COVID-19 Detection

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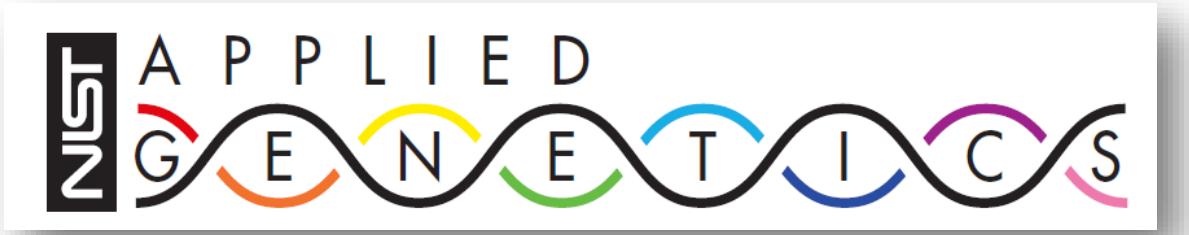
National Institute of Standards and Technology
U.S. Department of Commerce

Introduction

Applied Genetics Group – Biomolecular Measurement Division

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 in FY20!)*

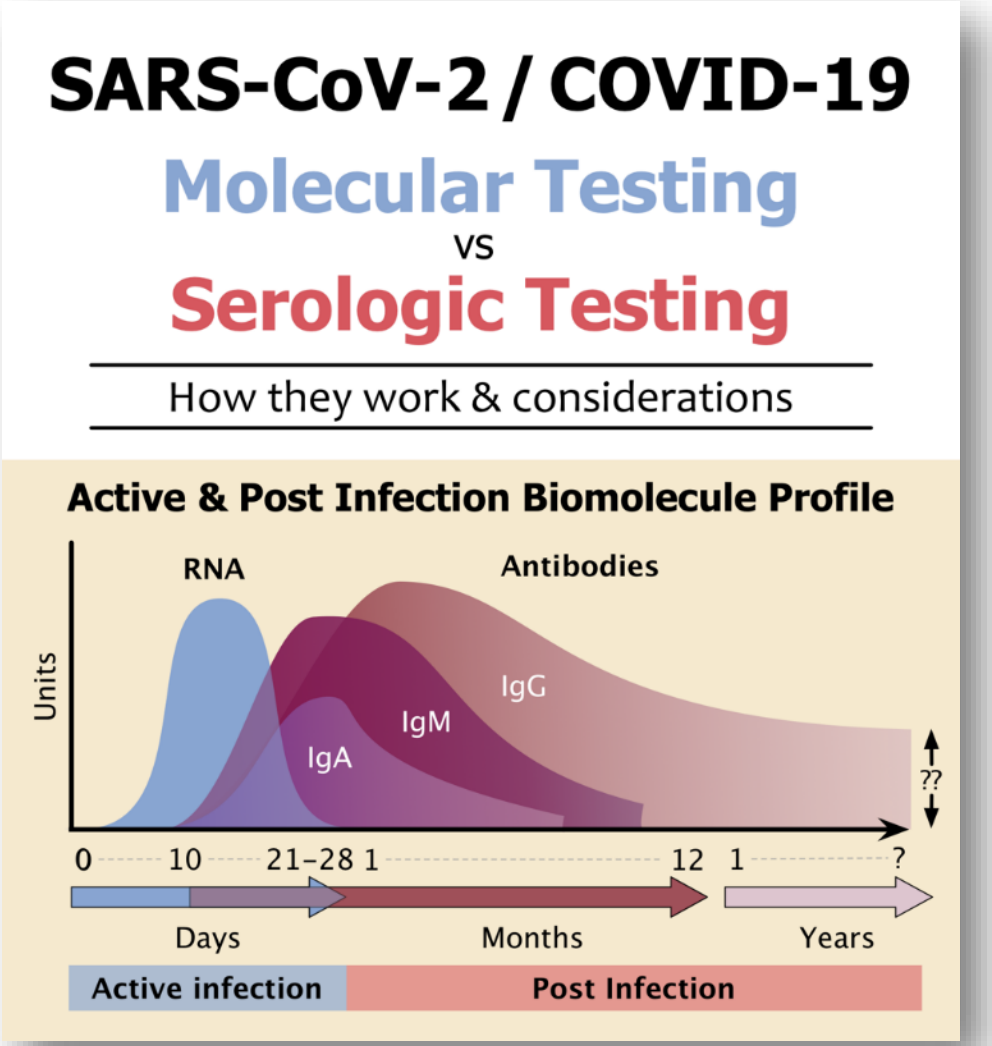
Variations on the polymerase chain reaction (PCR) technique such as **rapid PCR, multiplex PCR, real-time PCR, and digital PCR** are used to **genotype, sequence, and provide quantitative information** pertaining to an organism's genome.

Initial questions

Detection: Focusing on molecular Dx (viral RNA detection).
How are labs performing the analysis?

Controls and Standards: Survey existing commercial controls: format, concentration characterization

Stakeholders: Who can we work with? Working groups, other NMIs, industry, government agencies.



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

Initial ideas and measurements

Detection: Develop competency on RNA detection assays (RT-qPCR). Bring WHO and U.S. CDC assays in house.

Controls and Standards: What would a NIST SARS-CoV-2 material look like?
How fast can this be carried out?

Interlaboratory and collaborative studies: Participation and design of SARS-CoV-2 detection studies.

https://www.cdc.gov/coronavirus/2019-ncov/images/coronavirus-testkit-sample-5.jpg

All evolving in real-time

Research Grade Testing Material

Creating RGTM 10169

Goal: to send the RGTM out to commercial makers of controls and Dx assays, metrology institutes, and government agencies

Target: by the end of June 2020

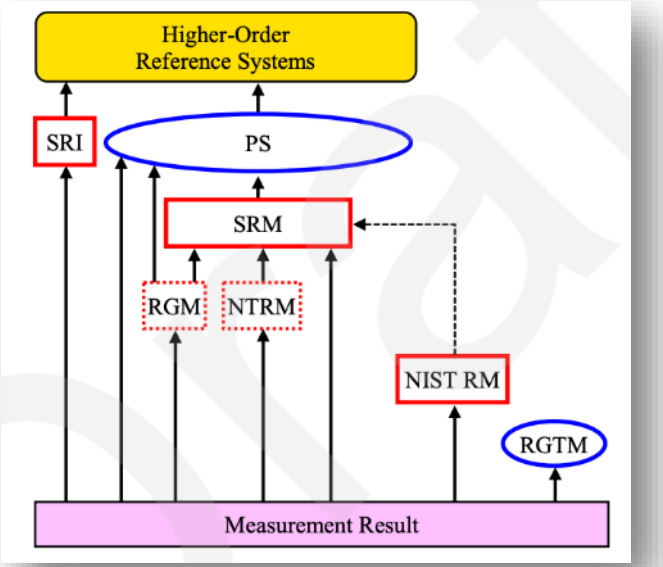
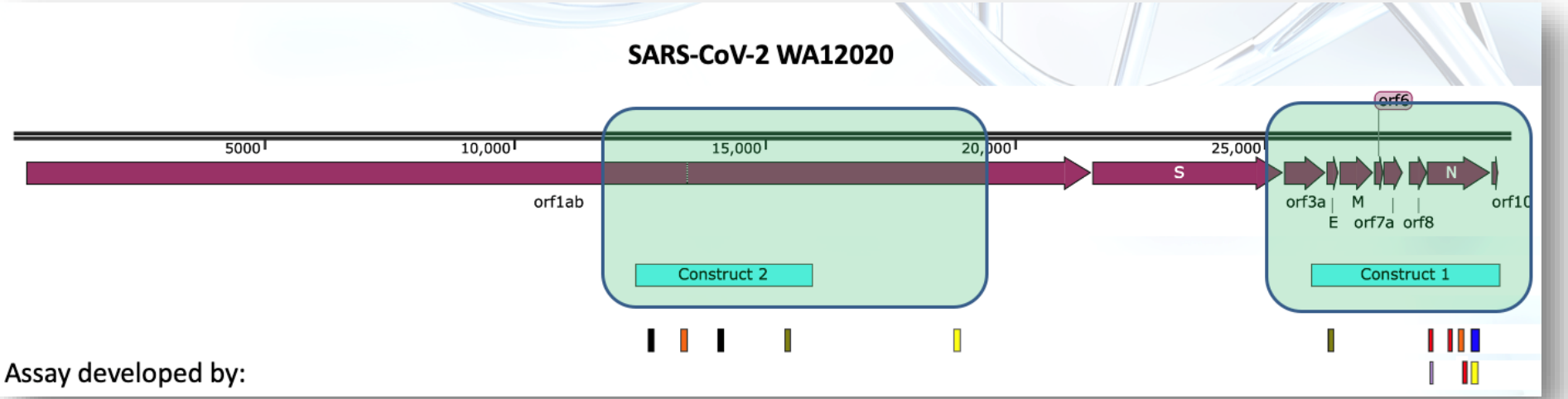


Figure – David Duewer NIST

4kb RNA fragments of the SARS-CoV-2 genome characterized using digital PCR



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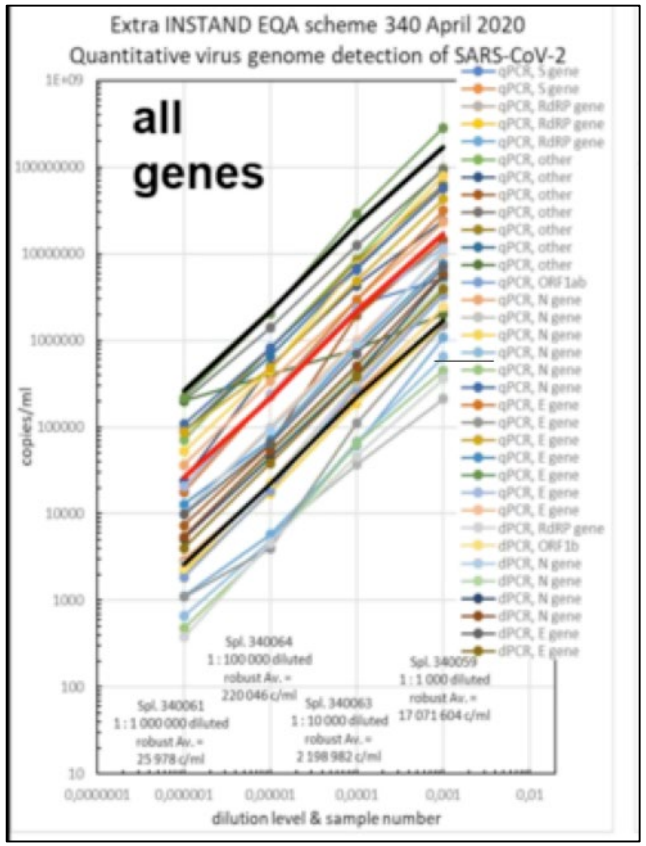
Interlaboratory Assessments

INSTAND: We participated in a global EQA – external quality assessment for SARS-CoV-2. Inactivated SARS-CoV-2 samples were extracted and analyzed. Over 450 labs participated. **Results were submitted** for EQA scheme 340 – summary can be downloaded at https://www.instand-ev.de/en/



CCQM: Nucleic acid working group Pilot study P199b will expand on capabilities demonstrated in the P199 study (HIV-1 RNA copy number quantification) for targeted RNA copy number concentration and viral gene quantification. (**Measurements will be carried out early this summer**)

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NML UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

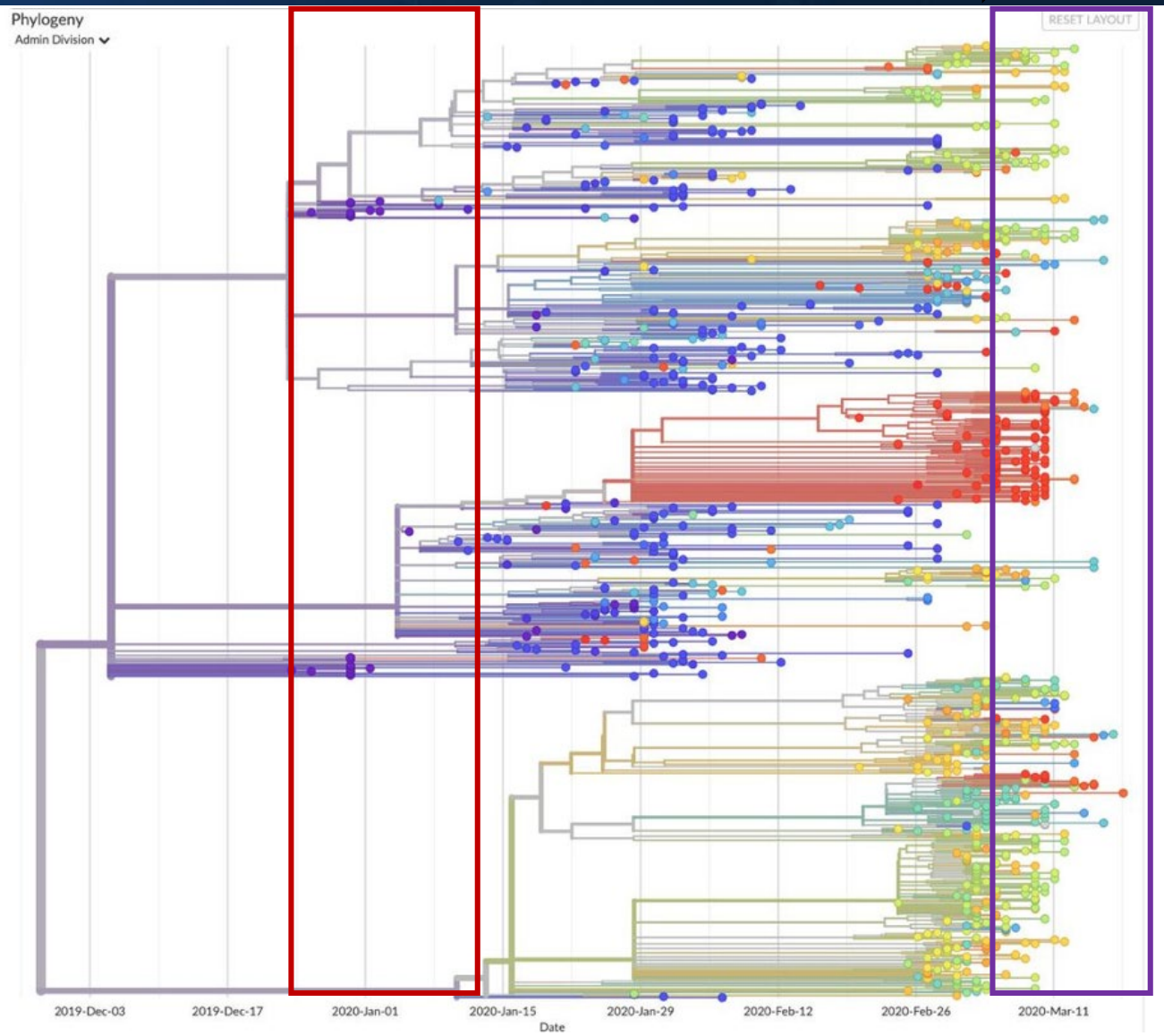
中国计量科学研究院 National Institute of Metrology,China

NIBSC Confidence in Biological Medicines

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

Dx Assay performance

- As the genome of SARS-CoV-2 develops mutations over the course of the pandemic, there is a likelihood that one will fall within the targeted regions of the CDC diagnostic tests
  - How will mutations affect qPCR assay performance?
  - How does digital PCR ‘tolerate’ mutations
  - **Materials in house, will continue after production of RGTM 10169**



https://www.gisaid.org/epiflu-applications/next-hcov-19-app/

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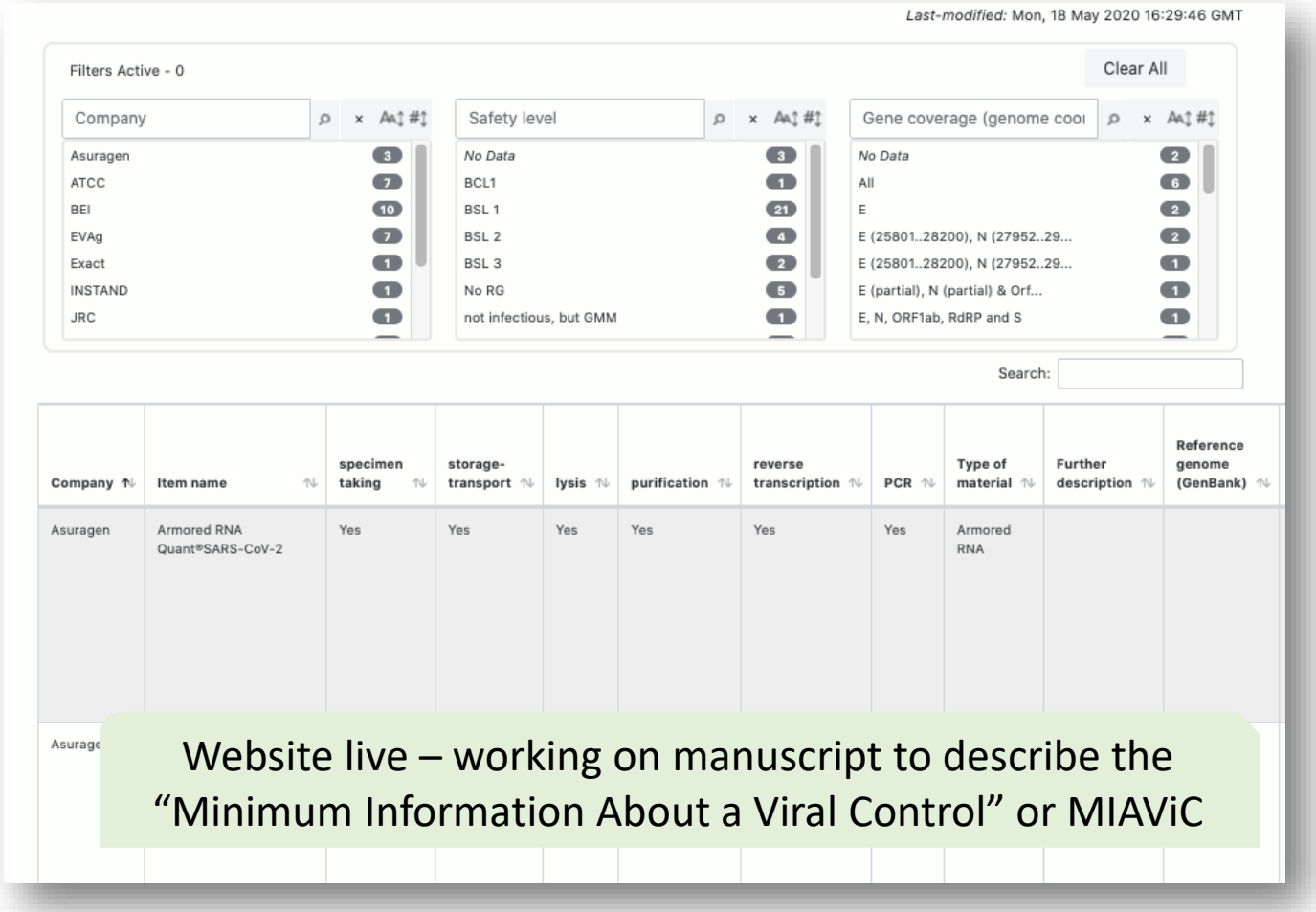
Controls and Standards

Working towards a minimum information standard for SARS-CoV-2 controls

- Type of material
- Genes represented
- Storage
- Amount
- Method of characterization
- Matrix
- Purpose



Website live – working on manuscript to describe the "Minimum Information About a Viral Control" or MIAViC

https://jimb.stanford.edu/covid-19-standards
https://poeli.gitlab.io/collated_vendor_info/

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qPCR Data analysis

In collaboration with the NIST ITL: Mathematical approaches to improve the sensitivity of qPCR detection methods

https://arxiv.org/abs/2004.05466

Improving Baseline Subtraction for Increased Sensitivity of Quantitative PCR Measurements

Paul N. Patrone,* Anthony J. Kearsley, Erica L. Romsos, and Peter M. Vallone
National Institute of Standards and Technology
(Dated: April 14, 2020)

Motivated by the current COVID-19 health-crisis, we examine the task of baseline subtraction for quantitative polymerase chain-reaction (qPCR) measurements. In particular, we present an algorithm that leverages information obtained from non-template and/or DNA extraction-control experiments to remove systematic bias from amplification curves. We recast this problem in terms of mathematical optimization, i.e. by finding the amount of control signal that, when subtracted from an amplification curve, minimizes background noise. We demonstrate that this approach can yield a decade improvement in sensitivity relative to standard approaches, especially for data exhibiting late-cycle amplification. *Critically, this increased sensitivity and accuracy promises more effective screening of viral DNA and a reduction in the rate of false-negatives in diagnostic settings.*

Keywords: qPCR, DNA Detection, Background Subtraction, Measurement Sensitivity

Quantitative polymerase chain-reaction (qPCR) measurements have had a long and of empirical models by directly leveraging the behavior of appropriate control experiments. The

Pre-print currently available

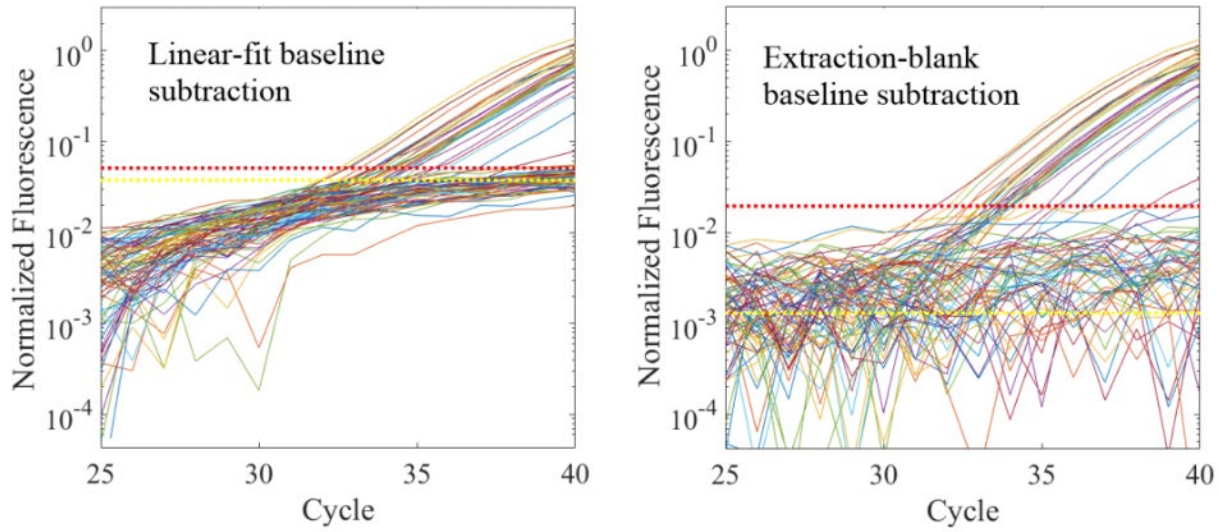

FIG. 2. Close-up of the bottom subplots of Fig. 1. In both sub-plots, the yellow dotted line is the mean value of all amplifications curves whose final value is less than 0.1. The red dotted line is 2 standard deviations away from the mean. Note that the extraction-blank baseline subtraction decreases the characteristic scales of the background by up to a decade.

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