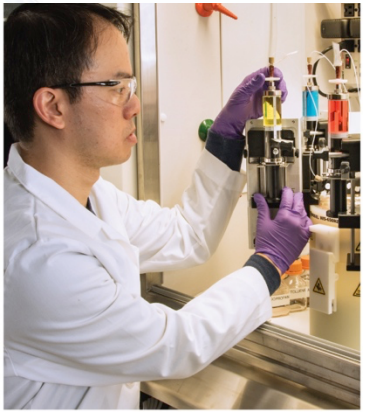
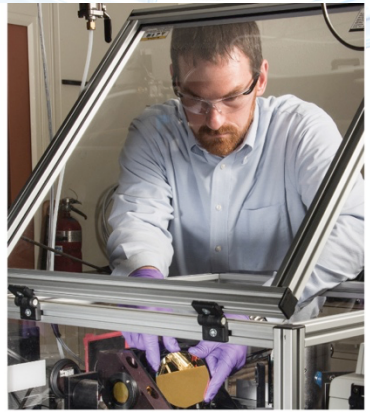




NIST Development of a SARS-CoV-2 Test Material

Peter M. Vallone
Applied Genetics Group

CSWG July 17, 2020

NIST
National Institute of
Standards and Technology
U.S. Department of Commerce

MATERIAL
MEASUREMENT
LABORATORY

Advancing technology and traceability through quality genetic measurements to aid work in Forensic and Clinical Genetics.

Forensic SRMs

- *PCR-Based DNA Profiling Standard (2391d)*
- *Human DNA quantitation standard (2372a)*
- *Mitochondrial DNA Sequencing (2392, 2392-I)*

Clinical SRMs

- *BK virus (2365)*
- *Cytomegalovirus (CMV) (2366a)*
- *Huntington's (2393)*
- *JC virus (coming soon!)*

NIST APPLIED GENETICS

Variations on the polymerase chain reaction (PCR) technique such as **rapid PCR, multiplex PCR, real-time PCR, and digital PCR** are used to **genotype, sequence, and provide quantitative information** pertaining to an organism's genome.

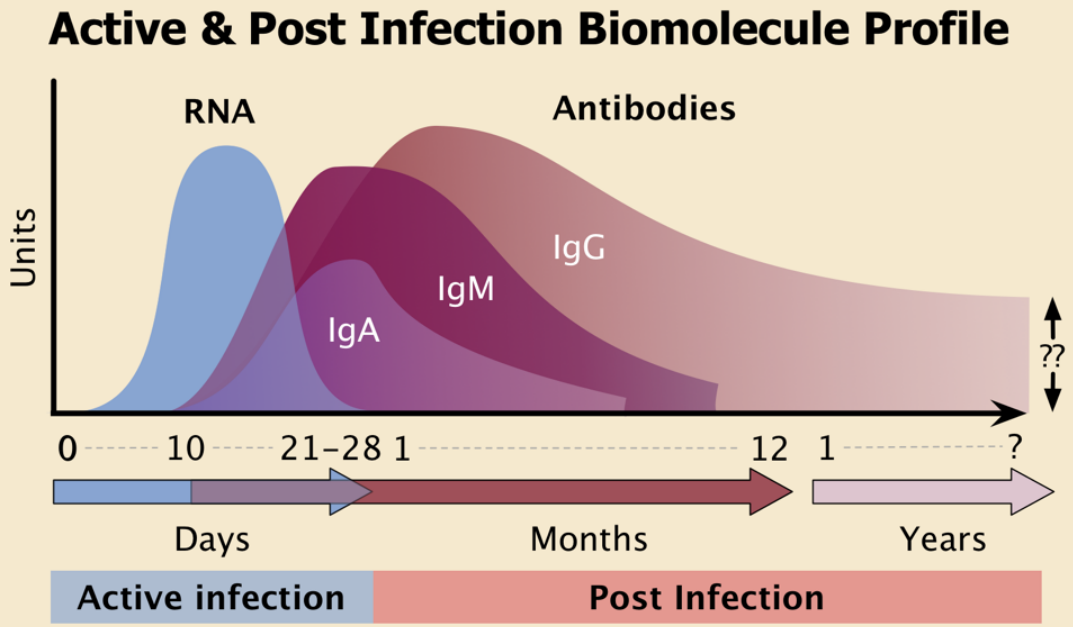
SARS-CoV-2 / COVID-19

Molecular Testing

VS

Serologic Testing

How they work & considerations

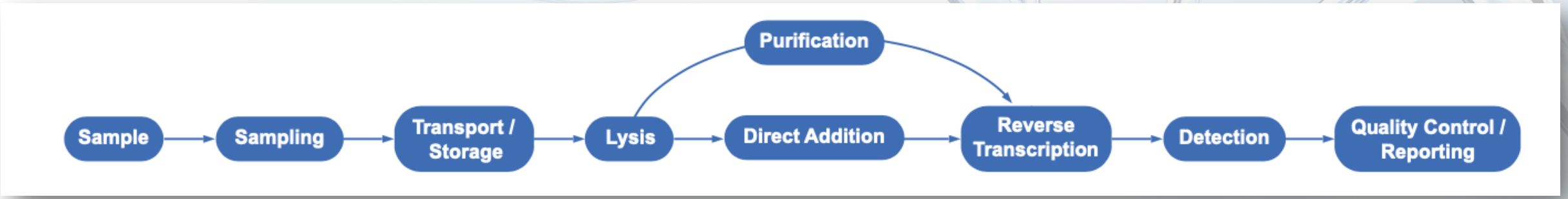


https://digitalworldbiology.com/blog/what-best-way-test-covid-19

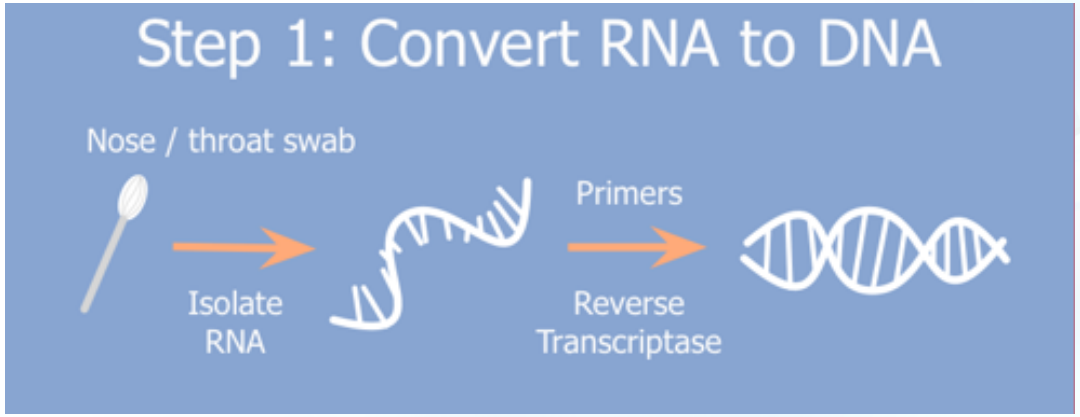
Molecular testing
testing for viral RNA present early in the infection

Serologic testing
testing for antibodies present post initial infection

General schematic of the molecular testing process (sample collection through RT-qPCR)



- Sample is collected onto a swab (nose – throat)
- RNA is extracted from the virus and purified
- DNA is produced through reverse transcription (RT)
- The DNA is then detected through (qPCR)

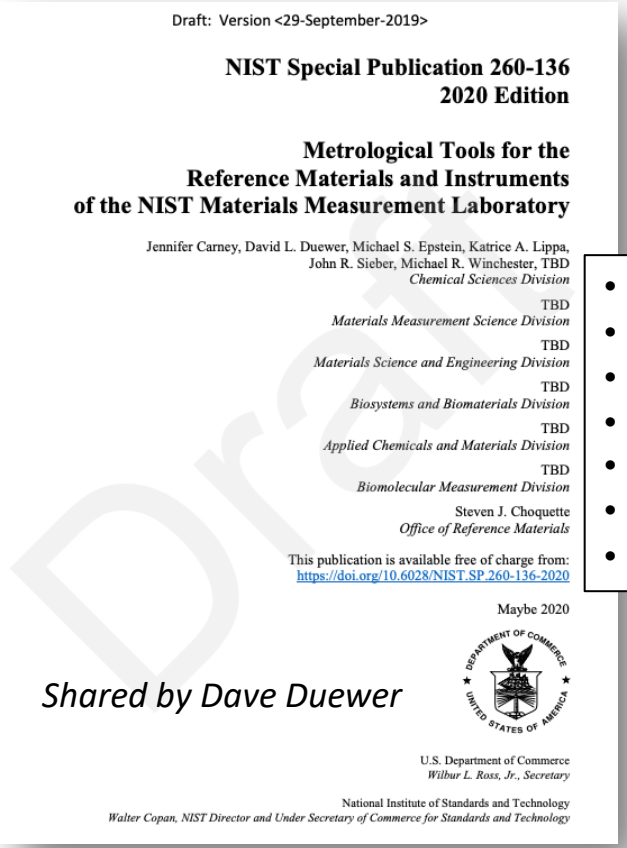


Preparation of a SARS-CoV-2 Research Grade Test Material (RGTM)

- What is a RGTM?
- What purpose will it serve?
- Design and development of the material
- Release and distribution of the material

Research Grade Test Material (RGTM)

Research-grade test materials (RGTMs) are fit for the purpose of exploring new measurement challenges



Draft: Version <29-September-2019>

NIST Special Publication 260-136
2020 Edition

Metrological Tools for the Reference Materials and Instruments of the NIST Materials Measurement Laboratory

Jennifer Carney, David L. Duewer, Michael S. Epstein, Katrice A. Lippa, John R. Sieber, Michael R. Winchester, TBD
Chemical Sciences Division

TBD
Materials Measurement Science Division

TBD
Materials Science and Engineering Division

TBD
Biosystems and Biomaterials Division

TBD
Applied Chemicals and Materials Division

TBD
Biomolecular Measurement Division

Steven J. Choquette
Office of Reference Materials

This publication is available free of charge from: https://doi.org/10.6028/NIST.SP.260-136-2020

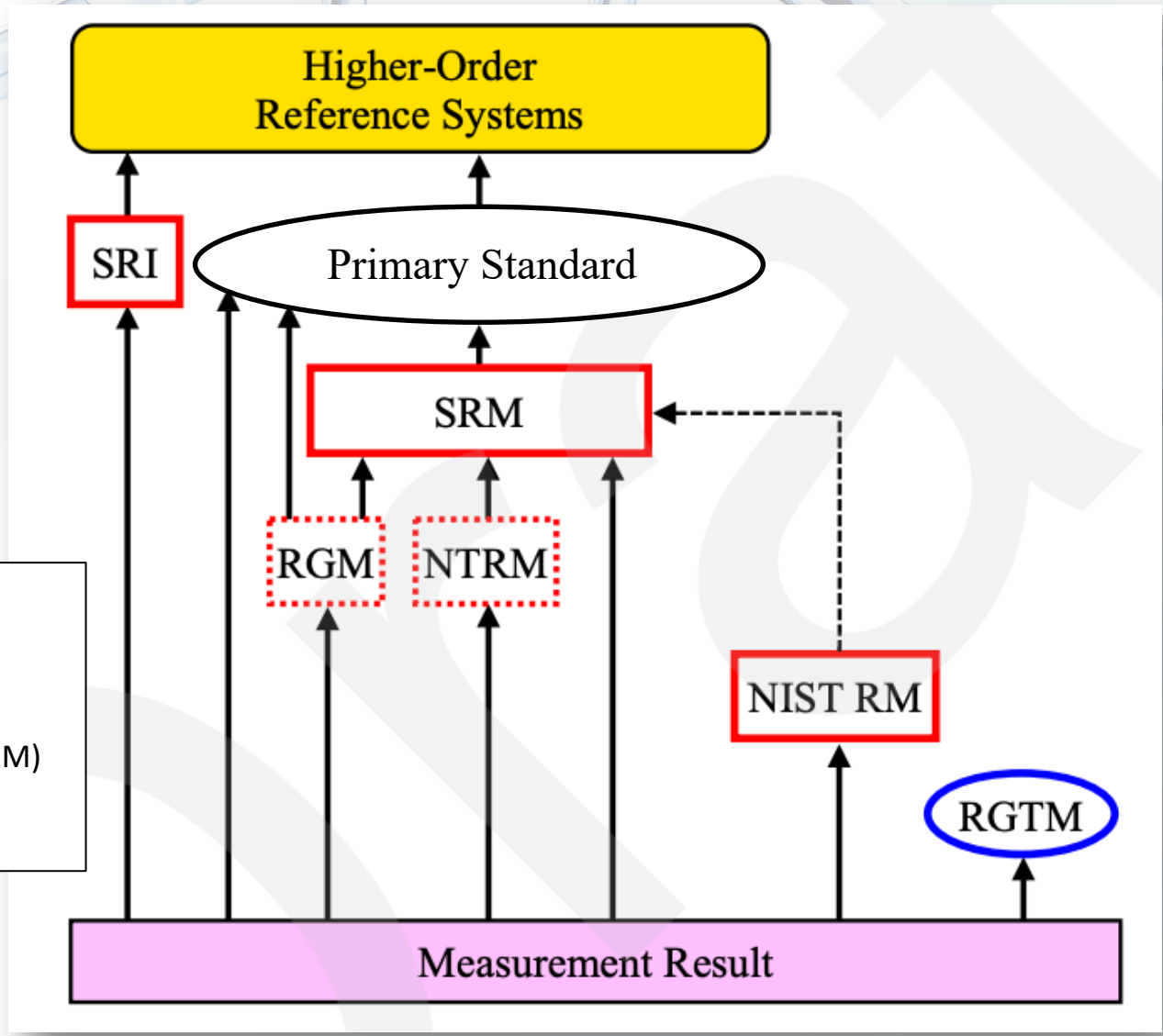
Maybe 2020

U.S. Department of Commerce
Wilbur L. Ross, Jr., Secretary

National Institute of Standards and Technology
Walter Copan, NIST Director and Under Secretary of Commerce for Standards and Technology

Shared by Dave Duewer

- Standard reference instrument (SRI)
- Standard Reference Material (SRM)
- Reference material (RM)
- Research gas mixture (RGM)
- NIST-traceable reference material (NTRM)
- Primary standard (PS)
- **Research-grade test material (RGTM)**



What purpose will the RGTM serve?

- For the quality control and benchmarking of existing NA-based assays
  - Limits of detection
  - Sequence specificity
  - Validation studies
  - Identify sources of bias
- Support the development of new assays
- Benchmarking other SARS-CoV-2 controls

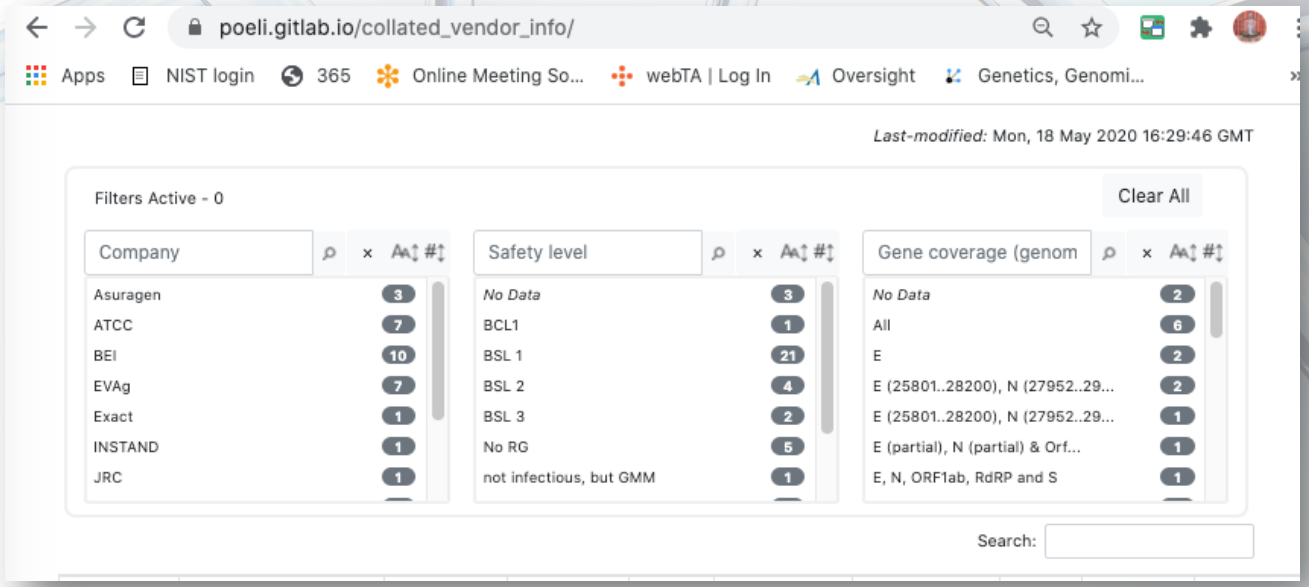
An element of immediate need – balance what can be done in 60-90 days versus the perfect material (RGTM is a good place for this)

RGTM 10169

- **A bridge to additional NIST - SARS-CoV-2 materials (or for other emerging viral agents)**
  - **The ideal nucleic acid-based RM may be extracted, native, viral RNA**
  - **Inactivated virus – or the SARS-CoV-2 genome in a surrogate 'container'**

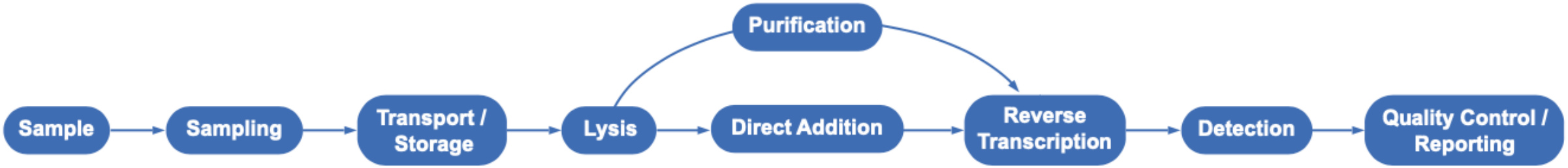
‘Flavors’ of currently available standards

- Infectious virus
  - BSL-2/3
- Inactivated SARS-CoV-2 virus
  - Actual virus
  - BSL-2
- The genome of the SARS-CoV-2 virus
  - 30 kb strand of RNA
  - BSL-2



https://poeli.gitlab.io/collated_vendor_info/

- Fragments of the SARS-CoV-2 virus genome that are targeted by the current tests
  - BSL-1
  - Can be prepared without working with the actual virus



Reference materials :

1. SARS virus or syntheic virus-like controls:

Controlled Steps:

Virus Culture, Patient Samples, Packaged Viral RNA

2. SARS nucleic acid controls:

Viral RNA, Synthetic RNA

Viral cDNA, Synthetic cDNA

3. Non-SARS controls:

Human RNA

Non-SARS Virus

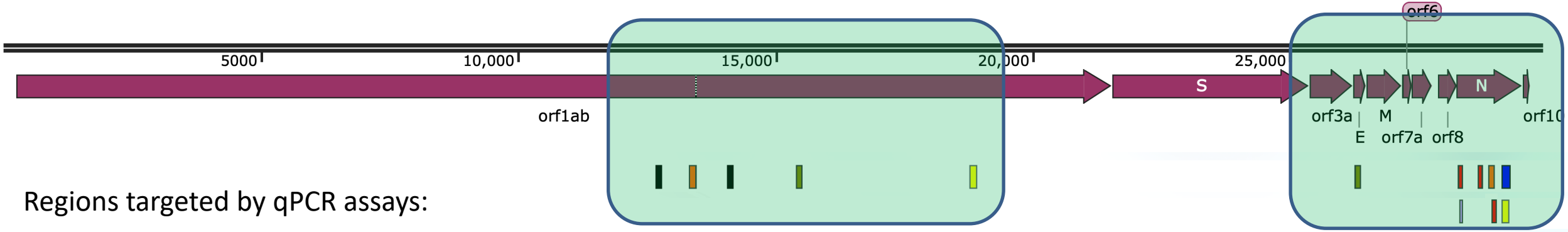
Non-SARS Nucleic-Acid

Non-SARS cDNA

Tim Mercer

Primers and protocols obtained from the WHO website

SARS-CoV-2 WA12020



Regions targeted by qPCR assays:

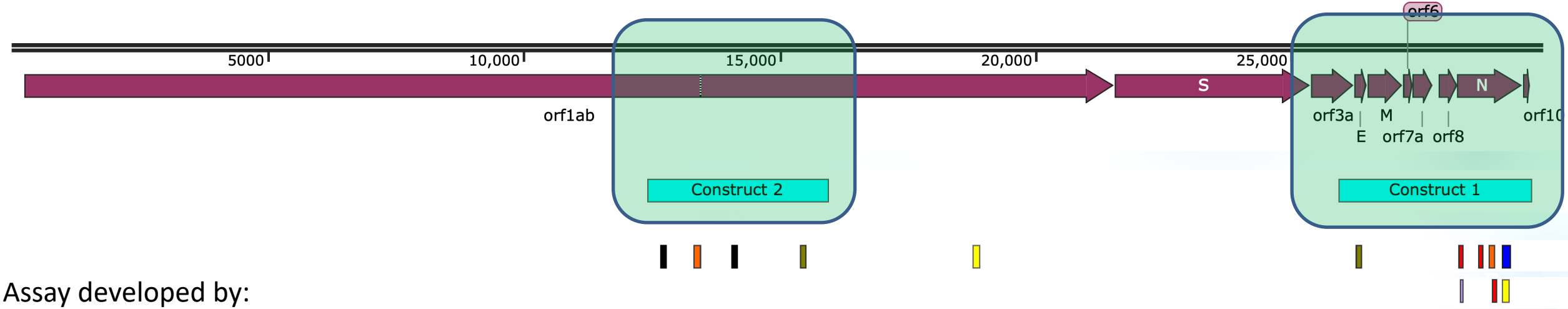
Assay developed by:

- Pasteur Institute
- Chinese CDC
- World Health Organization
- Hong Kong University
- U.S. CDC
- Thailand Ministry of Public Health
- Japanese Institute of Infectious Diseases

Initial strategy is to provide RNA fragments that would act as controls for the current WHO tests

Started in late March

SARS-CoV-2 WA12020

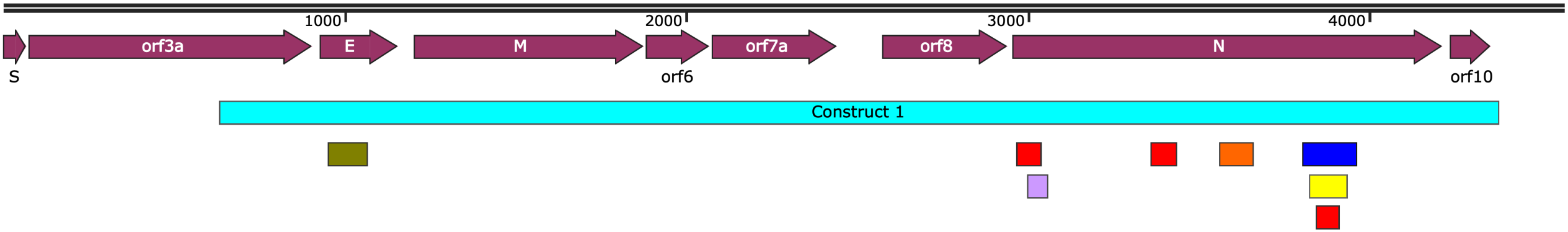


Assay developed by:

- Pasteur Institute
- Chinese CDC
- World Health Organization
- Hong Kong University
- U.S. CDC
- Thailand Ministry of Public Health
- Japanese Institute of Infectious Diseases

Constructs 1 and 2 were designed as candidates for the RGTM.

Zoom in on Construct 1



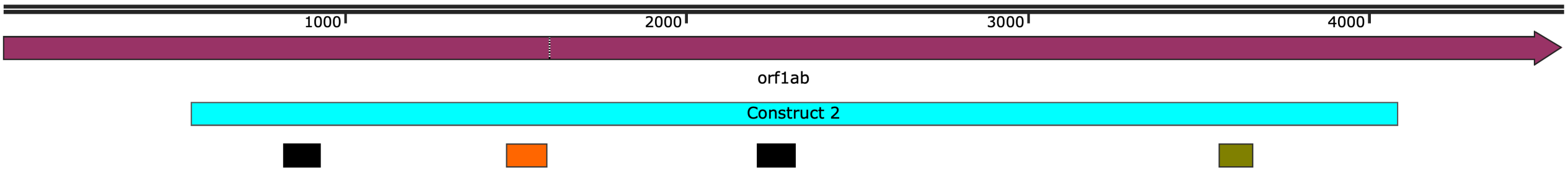
Assay developed by:

- Chinese CDC
- World Health Organization
- Hong Kong University
- U.S. CDC
- Thailand Ministry of Public Health
- Japanese Institute of Infectious Diseases

≈4 kb Fragment

Contains U.S. CDC assays N1, N3, N2 (red)

Zoom in on Construct 2

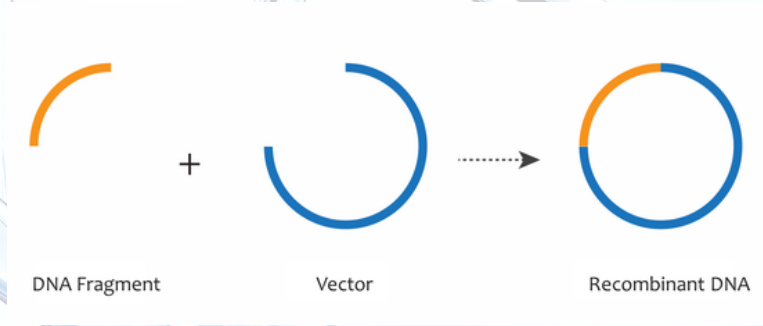


≈3.8 kb Fragment

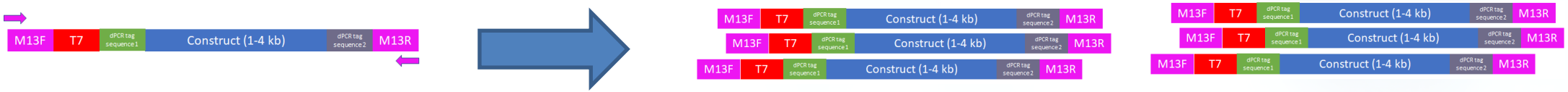
Assay developed by:

- Pasteur Institute
- Chinese CDC
- World Health Organization

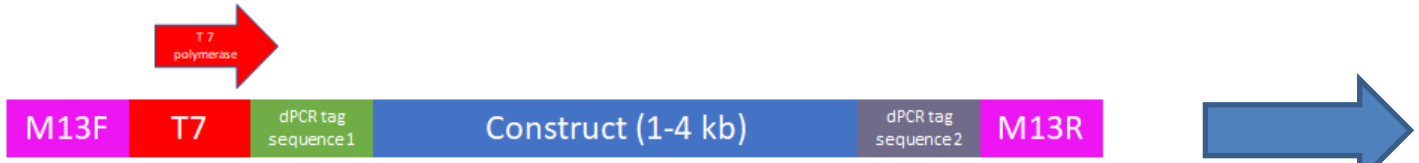
Production of RNA Fragments

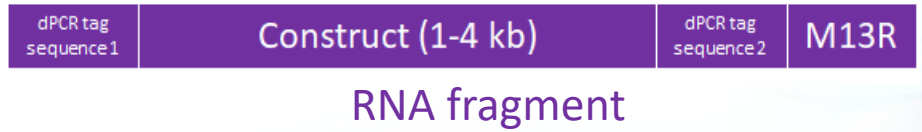


1) Purchase DNA fragment of interest inserted into a plasmid



2) PCR (using M13F/R primers) to generate copies of the construct that act as a template for RNA production





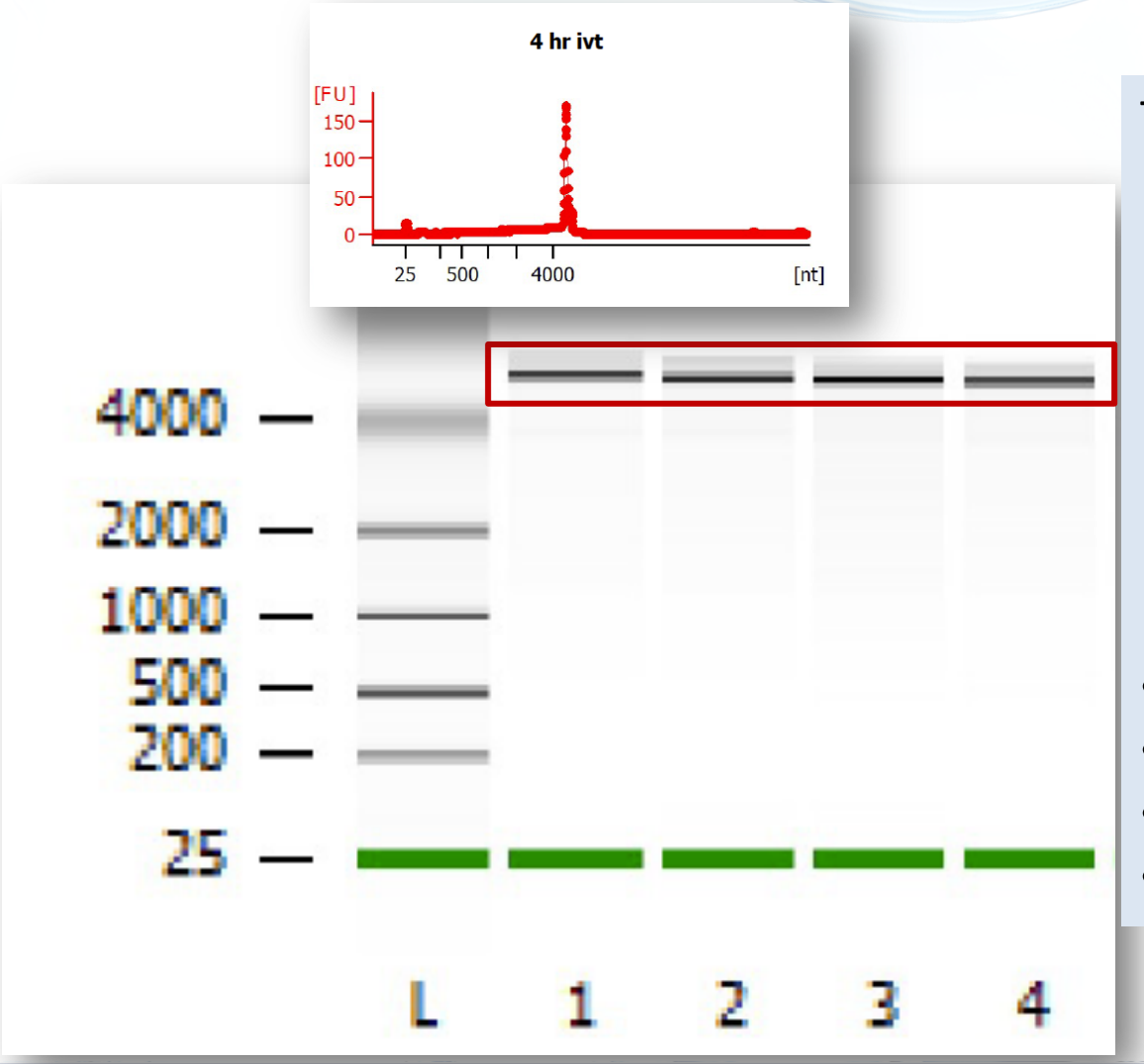
RNA fragment

3) T7 RNA polymerase for RNA production: products checked on a gel for the correct size and purified

4) Purified RNA fragments diluted in buffer and bottled followed by then RT-qPCR and RT-ddPCR measurements

Started in late April

Production of RNA Fragments – Example Fragment 1



Target goal:
Produce 1000 units, 110 μL, approx. 10^6 cp/μL
Buffer + 5 ng/μL of Jurkat RNA; -80°C
Examine homogeneity and stability
Measure copies/μL value to each fragment using digital PCR

Distribute at no cost to manufacturers of tests, controls, NMIs, FDA and other government agencies

- Fragment 1 bottled May 21
- Fragment 2 bottled June 6
- Homogeneity and concentration measurements performed
- RGTM 10169 released June 30

Digital PCR

Droplet digital (ddPCR) used for copy number determination

Does not require an external calibrant

Bio-Rad QX200 instrument

WHO RT-qPCR assays were adapted and optimized for ddPCR

Image credit: http://bio-rad.com/

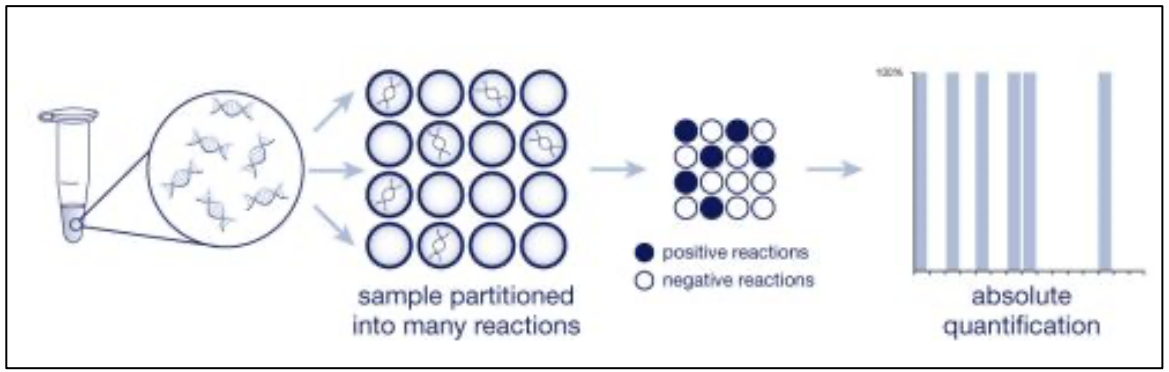
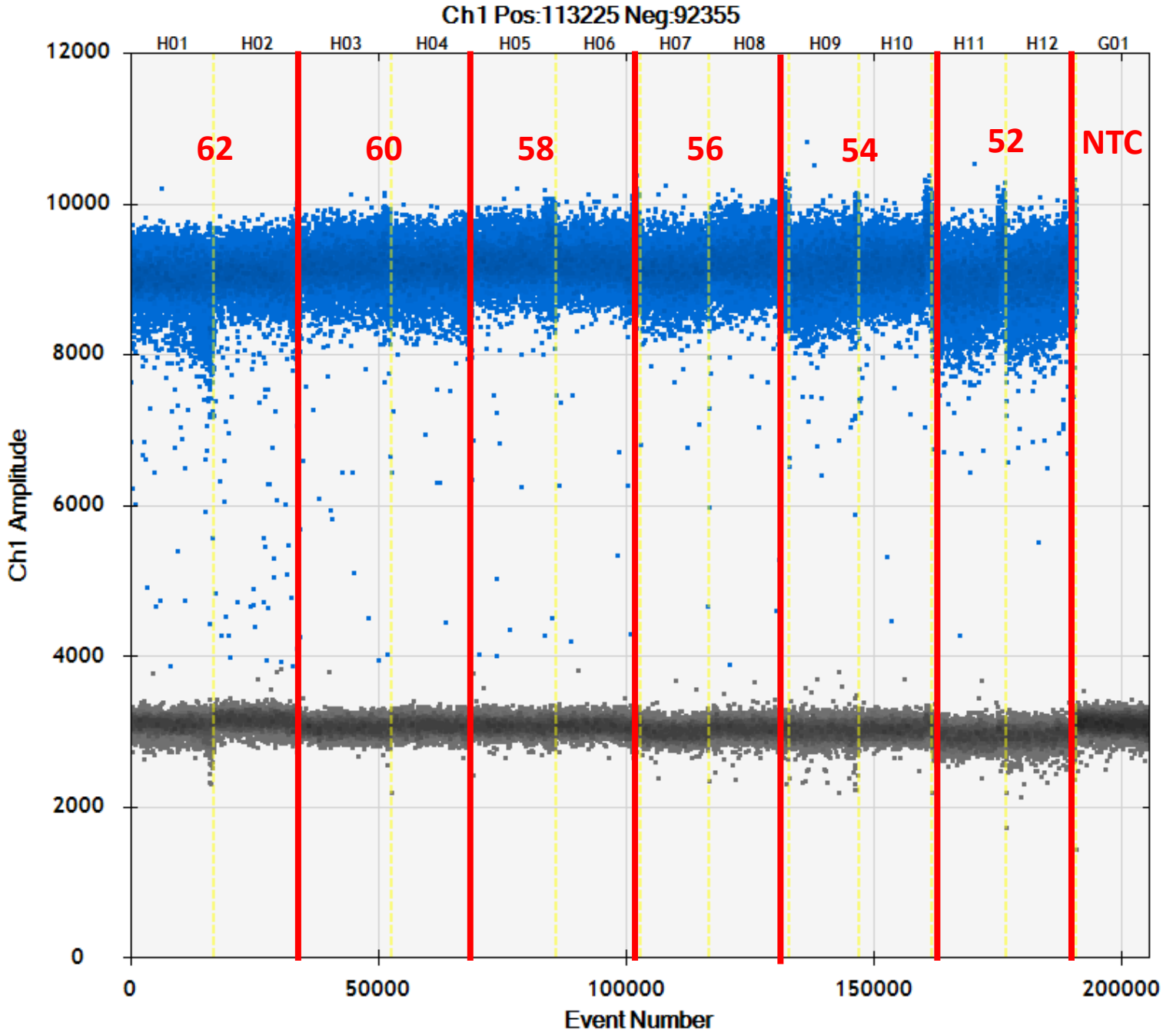


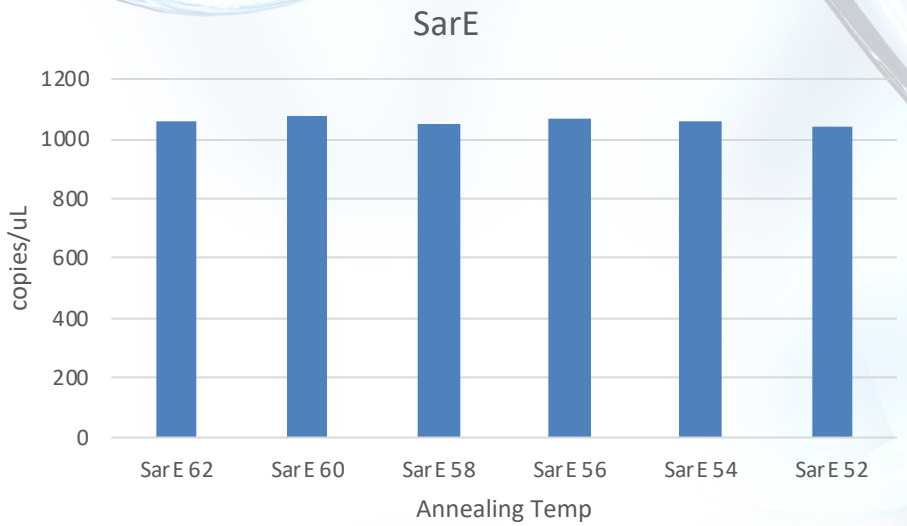
Image credit: http://digital-pcr.gene-quantification.info/

$\lambda = -\ln(1-\text{FractionPositive})$

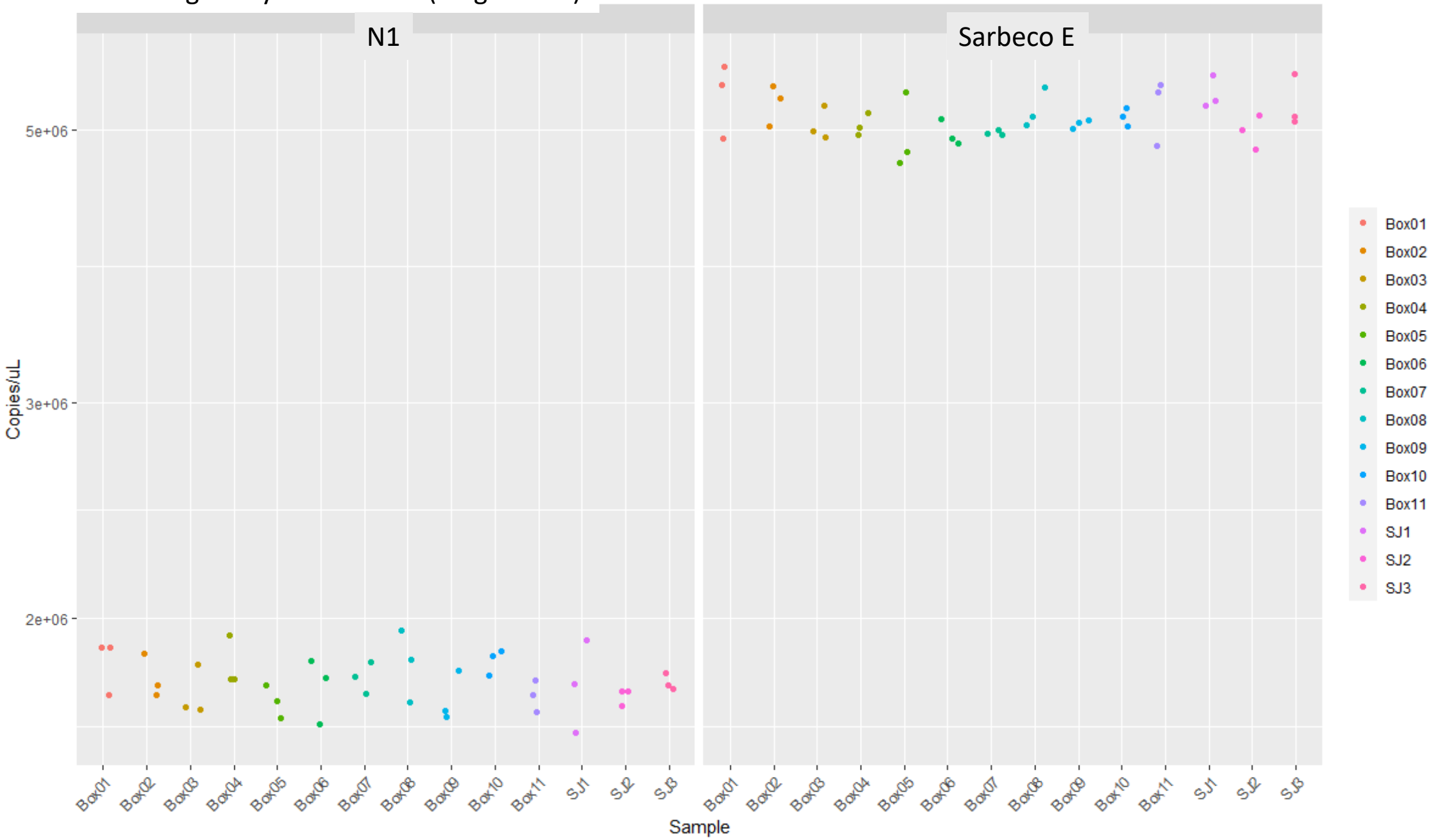
$\text{Copies}/\mu L = \lambda / (\text{Droplet Volume in } \mu L)$

Optimization of Sarbeco E assay

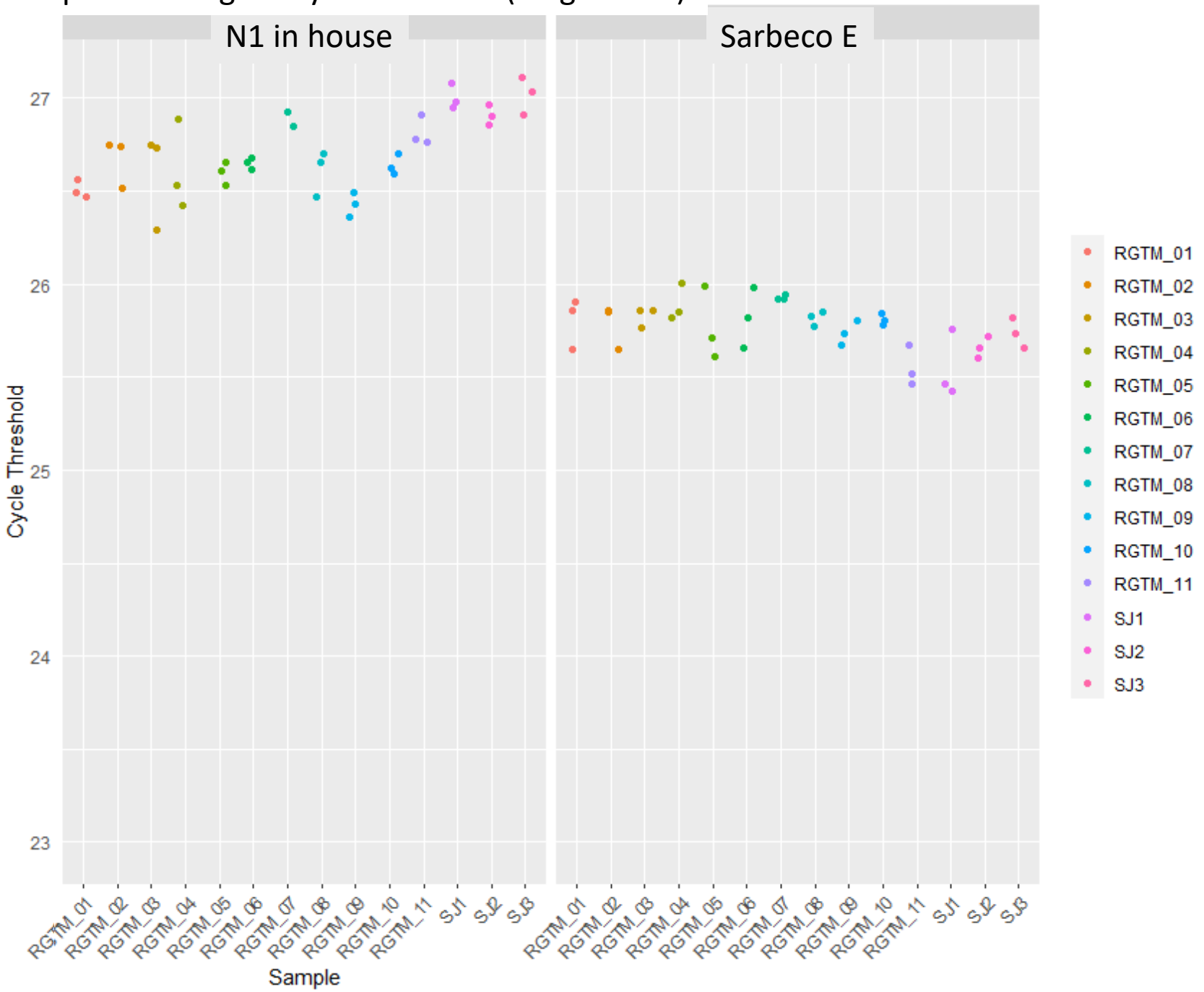




RT-dPCR Homogeneity Assessment (Fragment 1)



RT-qPCR Homogeneity Assessment (Fragment 1)

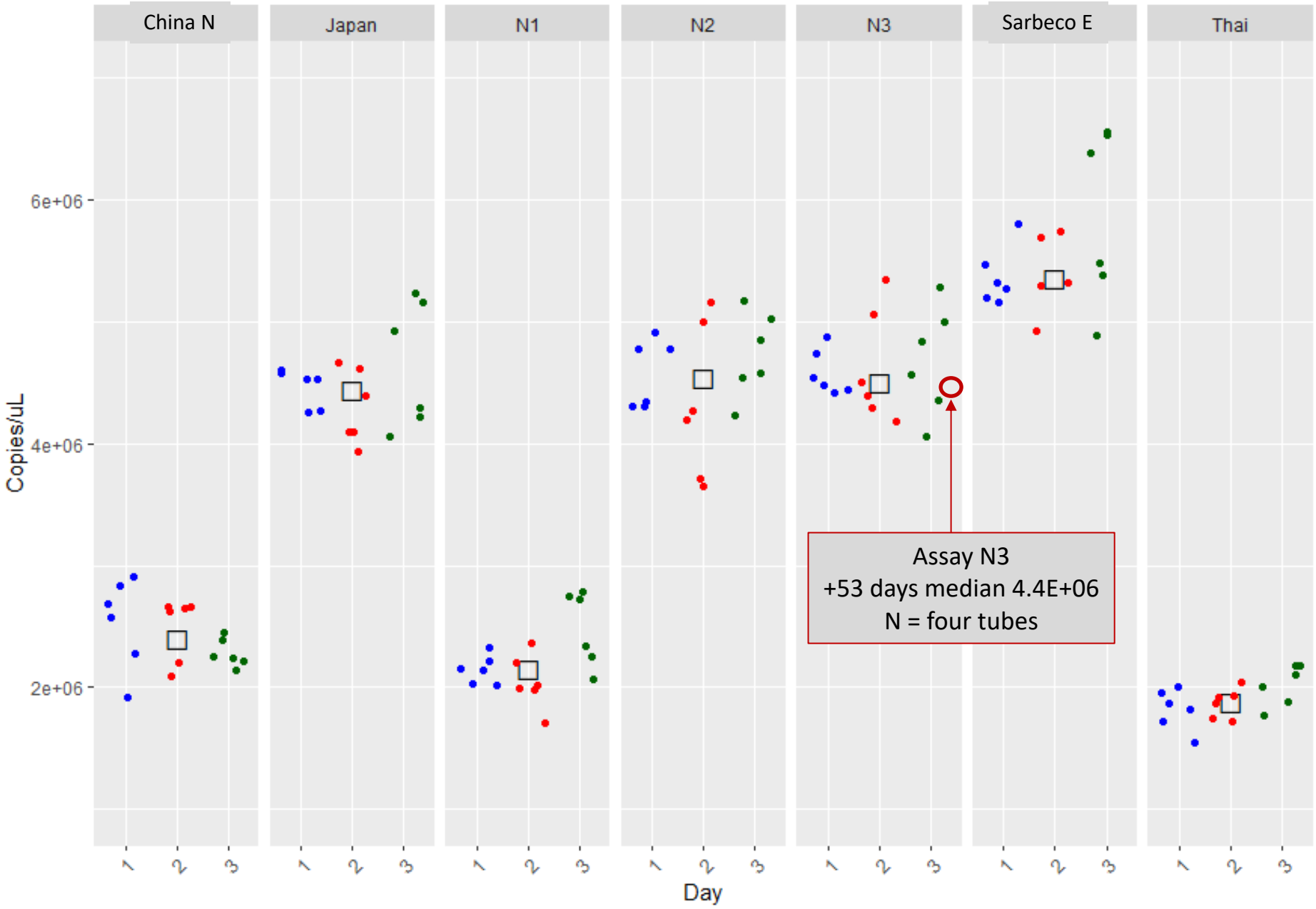


TaqPath 1-Step RT-qPCR Master Mix

7500 HID instrument with version 1.3 of the analysis software

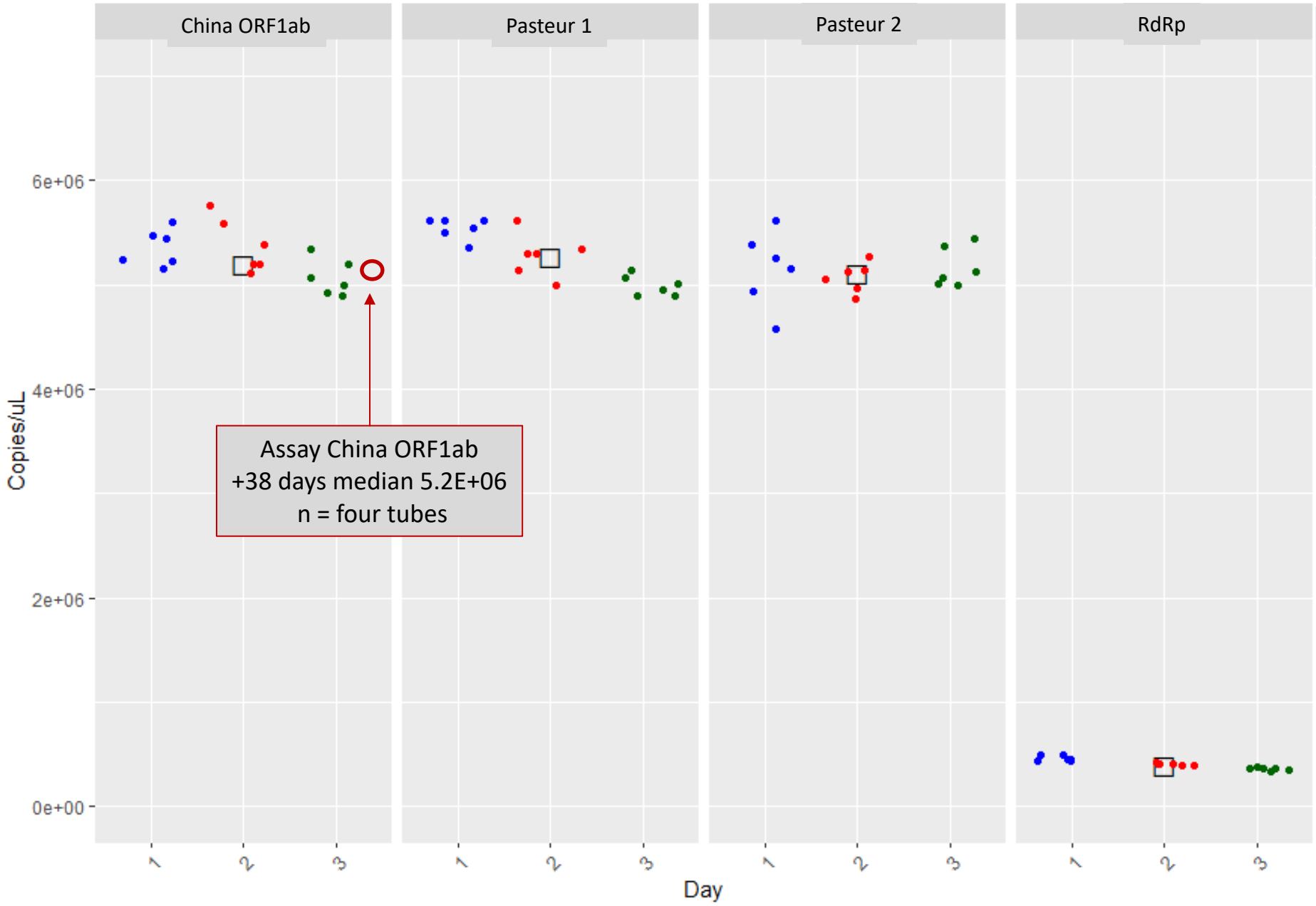
1:500 dilution of neat material

RT-dPCR Concentration Measurements (Fragment 1)



Fragment 1 Assay Name	Median concentration (copies/microliter)
China N	2.4 x 10^6
Japan	4.5 x 10^6
N1	2.2 x 10^6
N2	4.6 x 10^6
N3	4.5 x 10^6
Sarbeco E	5.4 x 10^6
Thai	1.9 x 10^6

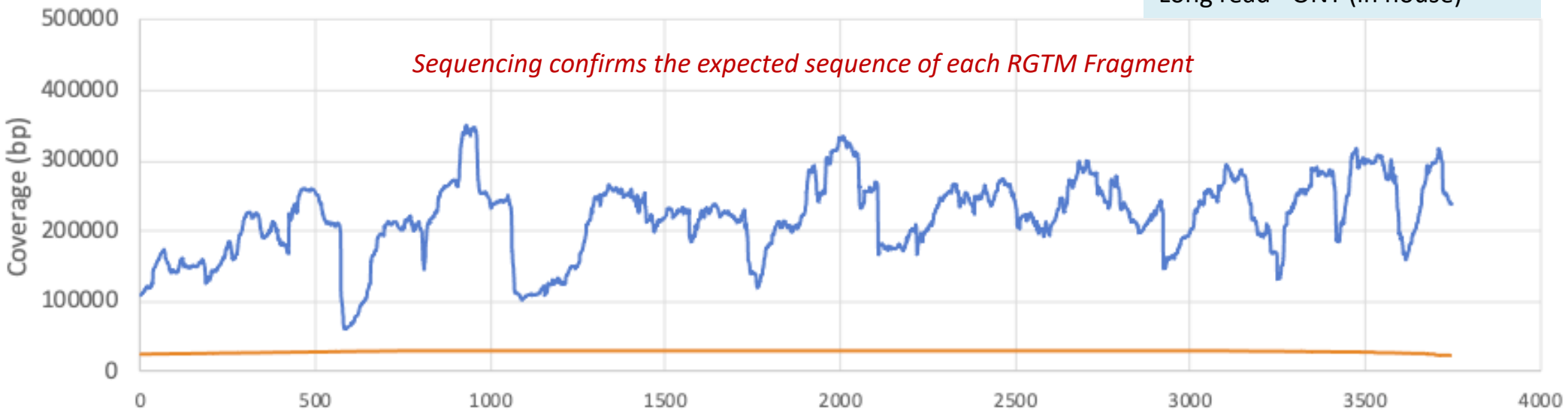
RT-dPCR Concentration Measurements (Fragment 2)



Fragment 2 Assay Name	Median concentration (copies/microliter)
China ORF1ab	5.2 x 10^6
Pasteur 1	5.3 x 10^6
Pasteur 2	5.1 x 10^6
RdRp	4.1 x 10^5

Short read - Illumina (GENEWIZ)
Long read - ONT (in house)

Sequencing confirms the expected sequence of each RGTM Fragment



https://www.nist.gov/programs-projects/sars-cov-2-research-grade-test-material

NIST

PROJECTS/PROGRAMS

SARS-CoV-2 Research Grade Test Material

DESCRIPTION

A new material from NIST can aid in the evaluation and development of RT-qPCR assays for SARS-CoV-2. We are offering a unit, free of charge, for evaluation in exchange for your feedback, which will help us improve and further develop the material.

Credit: Adobe Stock

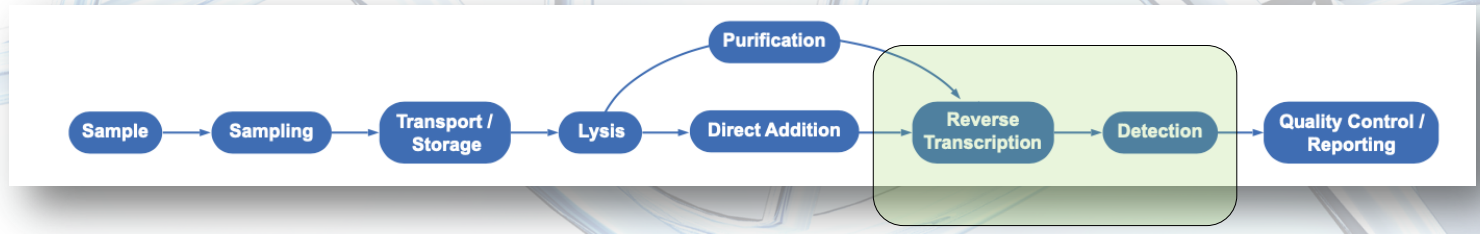
Available at no cost (link to order on webpage)
40 requests so far

Example of feedback that we request:
Suitability of material volume and concentration
Description of how the material was used
Concentration value estimates from quantitative and/or digital PCR methods
Impact of material on your work

https://github.com/usnistgov/RGTM10169

An attempt at a timeline to illustrate challenges

Each step exhibits variability that requires evaluation



Discover new virus

Sequence information (viral genome)

Tests are developed – now hundreds of tests

Standards and controls are created to develop new assays and harmonize results

Continual further understanding of the virus and disease

With SARS-CoV-2: this is all happening over days/week versus months/years

Staff with experience in producing and characterizing nucleic acid-based materials have come together to discuss ideas and share their knowledge

<u>Applied Genetics</u>
Megan Cleveland

Will Valiant
Erica Romsos
Becky Steffen

John Marino
Zvi Kelman
Brad O'Dell
Rob Brinson
Andrea Szakal
Scott Jackson
Jason Kralj
Hua-Jun He
Hari Iyer
Stephanie Servetas

<u>JIMB</u>
Marc Salit

<u>LGC (UK)</u>
Jim Huggett
Alison Devonshire
Eloise Busby
Alexandra Whale
Gerwyn Jones

Follow up questions
peter.vallone@nist.gov

COVID-19 CARES Act funding