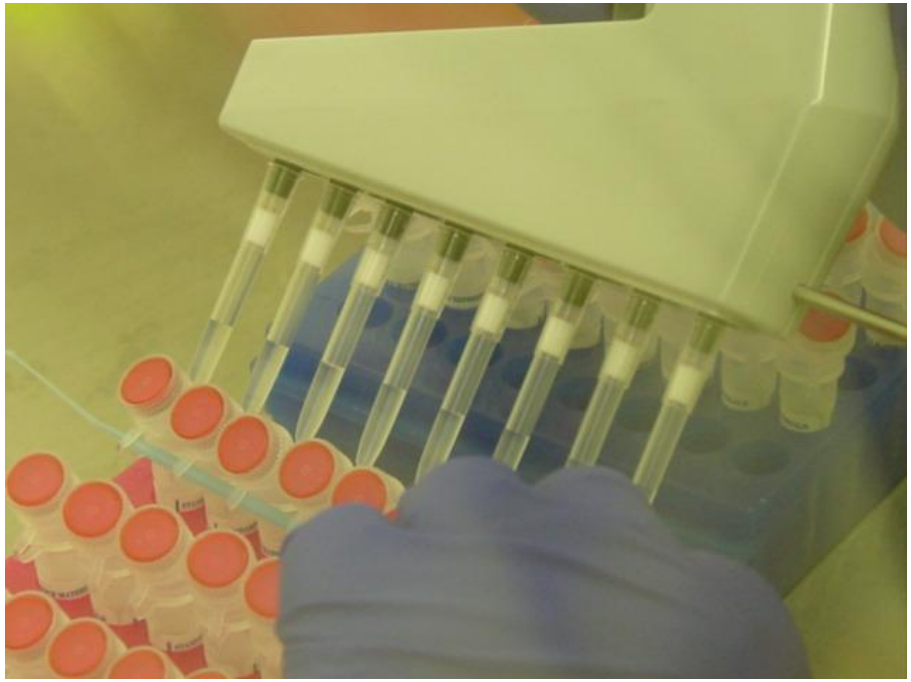
**NIST Special Publication 260-136** "Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements, Table 1 (p.2)

# Steps Involved in Production

- Tubes need to be purchased
- Boxes need to be put together
- Labels need to be ordered from the SRM Office for each component and the box ( 6 wk lead time)
- Samples need to be dispensed into separate tubes as uniformly as possible to maintain homogeneity
- Homogeneity studies need to be performed
- Data collected will be evaluated by a statistician from the NIST Statistical Engineering Division

# Bottling SRM 2372 Materials





Teflon container holding ≈ 250 mL of ***Candidate*** SRM 2372.

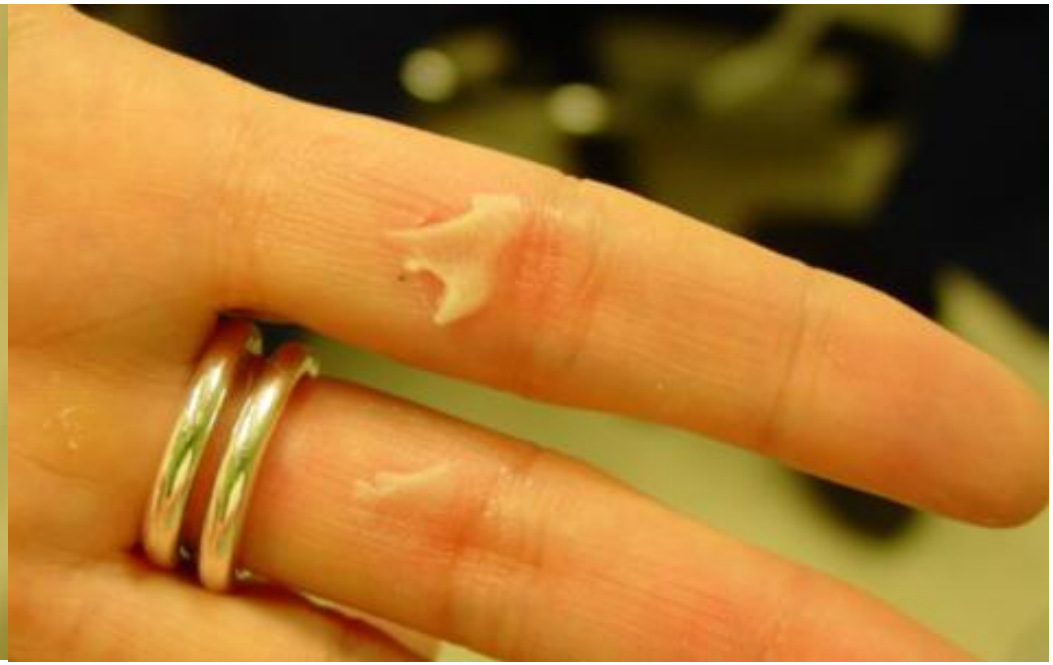
***It's not an SRM until it passes all testing.***

With a multi-channel pipettor 8 tubes can be filled at a time. That's ≈ 214 reps to fill 1700 tubes per component.

# The assembly line closing the recently filled tubes


Protecting the SRM Product from the Staff : Lab Coats, Masks and Hair nets or full face shields **P**ersonal **P**rotective **E**quipment (PPE) or **P**roduct **P**rotective **E**quipment.

# Safety Considerations:
## The Blister Brigade



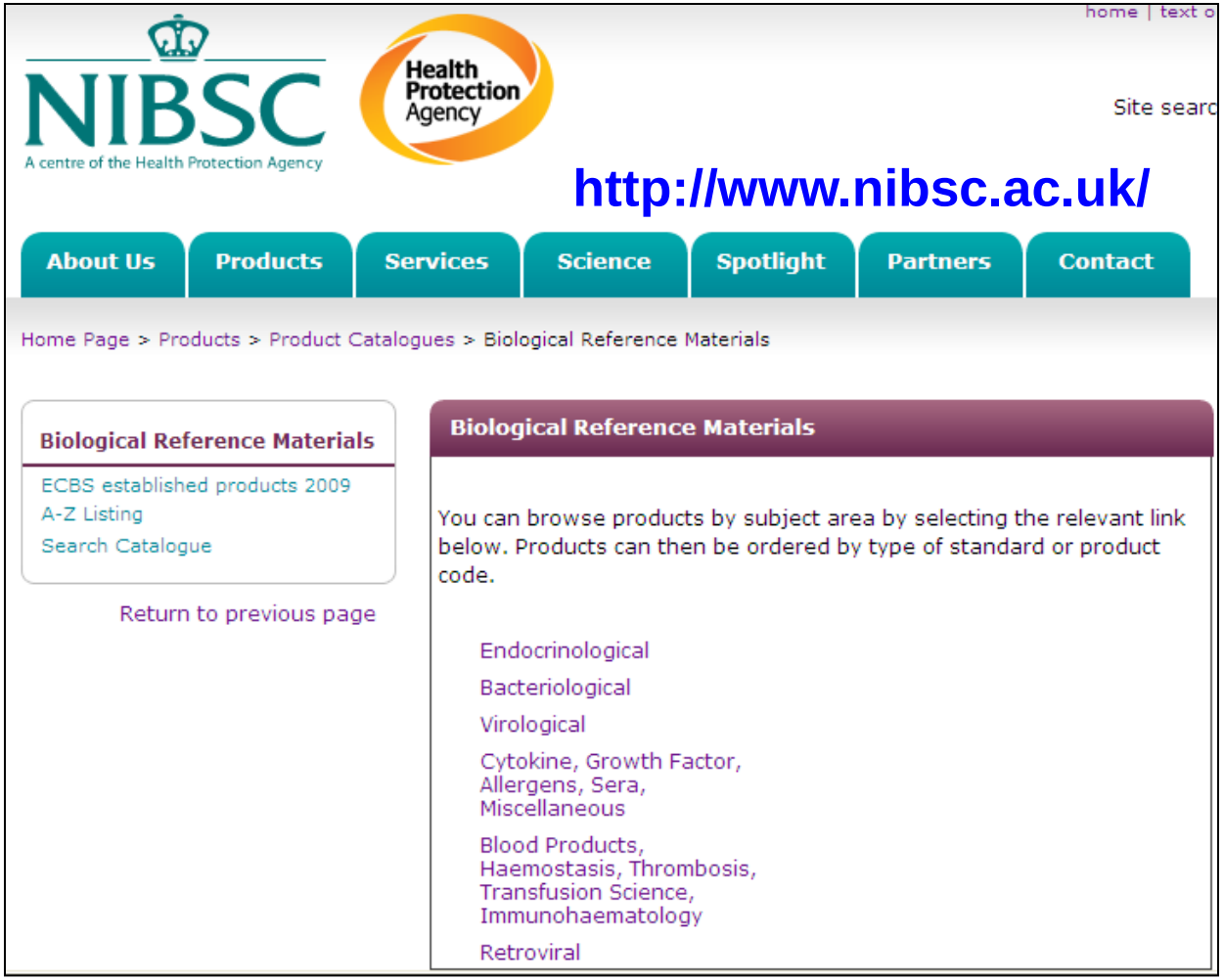
Closing the 1,700 component A tubes (SRM 2372) caused some blisters even while wearing gloves.

**Safety resolution:** Band-aids applied prior to closing SRM component tubes the next session helped reduce the number of blisters formed!

# World Health Organization (WHO)

http://www.who.int

**National Institute for Biological Standards and Control (NIBSC)**




http://www.nibsc.ac.uk/products/biological_reference_materials.aspx

# NIBSC

*National Institute for Biological Standards and Control*

- The World Health Organization established a program in biological reference materials with the intended use for vaccine production, immunological and biological assays. **NIBSC provides the International Standards (IS)**
- An important motivation for this work was the safety of the blood supply
- **They were initially designed to be qualitative standards**
- The first WHO International Standard, for HCV, was released in 1997
- IS include: HCV, HBV DNA, HIV1 RNA, HAV, HPV type 16 and 18, Parvovirus B19, CMV

# NIST and NIBSC have different models for reference materials production

| NIBSC | NIST |
| --- | --- |
| • Clinical material or whole cultured virus<br>• Consensus evaluation, assay dependent value<br>• Quantity expressed as International Units unique to that material<br>• No uncertainty | • Pure viral DNA<br>• Independent RM<br>• Units are genome copy number per volume, traceable to the International system of units or SI<br>• Reported uncertainty |

# WHO International Standards - IU/mL

- **An International Standard** is a collection of ampoules containing as far as is possible the same amount of the analyte in as stable a form as possible (e.g lyophilised, low moisture, low oxygen, stored at -20$^{o}$C)
- The material has a stable quantifiable biological activity. It is similar to the real life analyte.
- Stability studies are carried out before it is established and real time stability is monitored
- The lifetime is unspecified and depends on data or need. Replacement where necessary is an issue.
- The unit is arbitrarily defined as a fraction of the contents of an ampoule; when the collection of ampoules is exhausted the replacement is chosen to be as close to the old one as possible but there is uncertainty and strictly speaking the unit changes.
- *Information on this and the next few slides provided by Phil Minor, Head of Virology, NIBSC*

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# Process for establishing WHO International Standards

- Identify need for standard, including requirements for source material and formulation (in consultation with experts within the field)
- Proposal for development of standard presented to WHO ECBS for adoption into standardization program
- Source and evaluate candidate materials, process development (trial fills)
- Prepare candidate standard (definitive fills)
- Launch worldwide collaborative study to evaluate candidate standard, samples are sent to a number of high quality laboratories for their analysis
- Data return and statistical analysis
- Prepare report for approval by study participants
- Present report to WHO ECBS for establishment of standard (candidate standard assigned concentration in International Units (IU), usually based on the mean titer determined in the collaborative study)
- NIBSC acts as the custodian and worldwide distributor of established WHO International Standards

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# Summary of NIST & NIBSC/WHO

- Two standards developing organizations are working on primary reference materials for molecular diagnostics for infectious diseases with a current focus on viral infections of immune-compromised patients
- The organizations use two different models in the development of these reference materials
- NIST provides SRMs and NIBSC/WHO provide International Standards
- In the case of CMV, the NIST material is pure viral DNA (Towne strain) in buffer and the NIBSC material will be lyophilized intact virus (Merlin strain)
- The SRM will be certified for sequence and quantity traceable to the SI and with calculated uncertainties
- The IS will have an assigned unitage that is derived from a collaborative study consensus value
- The proposed standards are to be used as primary standards to calibrate secondary standards that will be subsequently used as calibrants

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# Clinical DNA SRMs:
## Current & Future

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Association for Molecular Pathology

# Search Results

**November 12, 2010**

SRM/RM Number: GO

Keywords: clinical DNA GO

D - Detail T - Table C - Certificate

## Archived Certificates

Full Certificate Archive >>

| | SRM | Description |
|---|---|---|
| C | SRM 918 | Potassium Chloride (Clinical Standard), January 22, 1971 |
| C | SRM 918a | Potassium Chloride (Clinical Standard), April 17, 1995 |
| C | SRM 919 | Sodium Chloride (Clinical Standard), August 6, 1973 |
| C | SRM 919a | Sodium Chloride (Clinical Standard), April 23, 2004 |
| C | SRM 928 | Lead Nitrate (Clinical Standard), Preprint, November 1, 1975 |
| C | SRM 933 | Clinical Laboratory Themometer |
| C | SRM 934 | Clinical Laboratoray Thermometer, June 18, 1990 |
| C | SRM 937 | Iron Metal (Clinical Standard), June 9, 1978 |

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# Clearly we need to improve our SRM offerings AND search engine!

Material Measurement Laboratory
Standard Reference Materials

NIST National Institute of Standards and Technology

Login | My Account | View Cart | Checkout

## Search Results

**November 12, 2010**

SRM/RM Number: GO

Keywords: molecular genetics GO

D - Detail T - Table C - Certificate

**Archived Certificates**

Full Certificate Archive >>

| | SRM | Description |
|---|---|---|
| C | SRM 705 | Polystyrene (Narrow Molecular Weight Distribution), February 1, 1963 |
| C | SRM 705 | Polystyrene (Narrow Molecular Weight Distribution), November 6, 1969 |
| C | SRM 706 | Polystyrene (Broad Molecular Weight Distribution), April 17, 1995 |

# Current NIST DNA Reference Materials

Date of release or certificate revision (r)

## Forensic Applications

- **STR PCR DNA Profiling** (SRM 2391b) – 1995, r2008
- Mitochondrial DNA Sequencing (SRM 2392-I, 2392) – 1999, 2003
- **Human Y-Chromosome DNA Profiling** (SRM 2395) – 2003, r2008
- RFLP DNA Profiling (SRM 2390) – 1992, r2001, *now obsolete*

## Clinical Applications

- Fragile X Human DNA Triplet Repeat (SRM 2399) – 2004, r 2007
- Huntington's Disease CAG Repeats (SRM 2393)
- Cytomegalovirus (CMV) Copy Number & Sequence (SRM 2366)

## Platform Testing

- **Human DNA Quantitation** (SRM 2372) - 2007
- Heteroplasmic mtDNA Mutation Detection (SRM 2394) - 2004
- DNA Sequence Library for External RNA Controls (SRM 2374)

***Several in development…***

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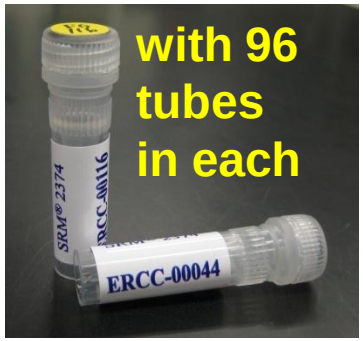
# SRM 2374 – *DNA Sequence Library for External RNA Controls*

**Work by another group at NIST**

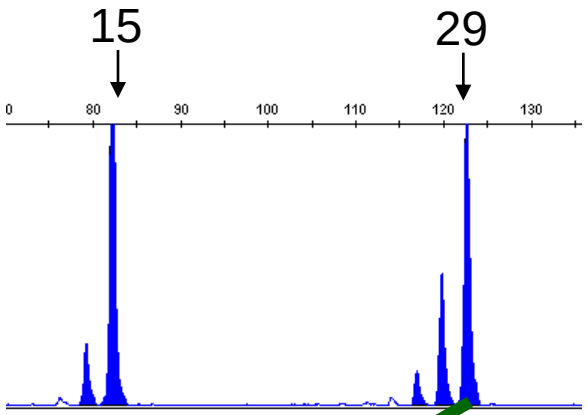

**External RNA Control Consortium (ERCC)**

- **NIST SRM to contain 96 unique control sequences inserted in common plasmid DNA**
  - engineered to be readily *in vitro* transcribed to make RNA controls
  - RNA controls intended to mimic mammalian mRNA
- Developed *sequence library* from submission by ERCC members, as well as synthesis
  - evaluated performance of RNA controls on variety of platforms
  - selected 96 well-performing sequences in collaborative study
    - from library of 176
- Array manufacturers modified products to include SRM sequences
- Prepared 400 units of SRM
    - 96 tubes in each

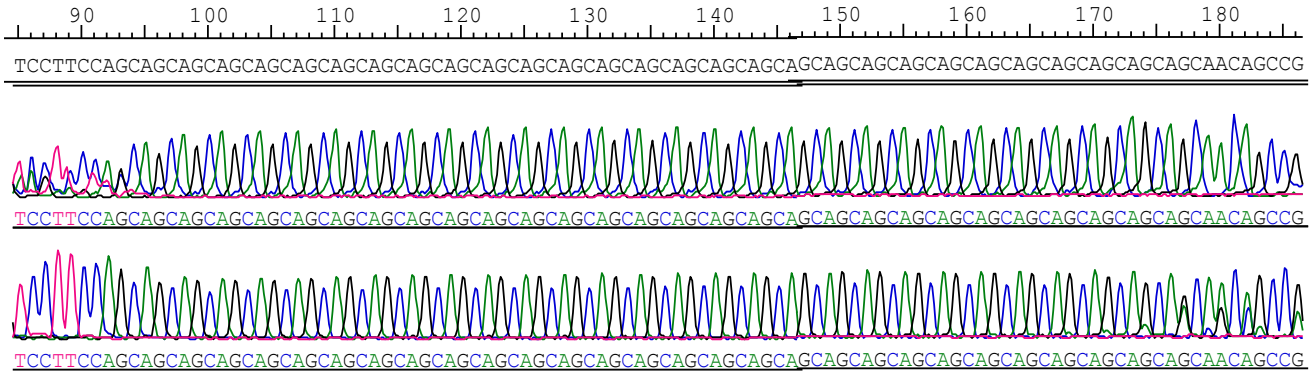
**Contact: Marc Salit to learn more**

# SRM 2393 HD Alleles

- Alleles included in the SRM are:
- 15, 17, 29, 35, 36, 39, 40, 45, 50, & 75




Forward and Reverse Sequencing 29 Allele



# NIST SRM projects for molecular diagnostics of infectious disease

**Cytomegalovirus (CMV)** – under development

**Epstein-Barr virus** and **BK virus** – in the planning stages

# Who are our customers for infectious disease standards?

- Reagent and calibrant manufacturers
- Clinical laboratories that prepare their own calibrants
- NIST standards should be primary standards traceable to the SI
- NIST standards should be used to establish traceability for secondary standards/calibrants
- A central reference point to link the standards in use in clinical laboratories

**Improved measurements through better calibration should lead to reduced variability among clinical labs**

# CMV Standard Reference Material

Type of material - **pure viral DNA in buffer**

- CMV DNA, Towne strain, cloned into a bacterial artificial chromosome (Towne Δ147)
  - Developed by Dr. Hua Zhu, New Jersey Medical School
- The BAC/CMV DNA is essentially a very large plasmid 240,000 bp, which is propagated and purified by a process that is similar to plasmids
- Advantages - Consistent genome size and ease of propagation

# Δ 147 Towne BAC

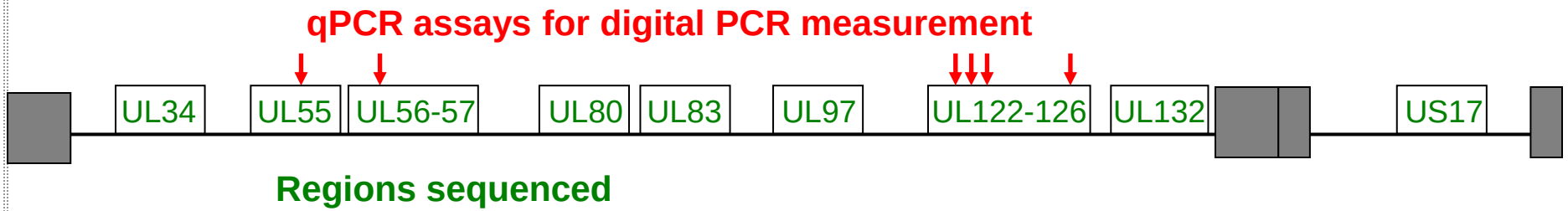
- BAC DNA is considered stable and can accommodate up to 300,000 base pairs
- DNA has been removed (20,000 base pairs) from the CMV viral genome to accommodate the BAC related DNA
- DNA that has been removed is <u>not</u> in the regions used for amplification

# Properties Being Certified

- **DNA sequence** of regions of the CMV genome that are used as targets of PCR amplification
  - The following regions have been Sanger sequenced – UL34, UL54, UL55-56, UL80, UL83, UL97, UL122-126, UL132, & US17
  - Sequence to date matches GenBank Towne strain sequence

- **Copy number by direct measurement** using digital PCR

# CMV DNA Sequence Characterization

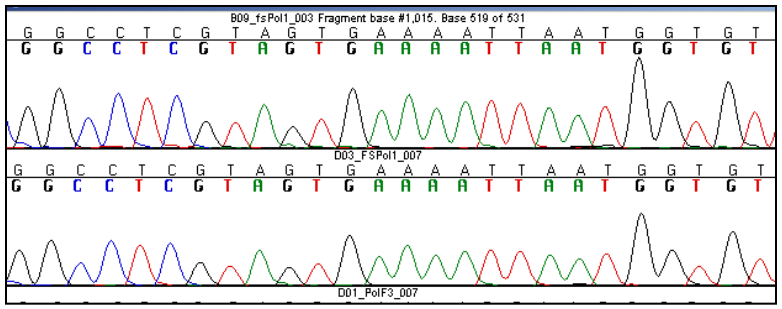



http://www.nist.gov/mml/biochemical/genetics/cmv_structure.cfm

Schematic map of the CMV genome. The CMV genome is organized as two regions of unique sequences, unique long (UL) and unique short (US), flanked by two sets of inverted repeats (light shaded boxes). *Kotenko et al. (2000) PNAS 97(4): 1695-1700*

**Sequence alignments from available GenBank CMV strains being compared against published PCR primers and probes – this information is being included on the NIST Clinical DNA Information Resource website**

**http://www.nist.gov/mml/biochemical/genetics/clinical_dna.cfm**




**Sanger sequence result obtained at NIST from UL54** (section shown is the reverse primer region for the CP1 assay used in digital PCR and matches the Towne strain and published primer)

# Certification of the CMV DNA - genome copy counting

**Digital PCR** – Quantify the amount of DNA (copies/volume) by counting amplification from single molecules

- Nano scale reactions (6 nL)
- DNA concentration where some reaction chambers are negative
- Based on Poisson statistics, number of copies is determined
- Thousands of replications/assay repeated with multiple assays targeting regions on the CMV genome
- Traceable to the SI via the Mole
- New tool, with active research at other National Metrology Institutes to validate this approach

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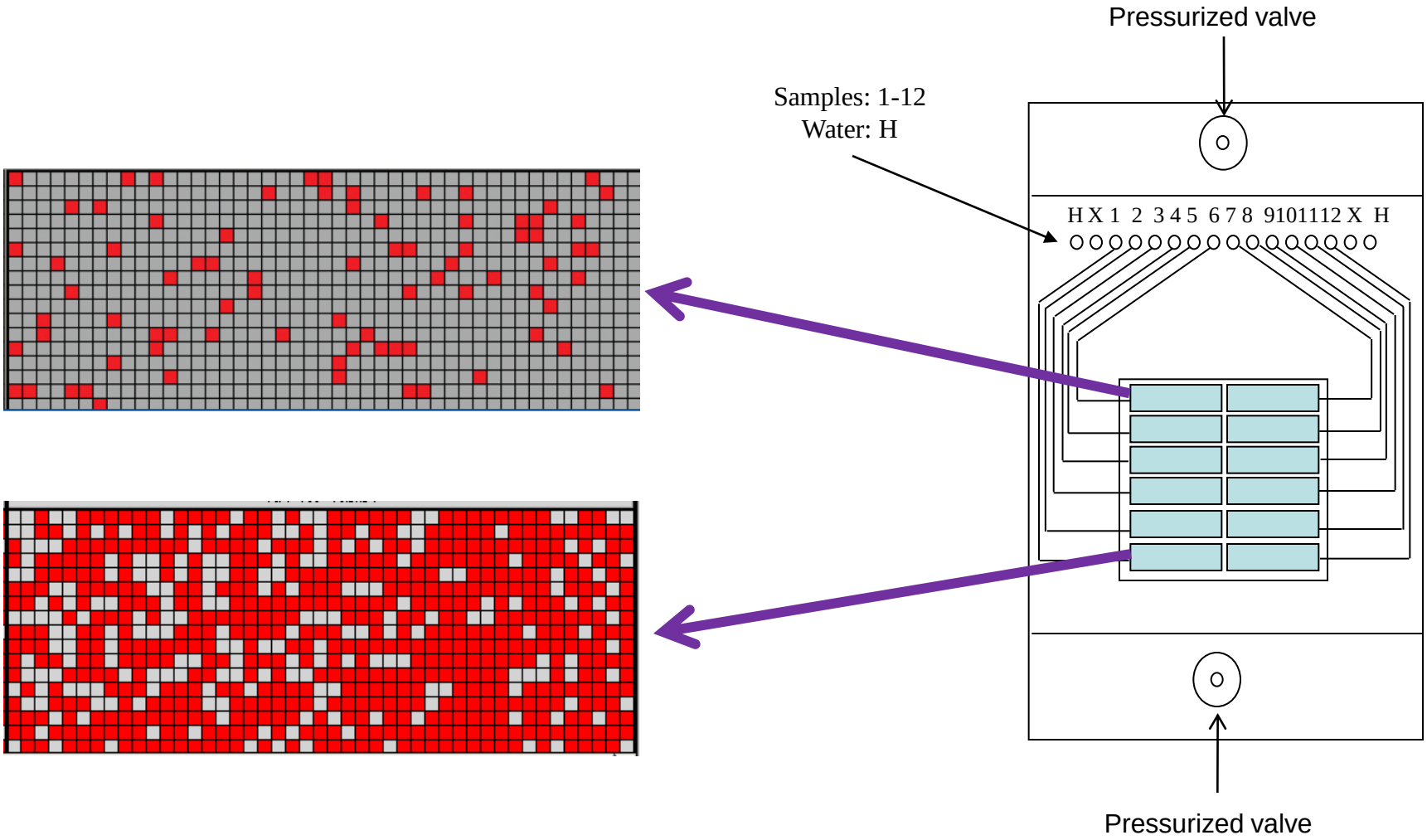
# NIST Digital PCR Instrument

# Digital PCR



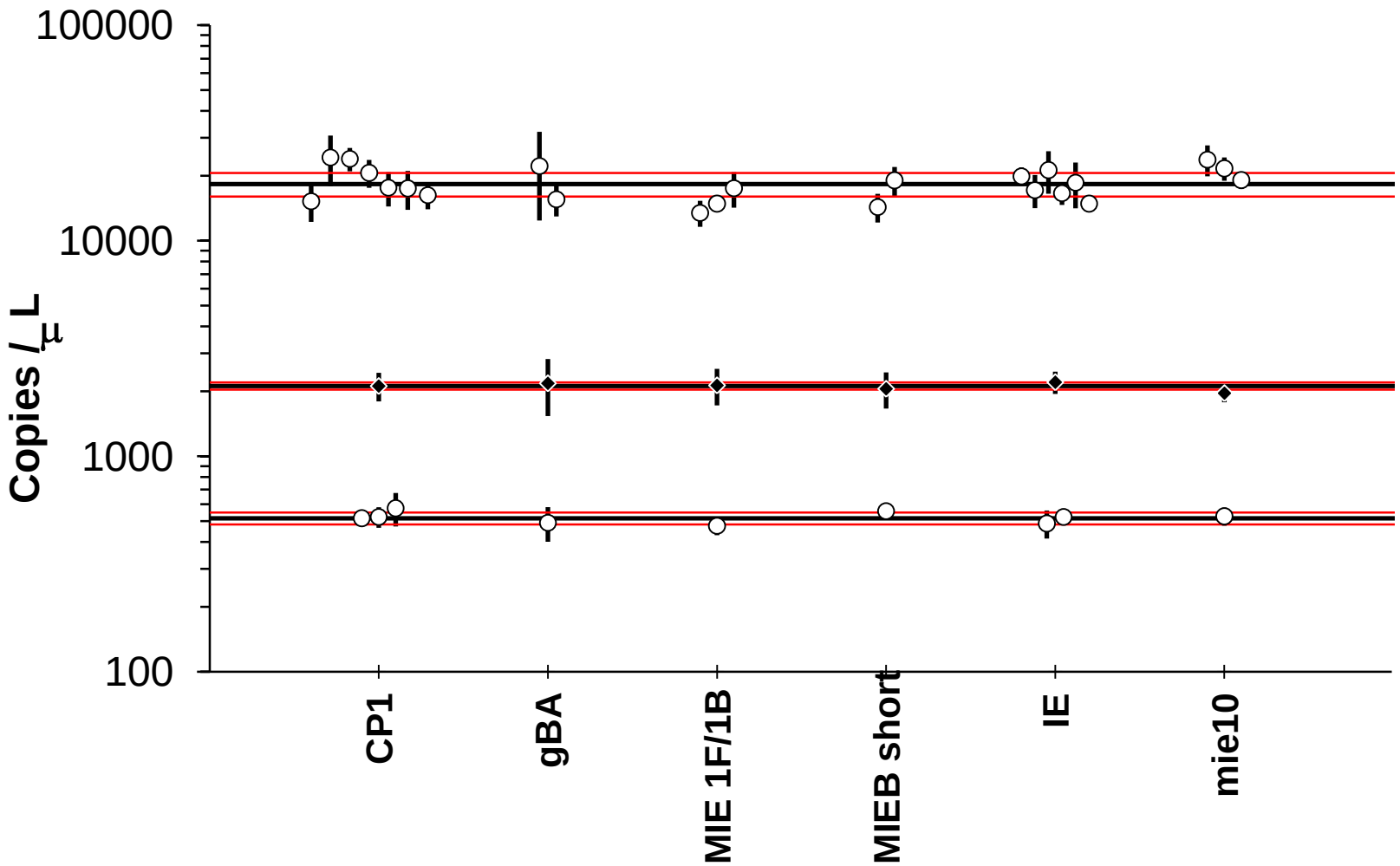

765 individual chambers / panel
12 panels/chip

# Preparation of the SRM

- Three levels of DNA concentration in buffer
- Packaging in Teflon tubes
- Certification for copy number/volume will be done on each of the concentrations
- Monitoring for homogeneity and stability for the life of the SRM (5 years from time of issue)
  - We currently have a stock that we have been monitoring for 18 months at three temperatures

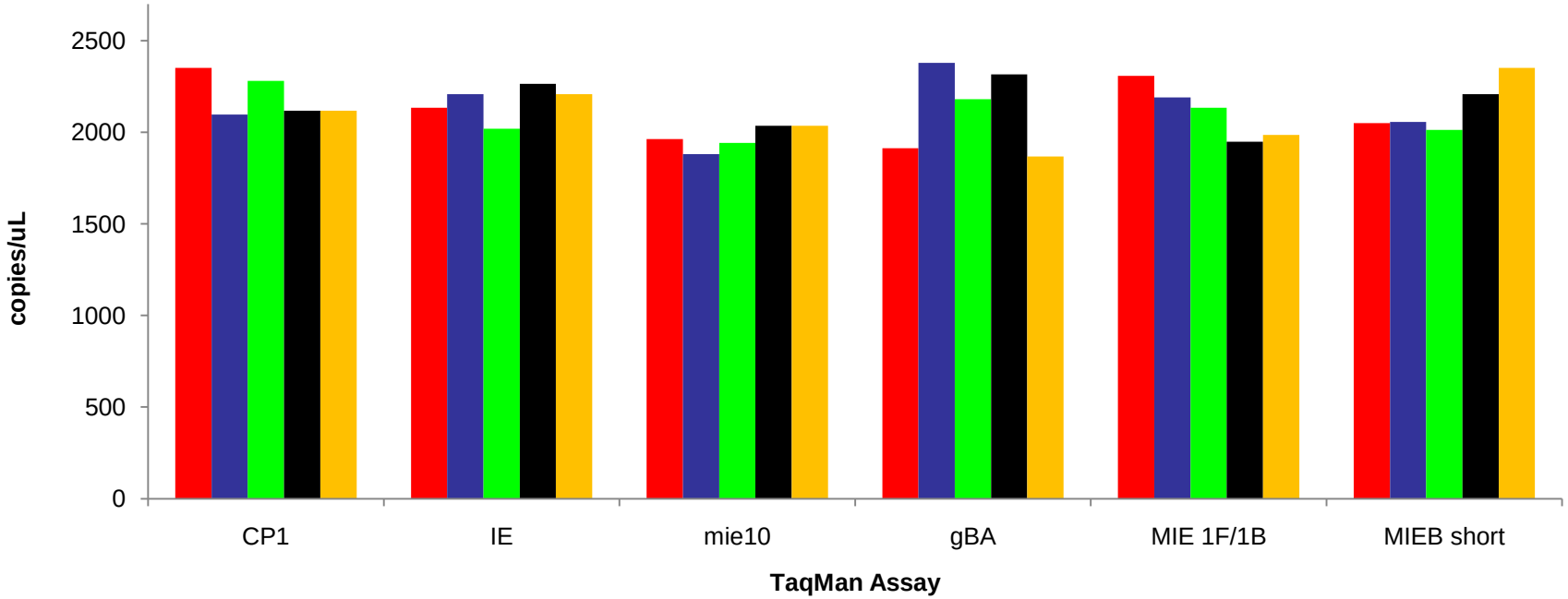
# Variability in Digital PCR Measurements Across Different CMV Targets

## (all three candidate components for SRM 2366)



# Variability in Digital PCR Measurements Across Different CMV Targets

## (single candidate component of SRM 2366)




ANOVA of the data showed no significant differences between groups

| Average across all groups | | |
|---|---|---|
| Average | 2118 | copies/μL |
| SD | 150 | copies/μL |
| Uk=2 | 300 | copies/μL |

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# QCMD CMV EQA
# 2010 Inter-laboratory Study

- NIST collaborated with Quality Control for Molecular Diagnostics (QCMD) for an inter-laboratory study conducted as part of the QCMD CMV 2010 EQA program
- NIST provided aliquots of CMV DNA, component B of the candidate CMV SRM, to QCMD
- A vial of DNA was sent out to each participating laboratory along with the 10 QCMD samples (lyophilized virus in plasma or VTM)
- While the QCMD samples required extraction, the NIST DNA was to be added directly to the assay. Participants were asked to run the assay in triplicate and report results in copies / mL.
- **181 data sets were submitted**

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# QCMD Data Analyzed by Assay Type

| QCMD CMV EQA - Participants and assays | # Data sets | $Log_{10}$ (copies/mL) Median | MADe |
|---|---|---|---|
| Total Datasets | 181 | 5.900 | 0.486 |
| Conventional Commercial | 5 | 5.854 | 0.872 |
| Real-Time Laboratory developed - Total | 78 | 6.002 | 0.650 |
| Real-Time Commercial - Total | 96 | 5.826 | 0.451 |
| Argene CMV HHV6,7,8 R-gene | 6 | 5.864 | 0.150 |
| Argene CMV R-gene | 15 | 6.205 | 0.332 |
| Nanogen Q-CMV Real time Complete Kit | 21 | 5.733 | 0.794 |
| QIAGEN artus CMV PCR Kit (RG, LC,TM) | 28 | 5.821 | 0.326 |
| Roche LightCycler CMV Quant Kit | 12 | 5.776 | 0.298 |

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# Information Resources for the Clinical Genetics/Healthcare Community

**http://www.nist.gov/mml/biochemical/genetics/clinical_dna.cfm**

**http://www.nist.gov/mml/biochemical/genetics/index.cfm**

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# Clinical DNA Informational Resource (CDIR)

**http://www.nist.gov/mml/biochemical/genetics/clinical_dna.cfm**

- In 2009, we started an internet based information resource – the goal is to provide information on DNA sequence alignments and primer and probes for clinical assays as a start

- We plan to load this resource with accessible and useful information for the clinical diagnostics community

- We welcome input from you on additions and changes to this information resource

# Clinical DNA Informational Resource Website

**http://www.nist.gov/mml/biochemical/genetics/clinical_dna.cfm**

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Chemical Science and Technology Laboratory

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NIST Home > CSTL > Biochemical Science Division > Applied Genetics > Clinical DNA Online

**Welcome to the NIST Biochemical Science Division Clinical DNA Informational Resource**

SHARE

**General Information**

- Publications/Presentations

## Standard Reference Materials (SRMs)

**Clinical DNA SRMs**

- Fragile X Human DNA Triplet Repeat Standard (SRM 2399)

**SRMs in Progress**

- Cytomegalovirus for DNA Measurements (SRM 2366)
- Huntington's Disease (SRM 2393)

**Detection System SRMs**

- Mitochondrial DNA Sequencing (SRM 2392)
- Mitochondrial DNA Sequencing (Human HL-60 DNA) (SRM 2392-I)
- Human DNA Quantitation Standard (SRM 2372)

**Other DNA Based SRMs**

- PCR-Based DNA Profiling (SRM 2391b)
- Human Y-Chromosome DNA Profiling (SRM 2395)

**Additional Materials under Consideration**

- BK virus
- Epstein-Barr virus

Please send comments or suggestions for this website to Ross Haynes

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Chemical Science and Technology Laboratory

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NIST Home > CSTL > Biochemical Science Division > Applied Genetics > CMV Homepage

**Cytomegalovirus**

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Return to Clinical DNA Informational Resource

**General Information**

Project description

How to use SRM 2366

Structure of CMV genome

**PCR Detection Specific Information**

Sequence alignments

Primer and probe sequences with citations (pdf)

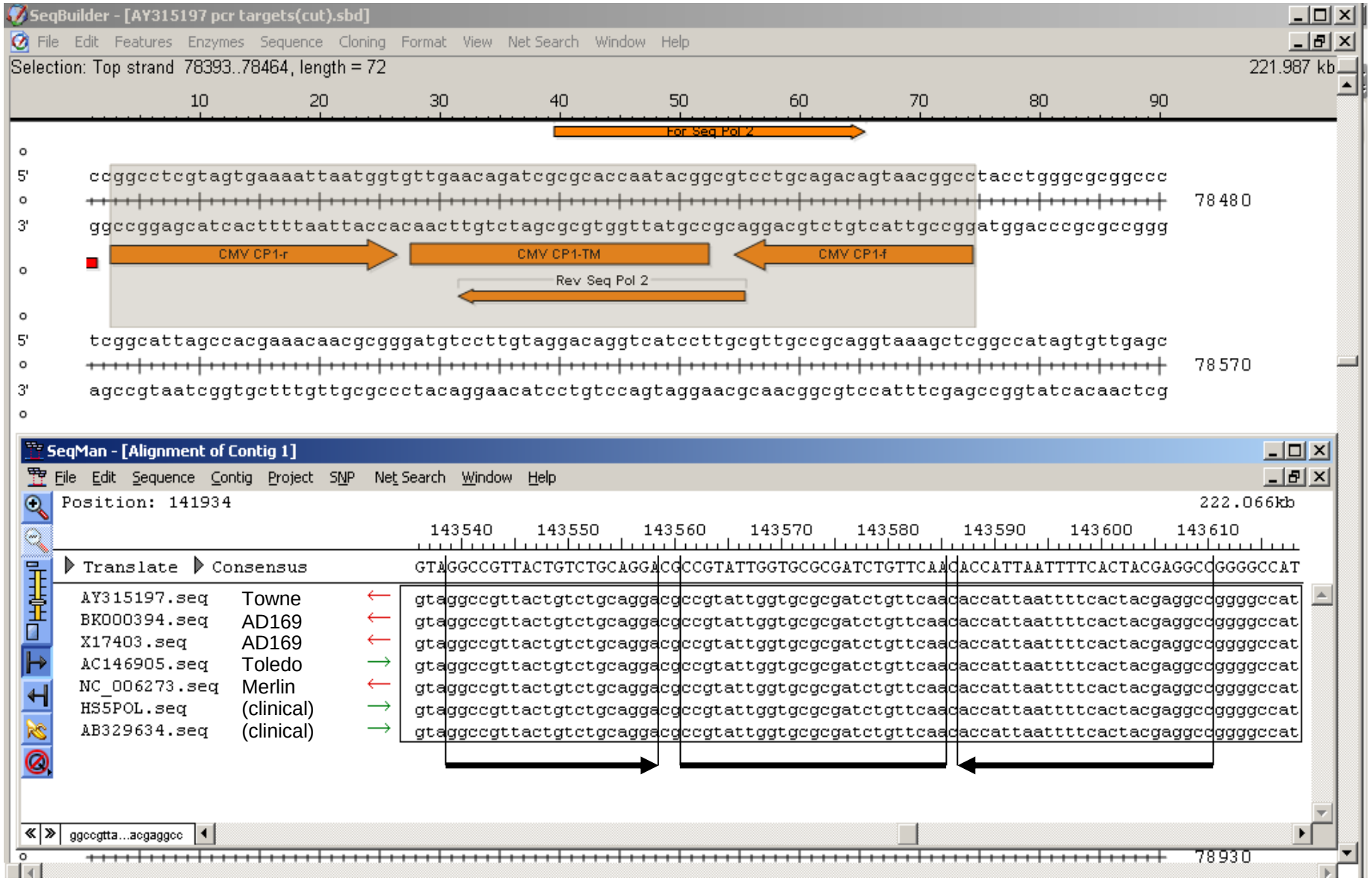
Location of primers and probes (full.pdf or cropped.pdf)

For suggestions of additional information to include on this site click here.



Date created: November 3, 2009 | Last updated: April 7, 2010 Contact: **CSTL Webmaster**

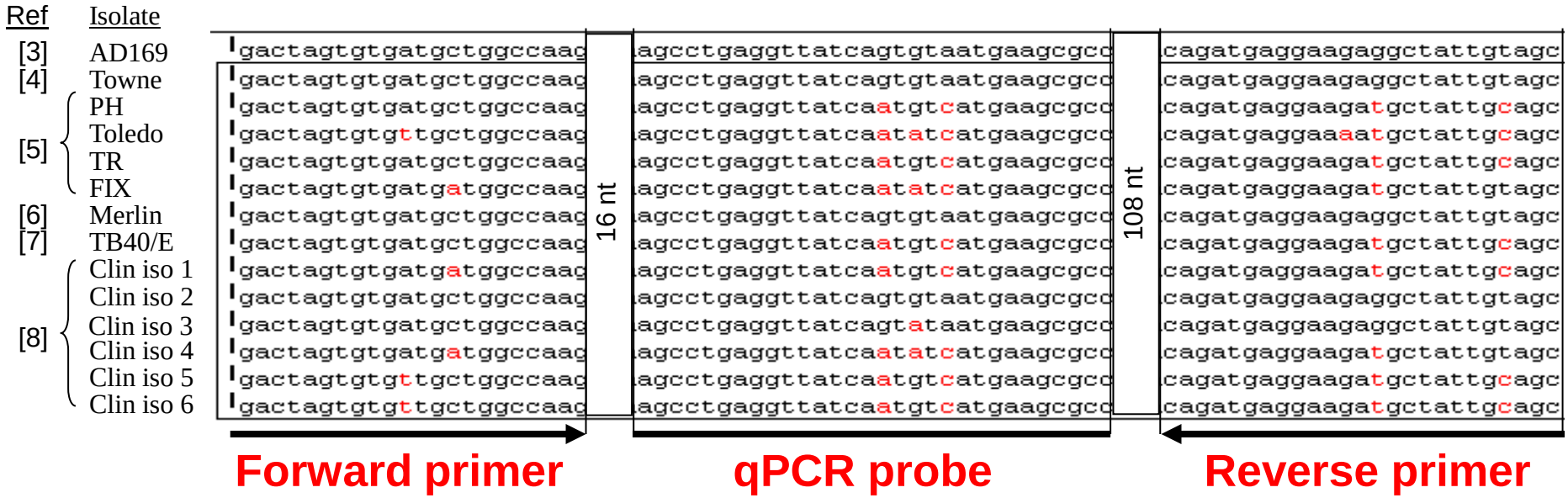
# Sequence Alignments for CMV Strains



# Sequences from 14 CMV Strains

## Sequence alignments

The qPCR assay below (Tanaka 2000 Journal of Medical Virology 60:455–462) has a large number of mismatches that cause a false negative rate of 24%. A little more time in the library, probably would have lead this researcher to redesign the primer and probe binding sites.



# Summary

- We are asking for your input on what we are currently doing and the relevance to the clinical community
- This is the first time that NIST has worked on standards for clinical diagnostics of infectious disease – we want to get it right
- Please come and talk to us during the meeting: we have three posters G09, ID19, and OTH05.

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# NIST Presentations at AMP 2010

Margaret Kline

Marcia Holden

Ross Haynes

- **G09 (poster): Characterizing the Electrophoretic Mobility of Huntington Disease Alleles 13-77: Are the Deviations from the Theoretical Values Intrinsic or Internal Sizing Standard Artifacts?**
- **ID19 (poster): NIST Candidate Standard Reference Material (SRM): Cytomegalovirus DNA**
- **OTH05 (poster): Cautionary Considerations when Exploring Cell Lines as Potential Reference Materials**

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# Contact Information

## Group Members Working on Clinical DNA Projects

- Marcia Holden (x4162)
- Margaret Kline (x3134)
- Ross Haynes (x4469)
- John Butler (x4049) – Group Leader

Email: first name . last name @ nist.gov
Phone: 301-975-x

**http://www.nist.gov/mml/biochemical/genetics/clinical_dna.cfm**

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# Pipeline for SRM Production

**Your help needed**

1. Request for SRM and internal decision to go forward
2. Understanding needs and scope of SRM (research)
3. SRM design

   what formats and amounts are best?
4. Gather materials for SRM components
5. Characterization of components

   Measure amounts with uncertainties, homogeneity, stability
6. Complete paperwork

   Report of Analysis (ROA) and Certificate of Analysis
7. Paperwork reviewed and SRM approved
8. SRM released (http://www.nist.gov/srm)
9. Customers use SRM and provide feedback

**SD** (standards development) | **WCF** (working capital funds) | SRM office

AMP.org Association for Molecular Pathology

# Reference Materials Requested
## in Karen Mann's Congressional Testimony (Feb 24, 2010)

- Immediate
  - CMV
  - BCR/ABL
  - KRAS
  - EGFR
- Short term
  - BK virus
  - Epstein Barr virus
- Medium term
  - Certified Gene Sequence Databases
  - Infectious agents: adenovirus, enterovirus, Hepatitis B virus, Herpes simplex, JC virus, …

Source: http://www.amp.org

# CMV

- Materials being characterized for sequence and amount
  - Material: Towne strain
  - Components: 3 vials of liquid DNA containing the same sequence but different concentrations

# BCR/ABL

- **Specific Needs**:
  - What is the measurement issue?
- **SRM Design**:
  - What format (e.g., genomic DNA, amount)?
  - How many variants (e.g., different types)?
  - How many components desired?
- **Source of Materials**:
  - Who can supply appropriate starting material of sufficient quantity for SRM production?

# KRAS

- **Specific Needs**:
  - What is the measurement issue?
- **SRM Design**:
  - What format (e.g., genomic DNA, amount)?
  - How many variants (e.g., different types)?
  - How many components desired?
- **Source of Materials**:
  - Who can supply appropriate starting material of sufficient quantity for SRM production?

# EGFR

- **Specific Needs**:
  - What is the measurement issue?
- **SRM Design**:
  - What format (e.g., genomic DNA, amount)?
  - How many variants (e.g., different types)?
  - How many components desired?
- **Source of Materials**:
  - Who can supply appropriate starting material of sufficient quantity for SRM production?