The Heat Is On:

Development of the Next Generation of Forensic DNA Standard Reference Material: SRM 2372a

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Development of NIST SRM 2372a

- Review of SRM 2372 and why it benefits forensic laboratories
- Examination of the next generation of certification measurements
  - From UV absorbance to Digital PCR
- Overview of the development process of SRM 2372a
  - Where are we and what do we still have to do?

What is SRM 2372 Human DNA Quantitation Standard?

SRM 2372 was originally released in 2007

Component A: Single-source male
Component B: Multi-source female
Component C: Multi-source male/female mixture

Certified for spectroscopic traceability in units of decadic attenuance, D_{10}.

National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2372
Human DNA Quantitation Standard

Parameter	A	B	C
2012 DNA Mass Concentration	57 ng/µL	61 ng/µL	59 ng/µL

Forensic Need for SRM 2372a

Manufacturer assigned DNA concentrations for commercial DNA found within qPCR kits

Commercial DNA used to generate a standard curve

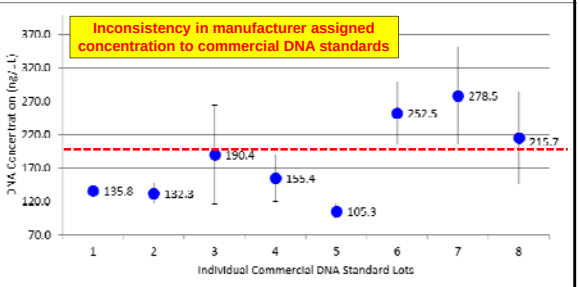
Concentration is assigned to unknown samples based on the standard curve

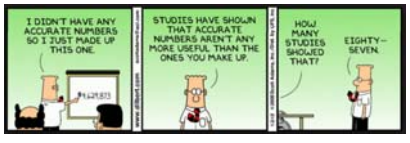
Is the manufacturer assigned concentration accurate?

8 Commercial DNA Standards

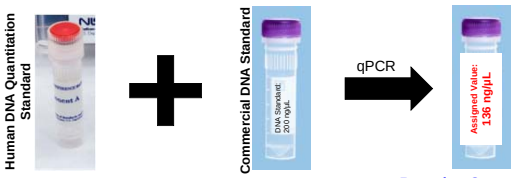
DNA Standard derived from a human cell line found in commercial qPCR kits

Nominal value assigned from manufacturer: 200 ng/µL





What can laboratories do to ensure more accurate quantitation results?



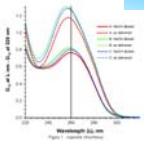
Standard Curve | **"Unknown"** | **Reassign Commercial DNA Standard**

Why is SRM 2372a being developed?

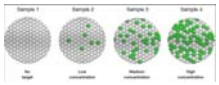
- As a successor to SRM 2372
  - Inventory may be depleted by mid 2017
  - Develop SRM 2372a now to ensure availability when needed
- Goal: to certify copies of DNA per microliter using Digital PCR
  - Groundwork has been laid for using dPCR as a certification method
  - Include information values for UV absorbance and genomic:mitochondrial ratio

What is changing in SRM 2372a?

UV Absorbance



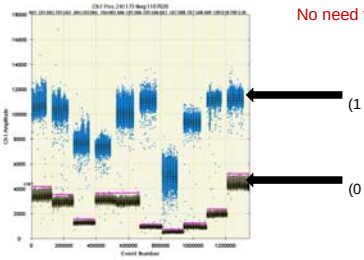
Digital PCR (dPCR)



What is Digital PCR?

Partitioning of samples into individual chambers or droplets

No need for a standard curve



Positives
(1 copy of the target)

Negatives
(0 copies of the target)

dPCR is counting *accessible* amplifiable targets

dPCR platforms at NIST

Droplet Digital - ddPCR

BIO-RAD QX100/200

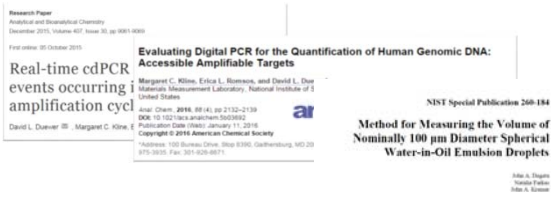
Chamber Digital - cdPCR

Fluidigm BioMark

"Absolute" Quantitation at NIST

NIST has developed and optimized >10 dPCR assays for absolute quantitation

Investigated volume as a source of bias



Real-time cdPCR events occurring i amplification cycl

David L. Duewer ✉ , Margaret C. Kline, I

Evaluating Digital PCR for the Quantification of Human Genomic DNA: Accessible Amplifiable Targets

Margaret C. Kline, Erica L. Romsos, and David L. Duew
Materials Measurement Laboratory, National Institute of S
United States

Anal. Chem., 2016, 88 (4), pp 2132–2139
DOI: 10.1021/acs.analchem.5b03692
Publication Date (Web): January 11, 2016
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*Address: 100 Bureau Drive, Stop 8390, Gaithersburg, MD 20 975-3935. Fax: 301-926-8971.

NIST Special Publication 260-184

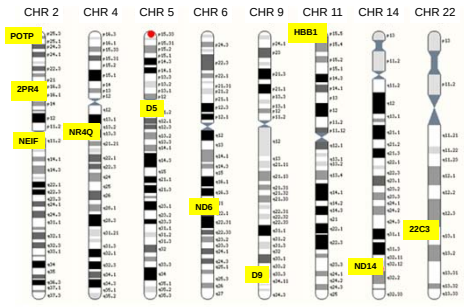
Method for Measuring the Volume of Nominally 100 μm Diameter Spherical Water-in-Oil Emulsion Droplets

John A. Dagata
Yoshio Farkas
John A. Kramar

Optimized Assays for SRM 2372a

10 assays across 8 different chromosomes

All assays are single copy, and Human, or Primate specific



Summary of Bio-Rad droplet measurements

Source	Date	Volume (nL)
Nominal		1.000
Bio-Rad [1]	2009	0.910
Pinherio et al [2]	2012	0.868
Corbisier et al [3]	2015	0.834
NIST with dUPT	2015	0.804
NIST without dUTP	2015	0.767

A volume difference of about 0.37 nL for dUTP/No dUTP observed with NIST measurement method

[1] Bio-Rad Laboratories Inc, Pleasanton, CA USA. (http://www.bio-rad.com/en-us/applications-technologies/introduction-digital-pcr
[2] Pinheiro et al. *Anal. Chem.* 2012, 84, 1003-1011
[3] Corbisier et al. *Anal. Chem.* 2015, 407, 1831

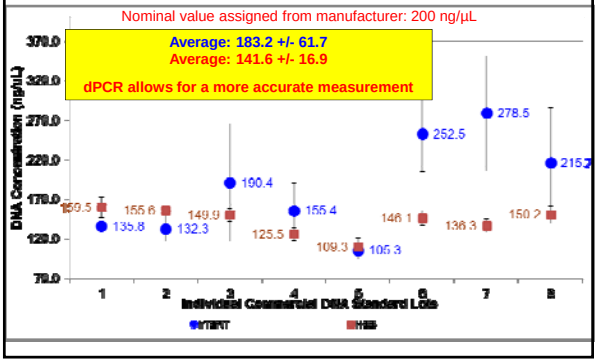
Why use dPCR for certification?

- No need for an external calibrant

- Multiple dPCR assays can be used for characterization
  - Establish reasonable estimates of uncertainty

- More accurate form of concentration measurement for end user

8 Commercial DNA Standards



Conclusions

- Groundwork has been laid to certify using dPCR
  - Uncertainties in instrumentation, volumes, assays, etc. all examined
- Homogeneity testing and stability testing are currently underway
- Certification measurements will begin after SED evaluates the homogeneity data

Our goal is to have SRM 2372a available for purchase by the summer of 2017

Thank you for your attention!

Questions?

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Acknowledgements

Margaret Kline

Dave Duewer

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