Strain evolution and host response in patients

@mason_lab

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October 15, 2020

Search

Bloomberg

What you need to know: Tracking the Reopening of New York City

Prognosis

Why New York Suffered When Other Cities Were Spared by Covid-19

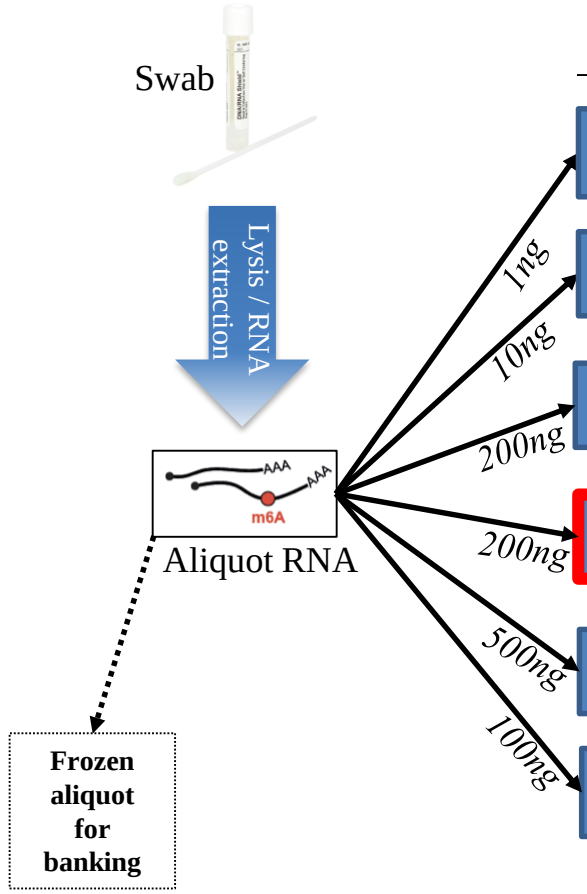
The mayor, the CDC and a New York disease expert weigh in

By Drew Armstrong, Henry Goldman, and Keshia Clukey
May 28, 2020, 9:51 AM EDT

>23,000 Deaths

https://www.bloomberg.com/news/articles/2020-05-28/why-was-new-york-hit-so-badly-with-covid-19

Deep characterization of COVID-19 samples has a short and long-term benefit



Assay	TAT	Measurements and Metrics
Loop-mediated Isothermal Amplification (LAMP)	20-30 minutes	*Polymerase and pH colorimetric output:* COVID-19 presence/absence
Quantitative real-time PCR (qRT-PCR)	3-4 hours	*Two Primer Sets for COVID-19:* The E (envelope) and S (spike) genes.
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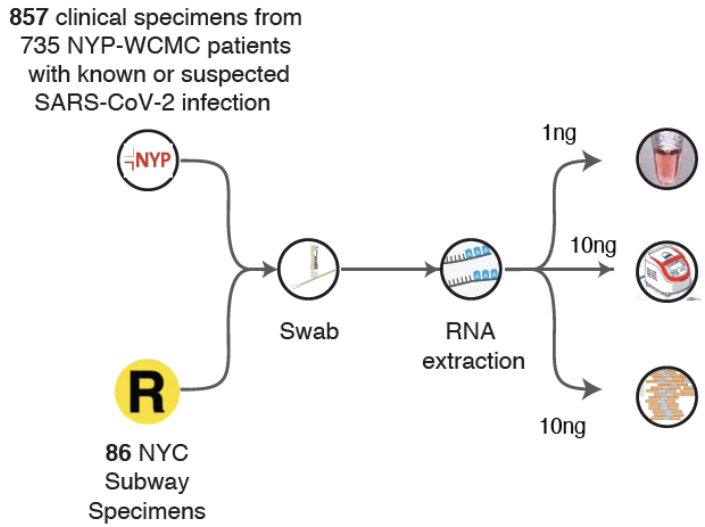
857 COVID-19 samples' total RNA-seq (63.2M 150x150 PE reads)



Assay	Time	Reagents	Result
Loop-mediated Isothermal Amplification (LAMP)	30-40 minutes	One primer sextet for SARS-CoV-2	Color change representing level of SARS-CoV-2 E (envelope) and N (nucleocapsid) gene in specimen
qRT-PCR	3-4 hours	Two primer sets for SARS-CoV-2	Ct representing level of SARS-Cov2 E (envelope) and S (Spike) gene in specimen
Total RNA-seq	8 hours - 2 days	Random Hexamers	Shotgun metatranscriptomes of all host and species RNA sequences in specimen

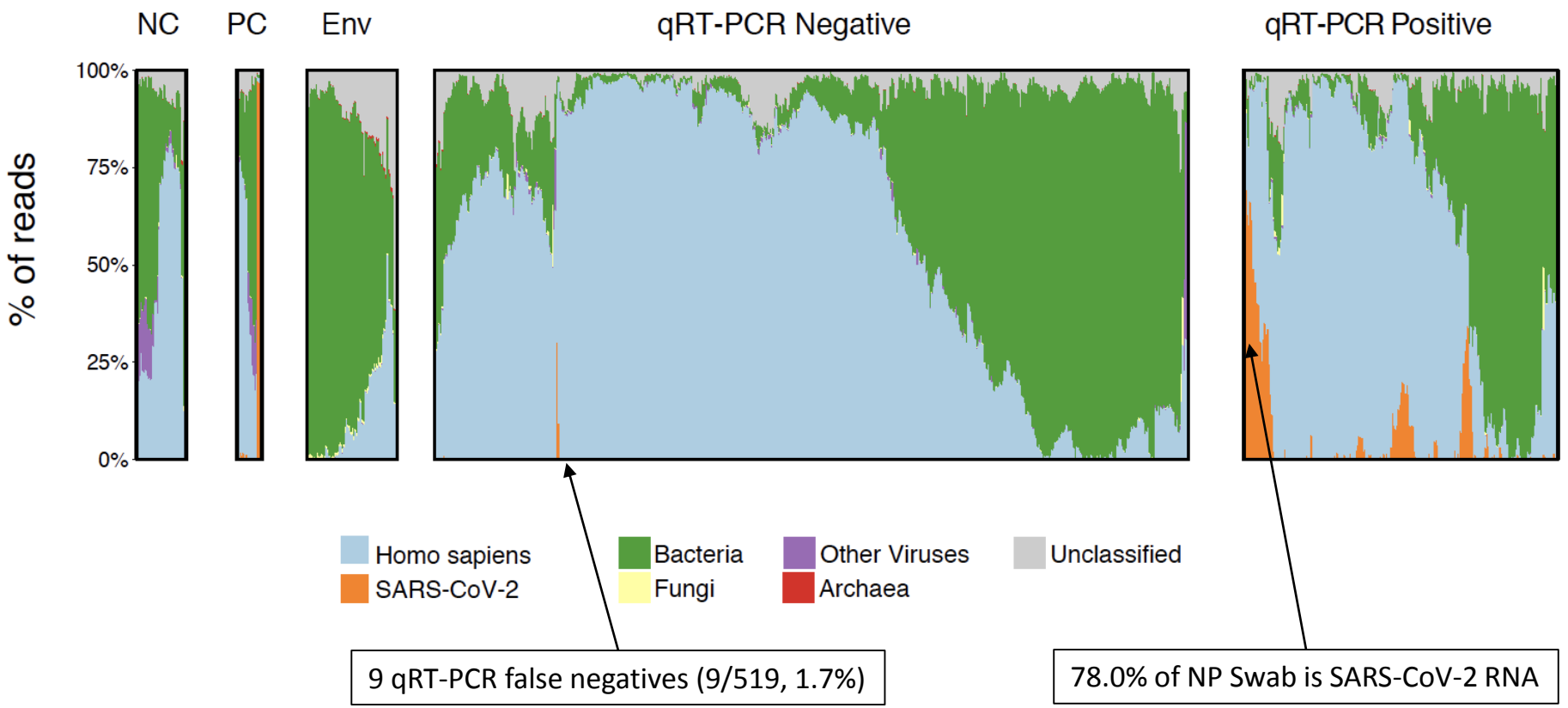
1) 735 suspected COVID-19 patients:
- COVID-19-POS, n=216
- COVID-19-NEG, n=519

2) 86 Environmental (54 sites)
- Grand Central
- Times Square

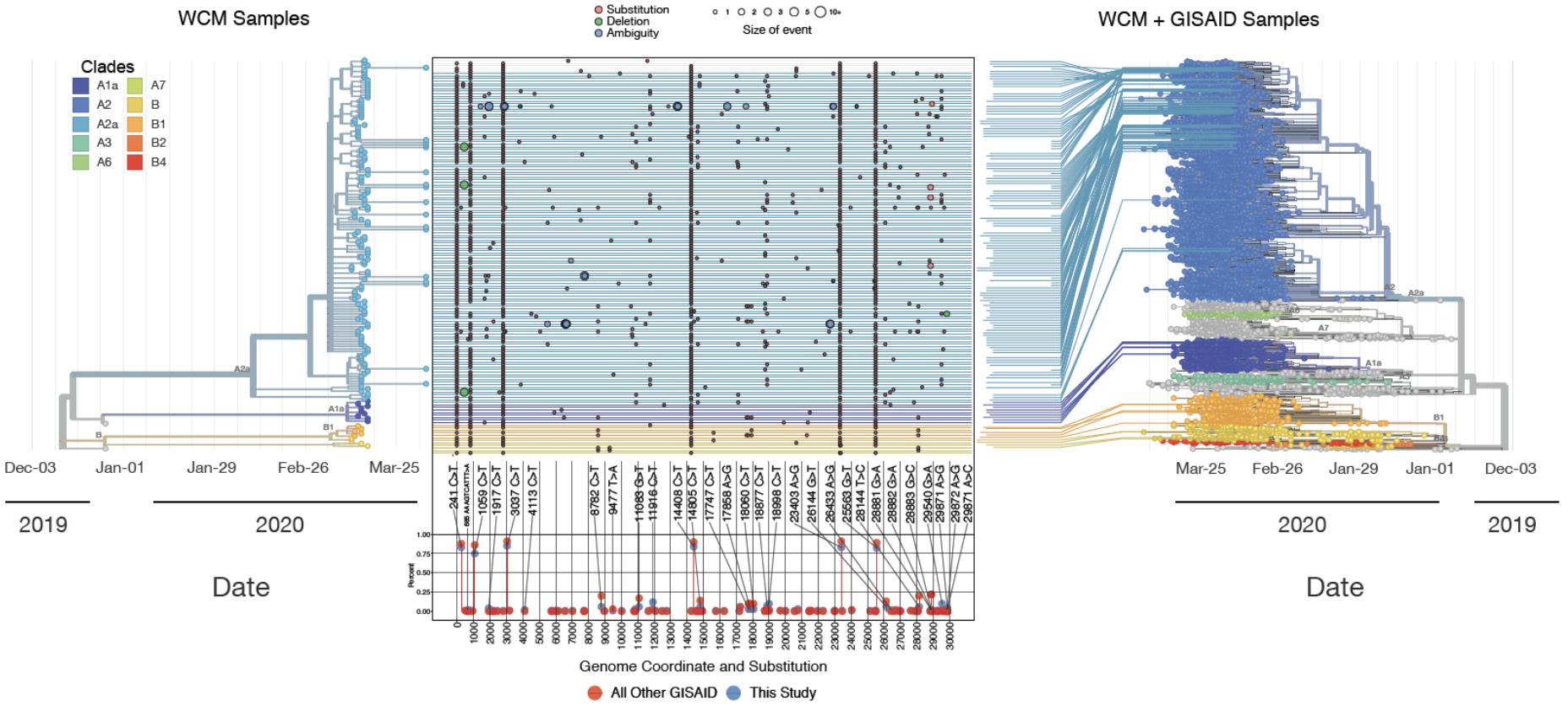
3) 36 Controls
- CP (Vero E6 Cells w/ SARS-CoV-2)
- Twist Synthetic RNA (2 strains)
- Negative Controls (TE buffer)

https://www.biorxiv.org/content/10.1101/2020.04.20.048066v5

Mostly human, bacterial, viral RNA in the NP swabs



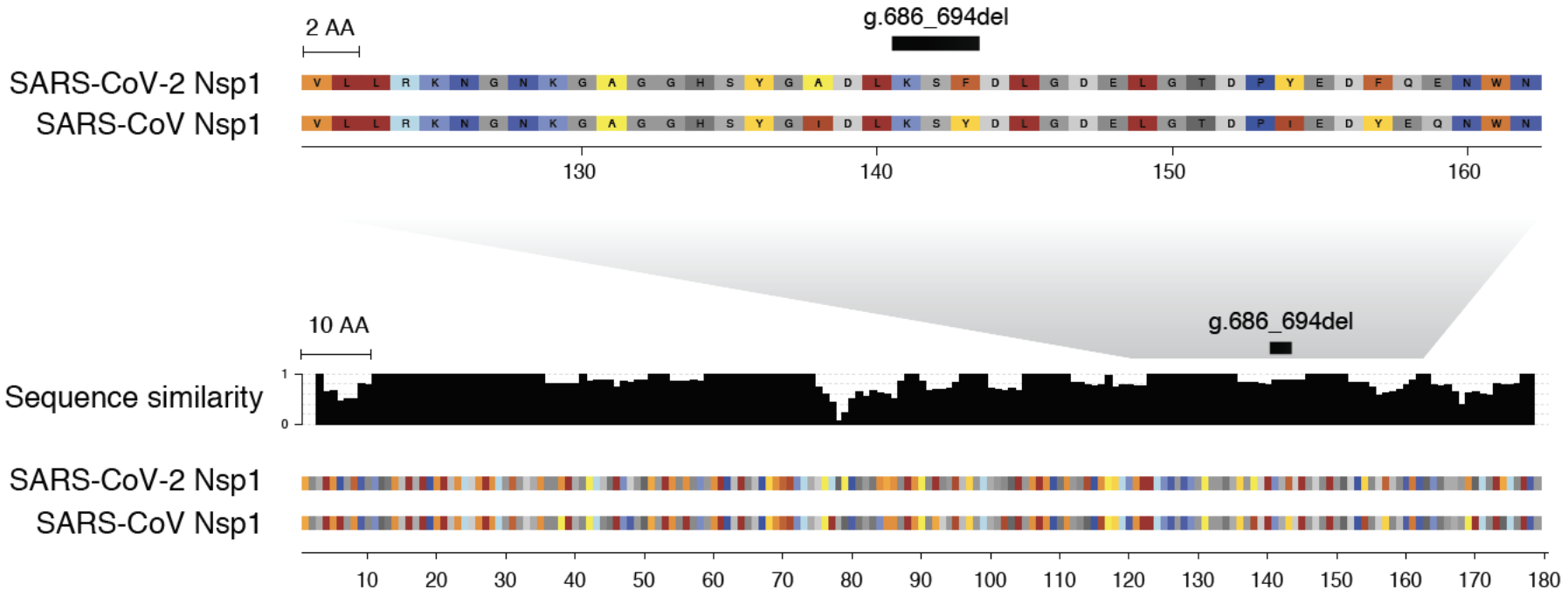
We can trace the evolutionary history of the virus



N=155 full covered and assembled genomes

https://nextstrain.org/ncov

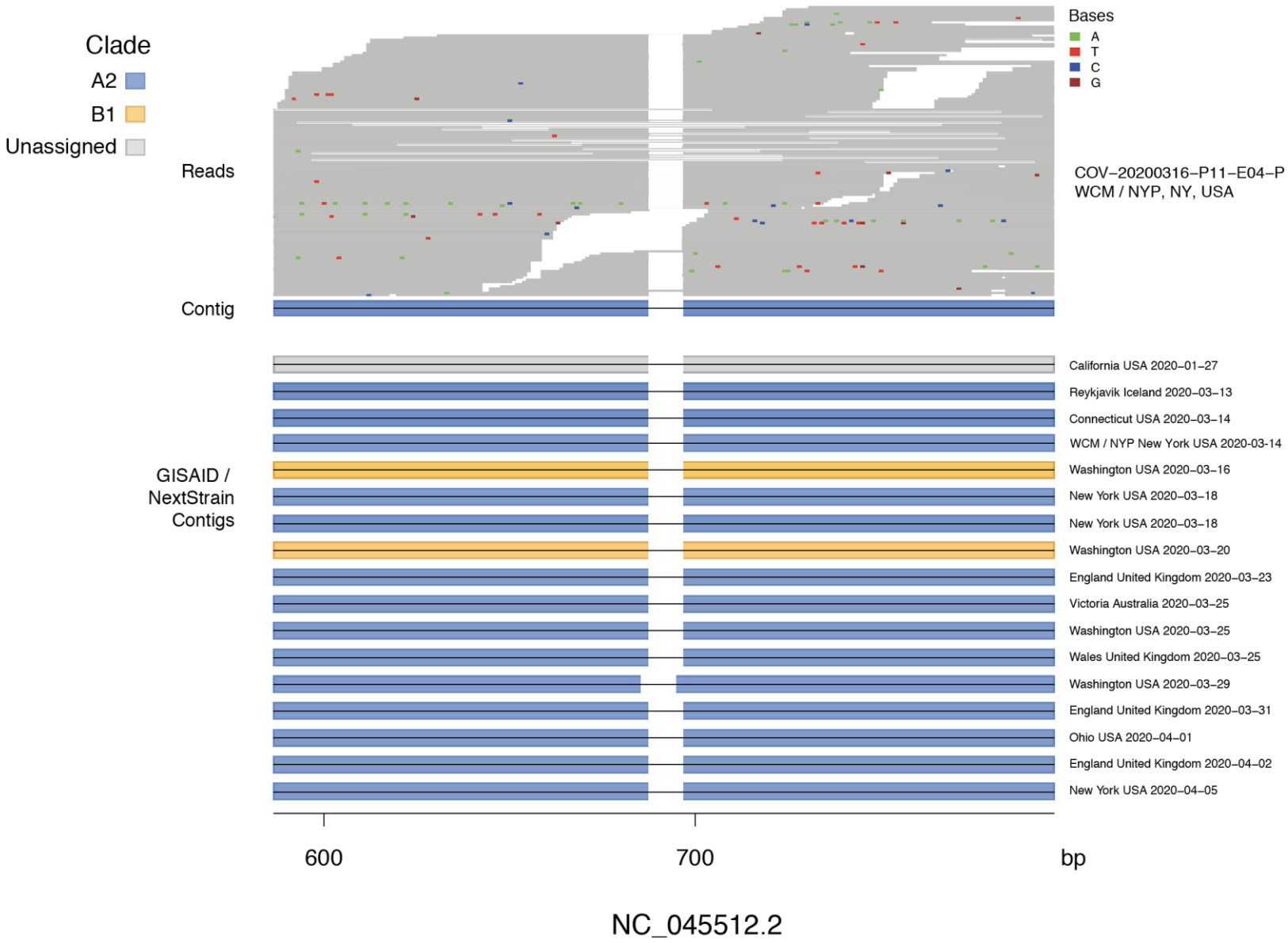
We see a unique deletion in three samples that removes a CoV-2 specific AA change relative to SARS CoV



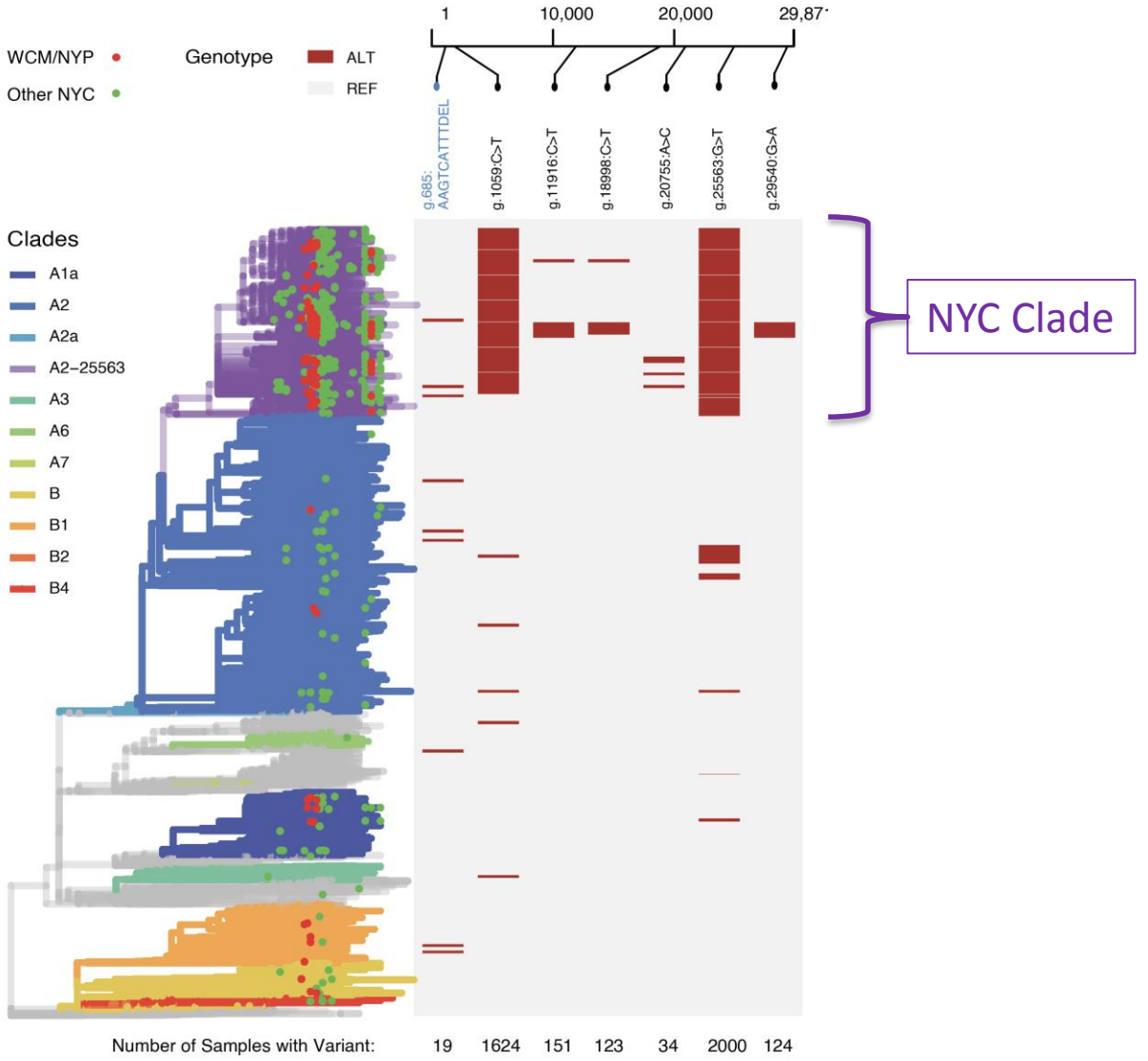
A conserved portion of the C-terminal region of NSP1, which has been linked to host chemokine dysregulation and translational inhibition in SARS-CoV.

Narayanan K, Ramirez SI, Lokugamage KG, Makino S. Coronavirus nonstructural protein 1: Common and distinct functions in the regulation of host and viral gene expression. *Virus Res.* 2015 Apr 16;202:89-100.

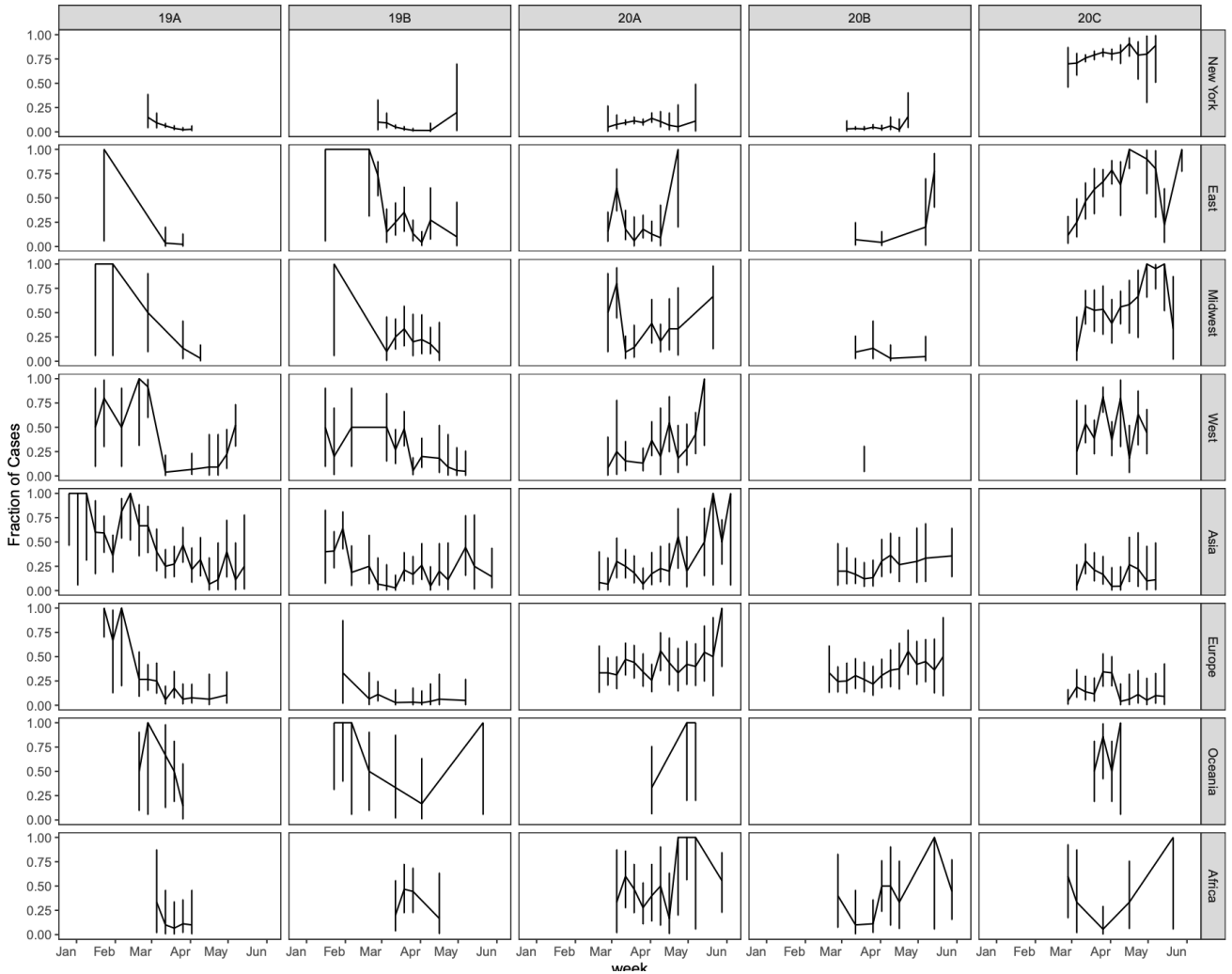
We aren’t the only ones



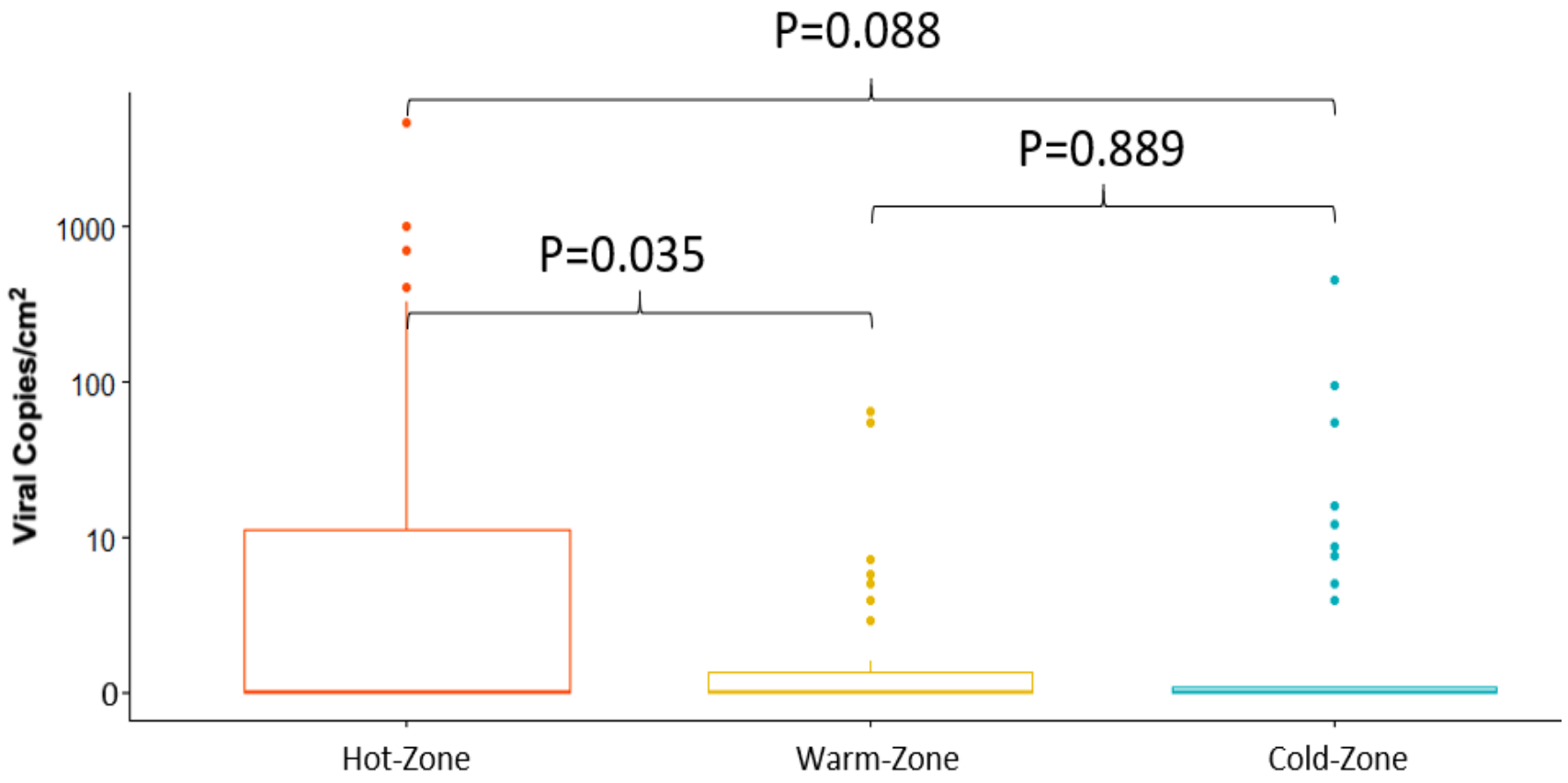
Our “NYC clade” also appears in NYU/Sinai strains



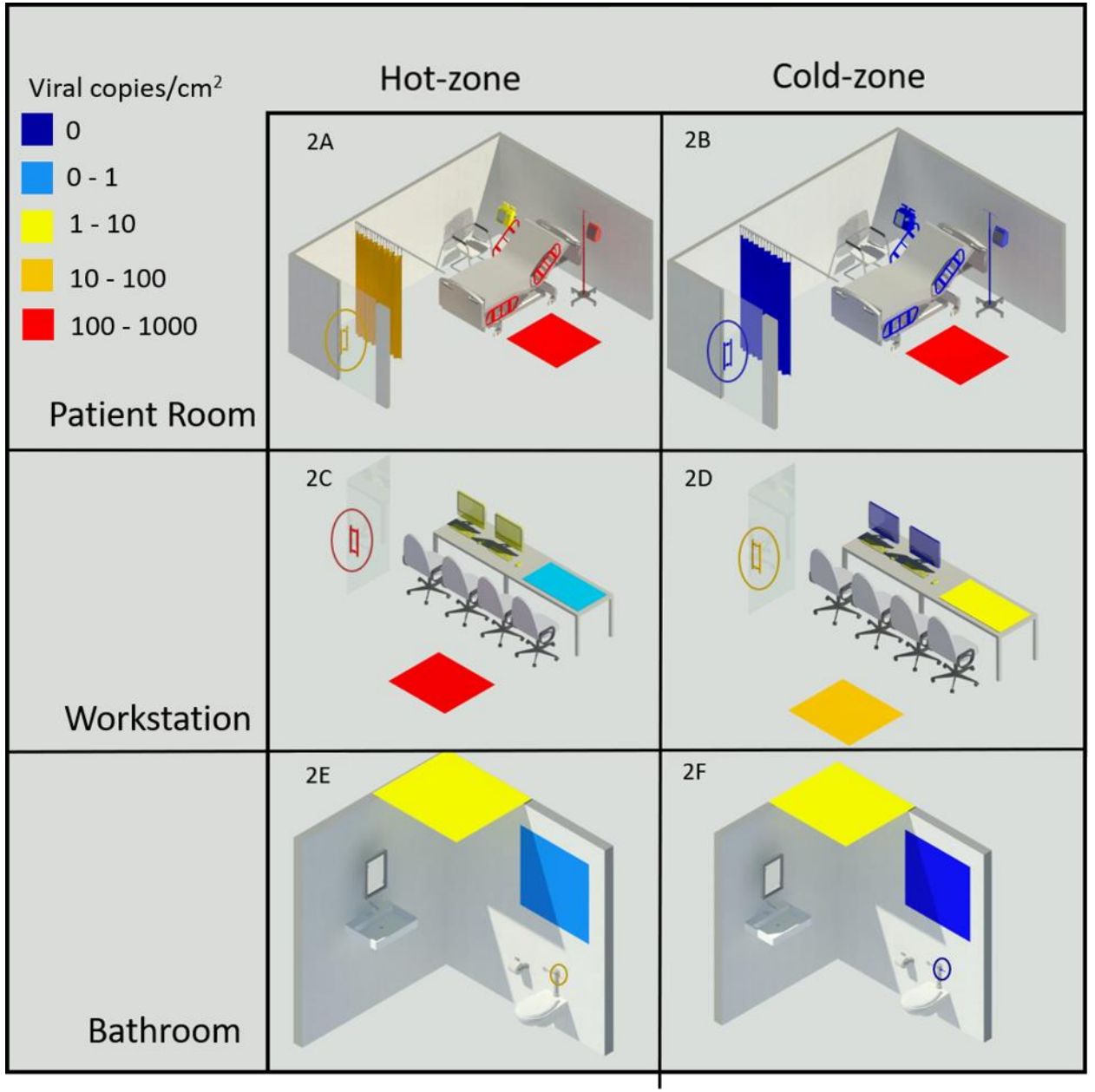
This NYC-enriched strain is highly dynamic around the world



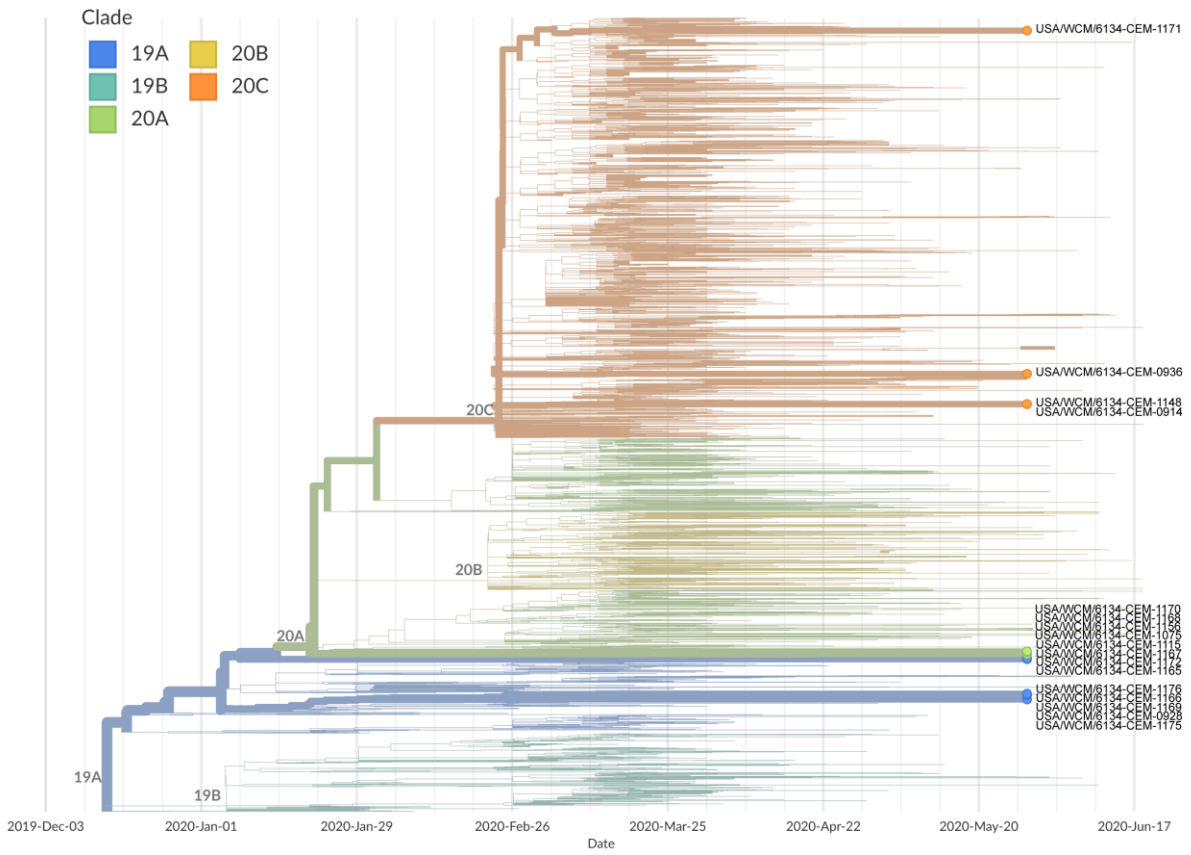
Hospital Surfaces with SARS-CoV-2?

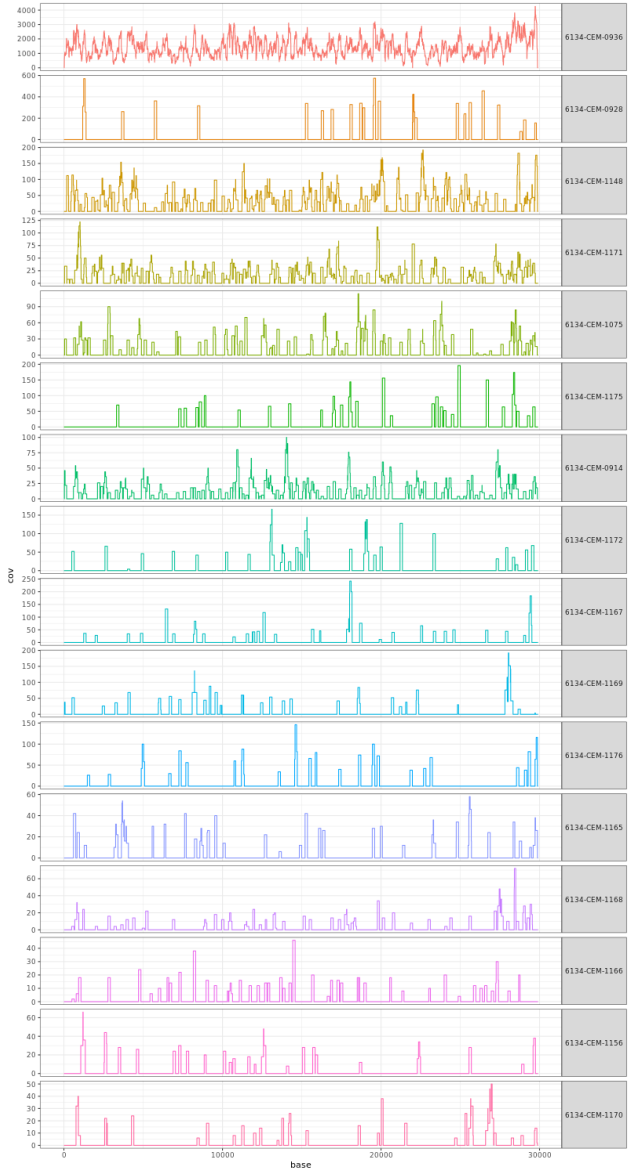


Hospital Surfaces Distribution



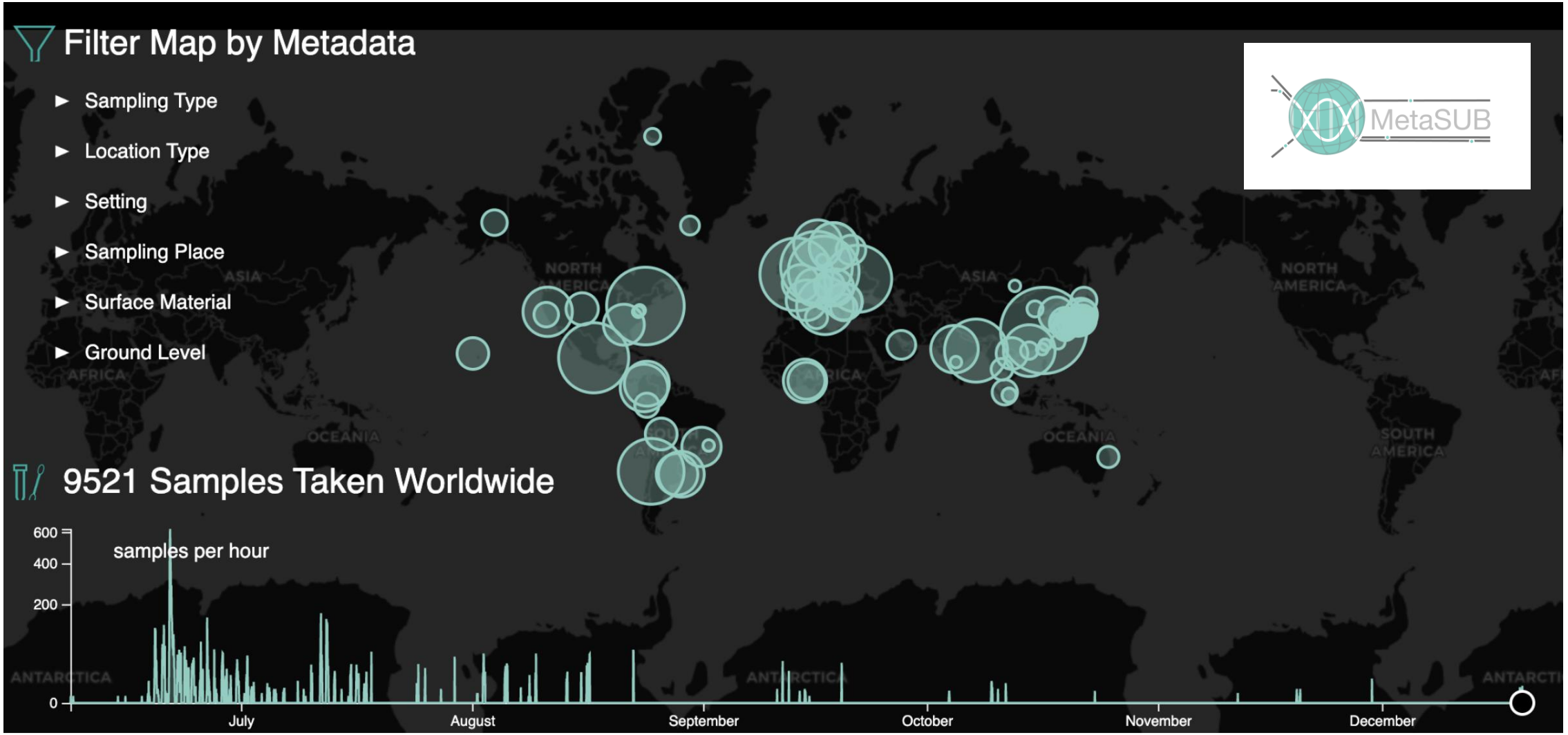
The hospital walls tell a story too; genome coverage and phylogenies





COVID and MetaSUB Leverage a global network of scientists

The International MetaSUB Consortium started sampling on Monday,
March 16th in public surfaces and hospitals



http://metasub.org

SARS – CoV-2 RNA Virus Tracking 2020 with MetaSUB

Sampling Kit includes:

- 1 Isohelix Bucal Swab
- 1 TFS Barcoded Tube
- 200ul Zymo DNA/RNA Shield

Sampling Kits for Pilot Projects:

17 x 96 = 1,632

Projected Sampling Kits for RNA Virus Tracking 2020:

- 10,272

Sampling Locations:

- 59 Cities (12 hospitals)
- 25 Countries
- 6 Continents

Bioinformatics and Analysis:

- 327 members from universities and industry

Preliminary list of RNA-based subway passengers:

TaxID	Taxon	TaxonName	rank	avg
0	x__unclassifi	unclassified	U	32062199
9606	d__Eukaryot	Homo sapiens	S	11329363
56947	d__Viruses|f	Choristoneura fumiferana granulovirus	S	5421.1233
12242	d__Viruses|f	Tobacco mosaic virus	S	3466.8062
11320	d__Viruses|p	Influenza A virus	S	3287.9371
162145	d__Viruses|p	Human metapneumovirus	S	2527.9019
1392231	d__Viruses|c	Streptococcus phage 20617	S	1788.0316
1969841	d__Viruses|c	Proteus phage VB_PmiS-Isfahan	S	1616.7909
1188792	d__Viruses|f	Phaseolus vulgaris alphaendornavirus 1	S	1095.1807
1414655	d__Viruses|p	Pepper chlorotic spot virus	S	552.81272
425279	d__Viruses|f	Rehmannia mosaic virus	S	422.59027
1980484	d__Viruses|p	Oxbow orthohantavirus	S	394.41437
2560751	d__Viruses|c	Serratia virus BF	S	384.72824
1979161	d__Viruses|p	Human rubulavirus 4	S	352.10111
929814	d__Viruses|c	Salmonella phage RE-2010	S	316.31429
129951	d__Viruses|f	Human mastadenovirus C	S	315.18038
2169967	d__Viruses|c	Escherichia virus DE3	S	309.16571
1141136	d__Viruses|c	Cronobacter phage vB_CsaM_GAP32	S	274.03831


Num. Reads
1e1
1e3
1e5
1e7
Baltimore, MD
Berlin
Daegu
Frederick, MD
Hong Kong
Krakow
Kusatsu
London
NYC
Sao Paulo
Seattle
Seattle, WA
Seoul
Silverspring
Tel Aviv
Tubingen

NATIONAL GEOGRAPHIC

ANIMALS | CORONAVIRUS COVERAGE

Tiger tests positive for coronavirus at Bronx Zoo, first known case in the world

The big cat is the first known case of a non-domesticated animal with COVID-19 symptoms—and is one of seven sick tigers at the New York zoo.

A Malayan tiger at the Bronx Zoo, photographed in 2017. In a first, one of the zoo's Malayan tigers, Nadia, has tested positive for the virus that causes COVID-19. Six other big cats are also showing symptoms of the illness.

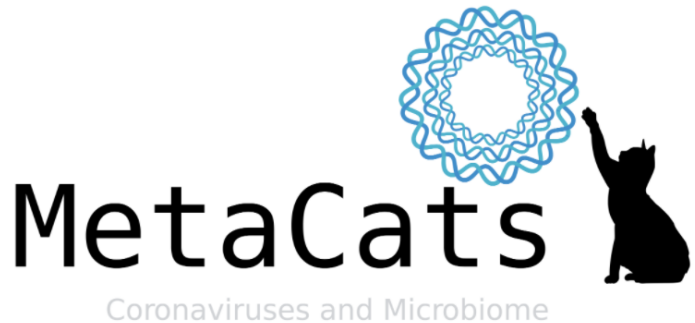
PHOTOGRAPH BY ANDREW LICHTENSTEIN, CORBIS VIA GETTY IMAGES

https://www.nationalgeographic.com/animals/2020/04/tiger-coronavirus-covid19-positive-test-bronx-zoo/#close

INTRODUCTION

MetaCats: Domestic Cats Coronaviruses & Microbiome

> The project, launched in 2020, aims at investigating the prevalence of SARS-CoV-2 and other coronaviruses in domestic cats, along with their microbial communities or microbiomes.
>
> While the COVID-19 coronavirus pandemic produced by the virus SARS-CoV-2 has been well studied to address the effect on human health, little is known about the effect that the virus might have on domestic animals, especially in cats.



BACKGROUND

Pet ownership has been pointed to as an important factor in determining the human microbiome, from skin to gut.

Recent studies suggest that pets can help to the development of a healthy immune system, while others point that they could act as potential carries of pathogens.

http://metasub.org/metacats/

STOOL SAMPLE

MOUTH SAMPLE

AIM: TO STUDY THE PREVALENCE OF **SARS-COV-2** AND OTHER CORONAVIRUSES IN *DOMESTIC CATS*, ALONG WITH THEIR MICROBIAL COMMUNITIES OR MICROBIOMES.

EASY

Registration is easy and will let you know if you and your cat qualift for the study

FREE

Participation is entirely free and all supplies will be shipped to your home for your use

SAFE

Our upmost priority is the safety of yourself and your cat. Sampling is safe and simple.

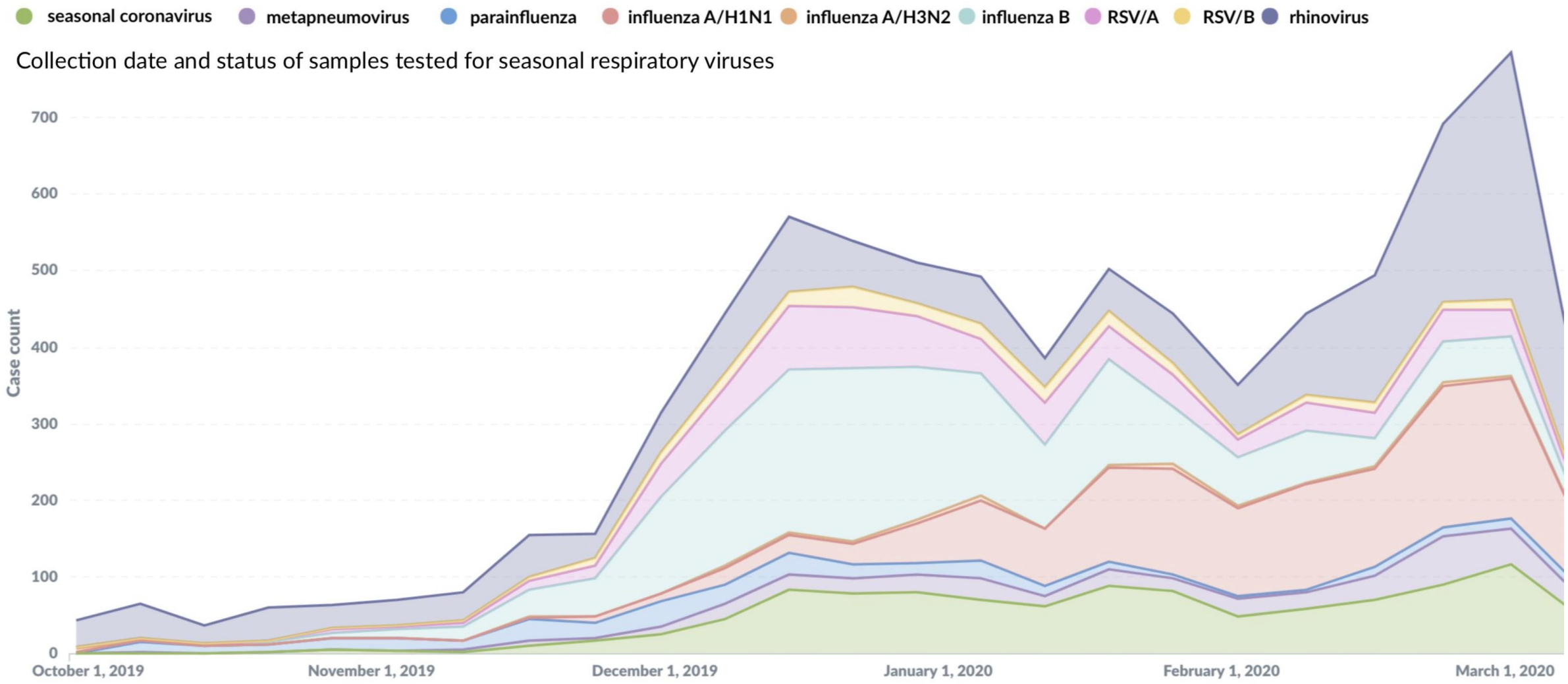
CITIZEN-SCIENCE

By registering for this project you are supporting citizen-science and contributing to important research.

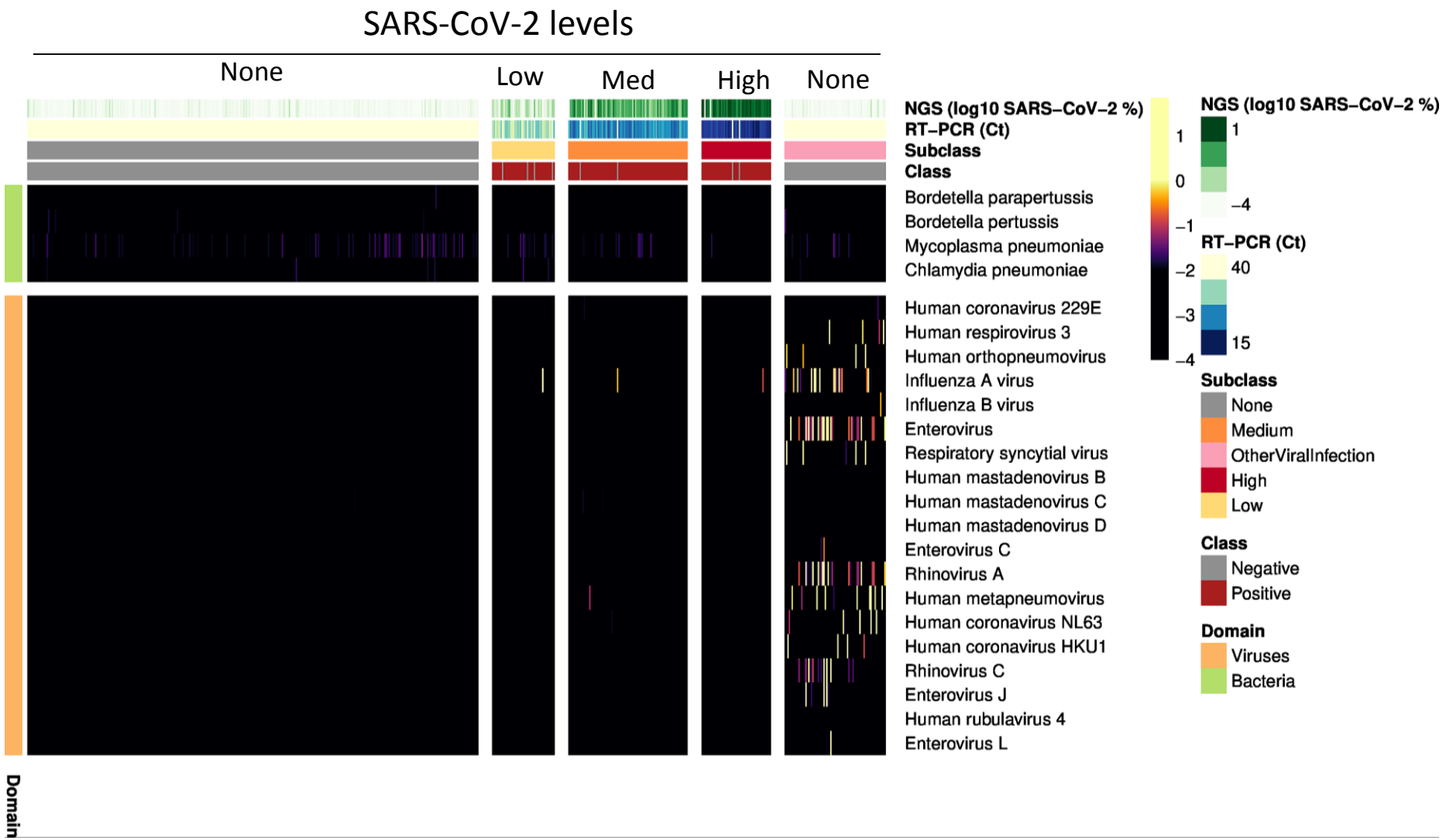
http://metasub.org/metacats/

Who else is there?

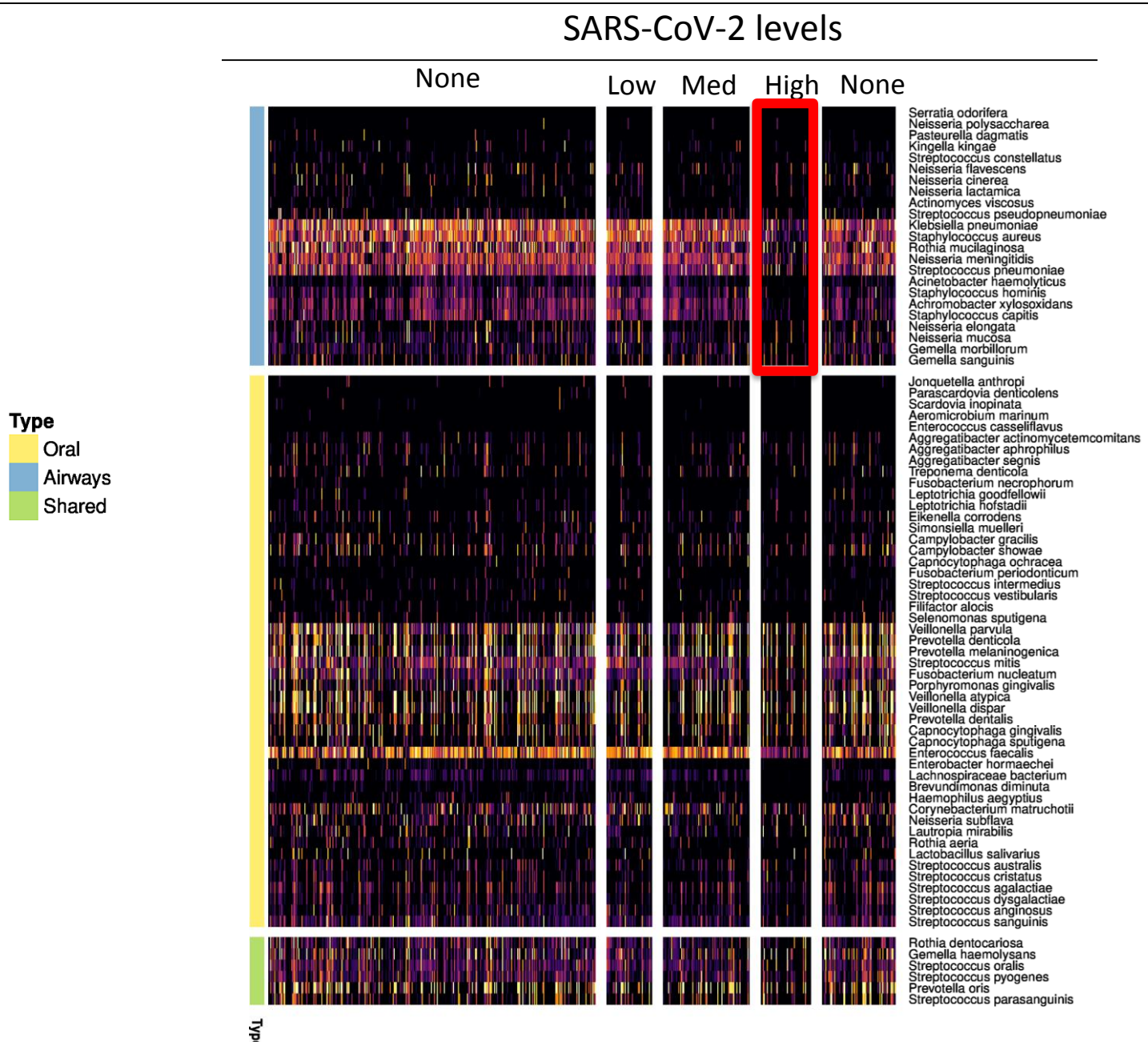
Data from @seattleflustudy and Trevor Bedford (@trvrb)



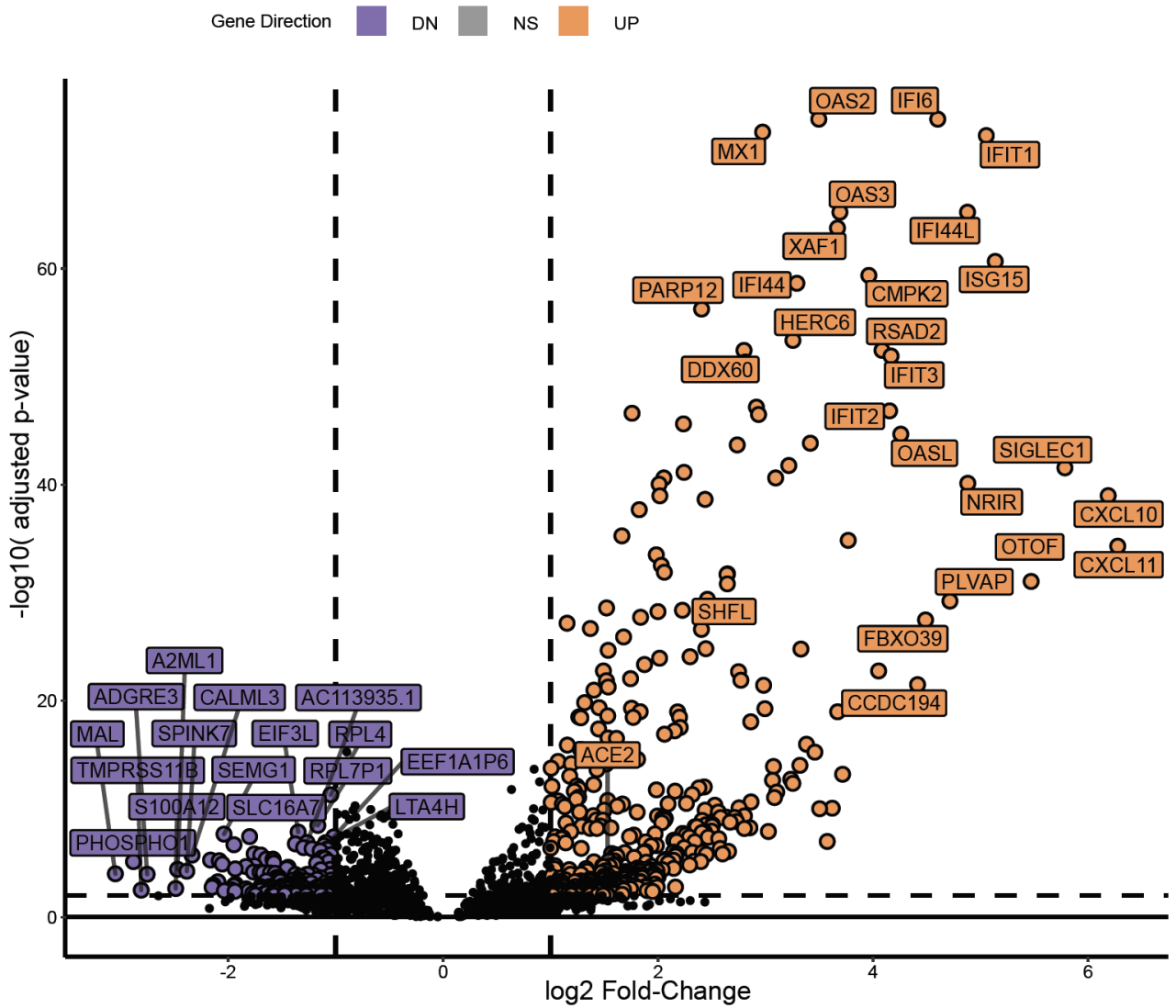
Co-infection is rare



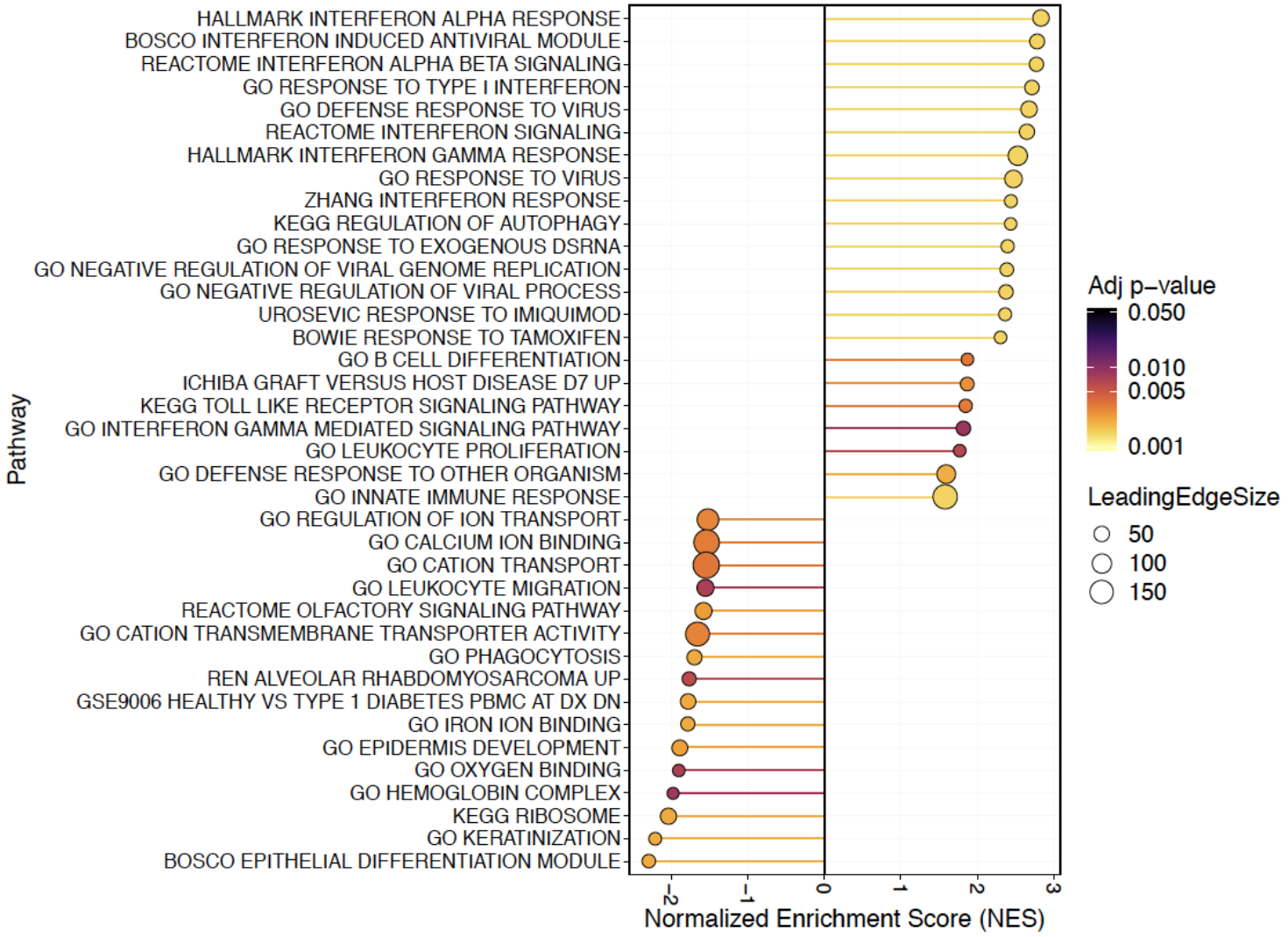
Microbiome Disruption in High Titer Patients



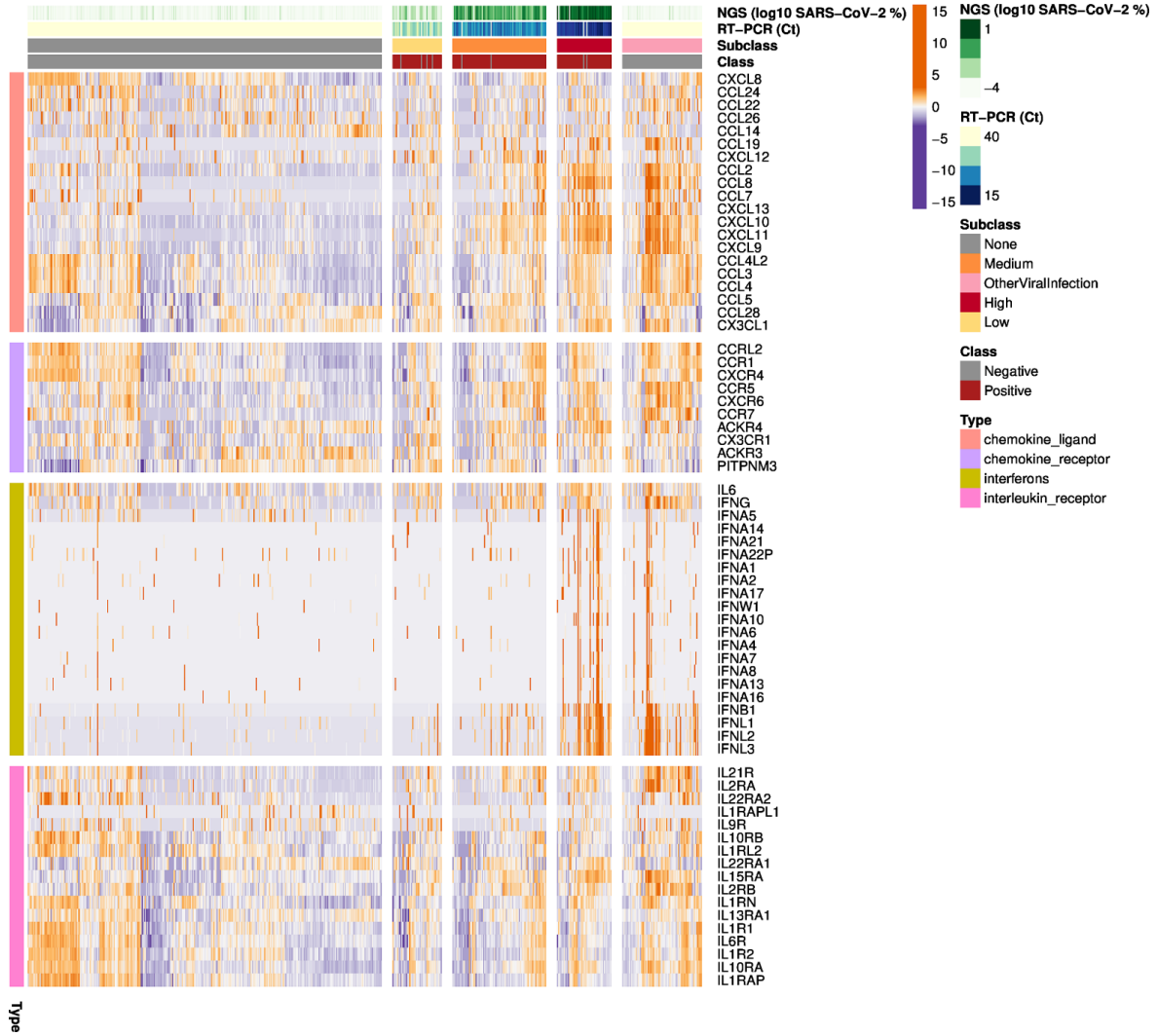
The human response:
Host DEGs (q<0.01, >1.5-fold)



Biological pathways feature familiar, and some new networks (including heme, olfaction)



Cytokine and interferon signaling especially pronounced for high titer



covidgenes.weill.cornell.edu

Weill Cornell Medicine COVID-19 Genes

- Information
- Single Gene Plot
- Multi Gene Heatmap
- Pathway Enrichment
- Settings

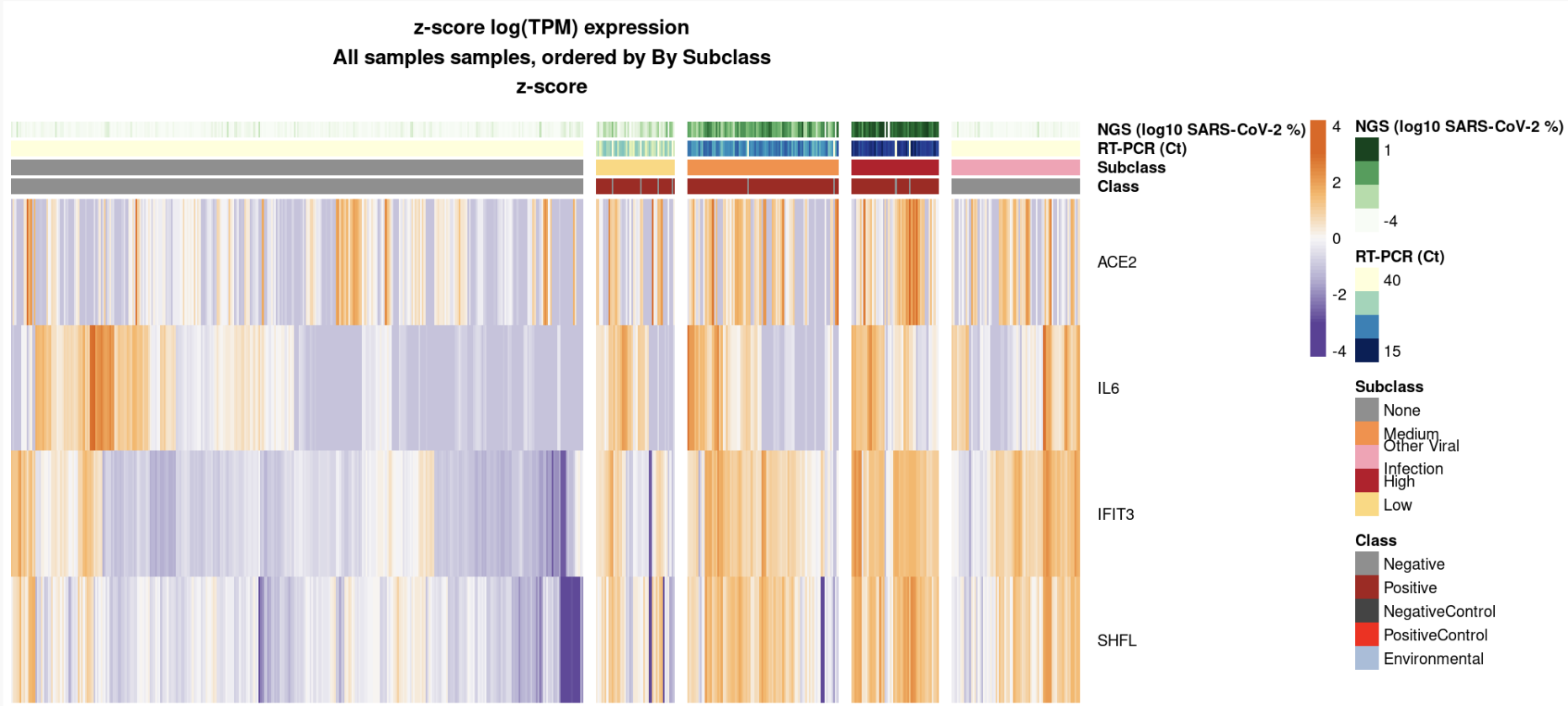
Enter/Paste gene symbols (comma, space, new line separated)

IFIT3 ACE2 SHFL IL6

Plot

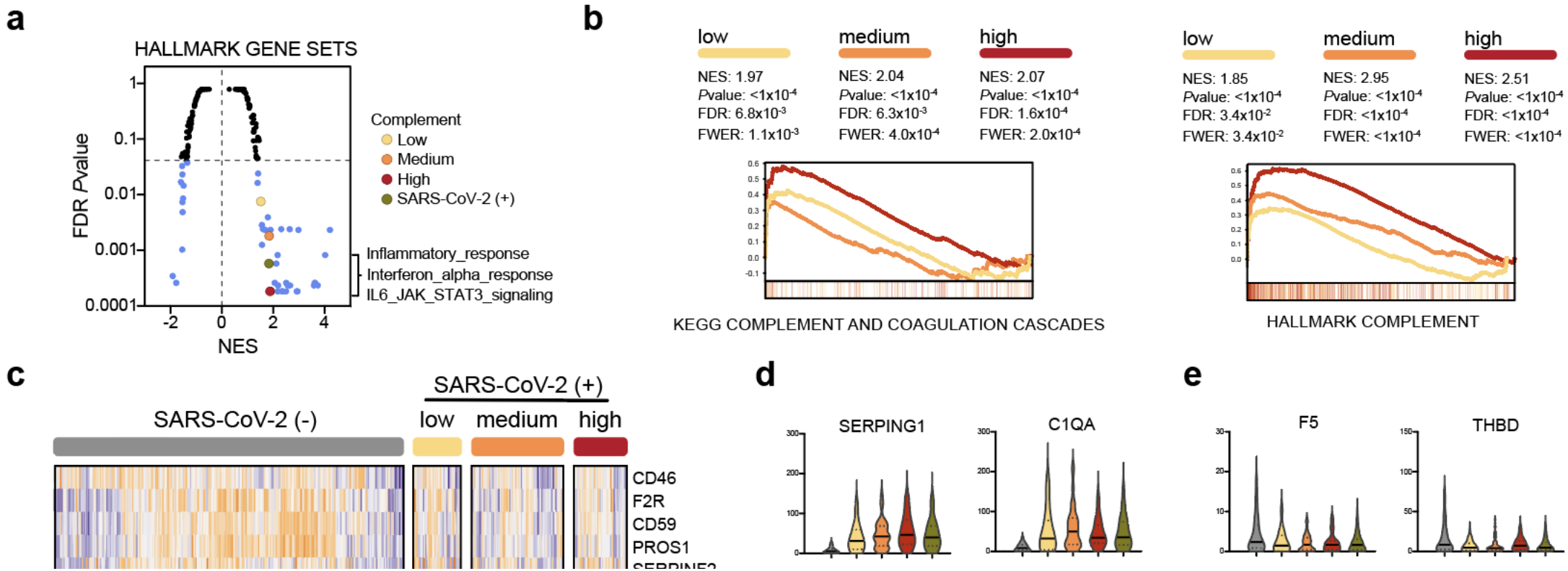
Multi gene heatmap

Heatmap



https://covidgenes.weill.cornell.edu/

Complement factor pathways disrupted as a function of viral load



Now online

nature medicine

Letter | Published: 03 August 2020

Immune complement and coagulation dysfunction in adverse outcomes of SARS-CoV-2 infection

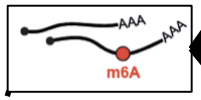
Vijendra Ramlall, Phyllis M. Thangaraj, Cem Meydan, Jonathan Foox, Daniel Butler, Jacob Kim, Ben May, Jessica K. De Freitas, Benjamin S. Glicksberg, Christopher E. Mason, Nicholas P. Tatonetti ✉ & Sagi D. Shapira ✉

Nature Medicine (2020) | Cite this article

66 Altmetric | Metrics

https://www.nature.com/articles/s41591-020-1021-2

Lysis / RNA extraction



Aliquot RNA

Frozen aliquot for banking

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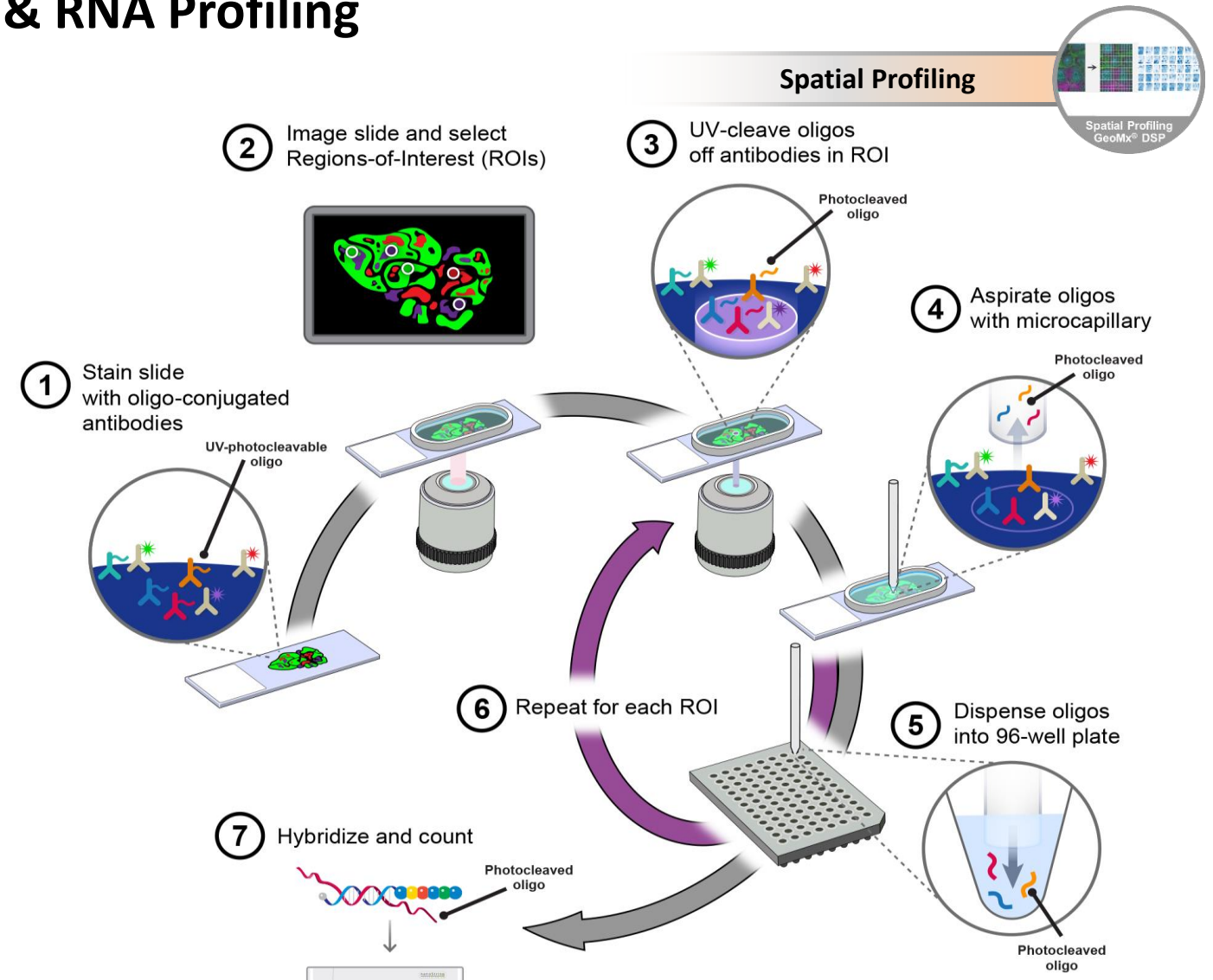
1ng
10ng
200ng
200ng
500ng
100ng

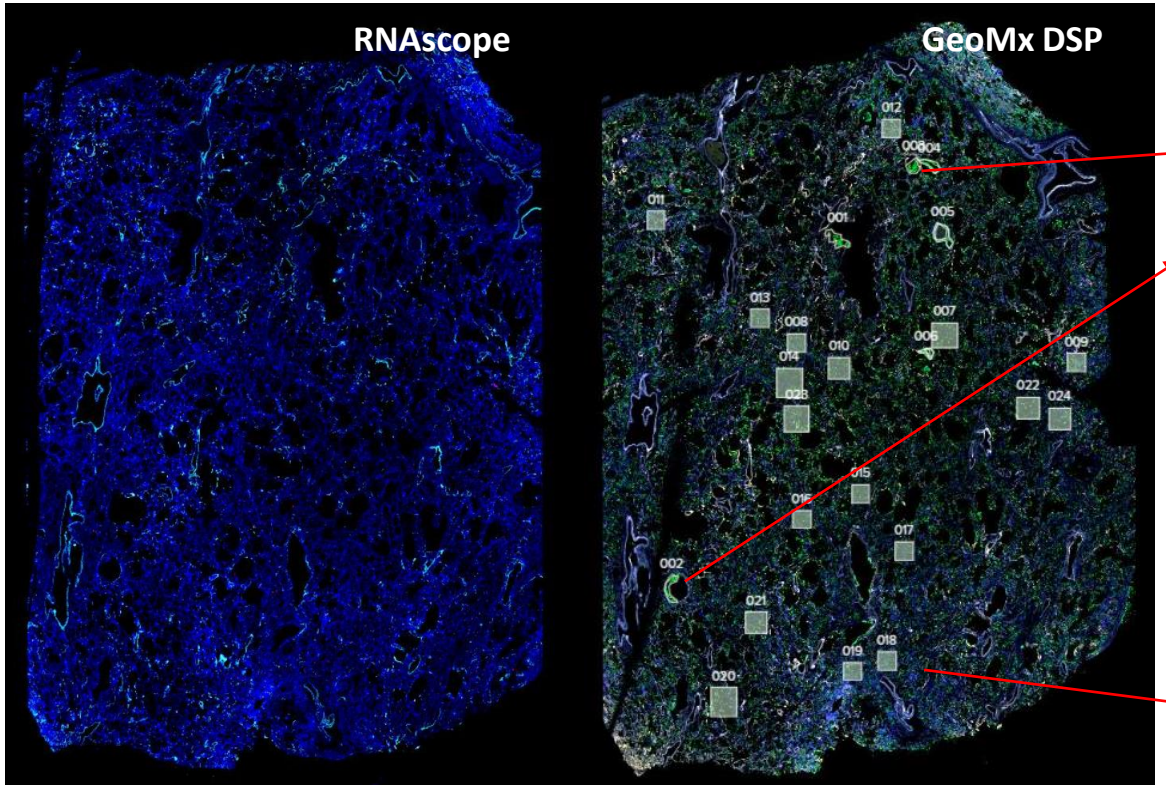
GeoMx DSP: Spatial, High-Plex Protein & RNA Profiling

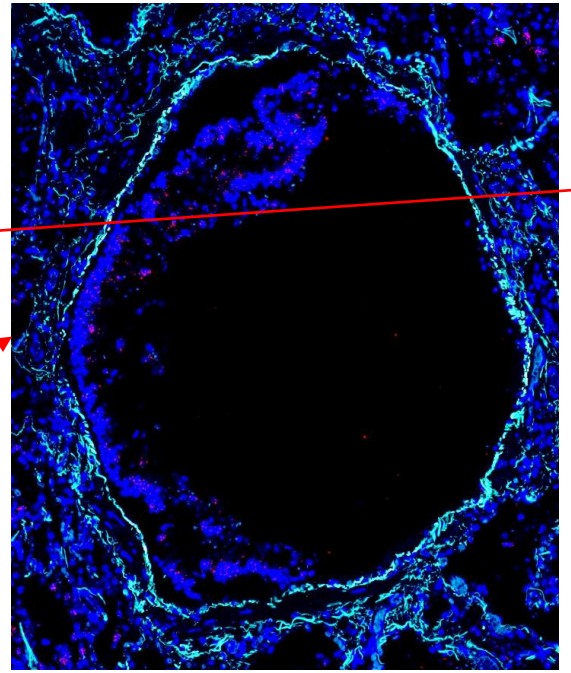
GeoMx™ Digital Spatial Profiler

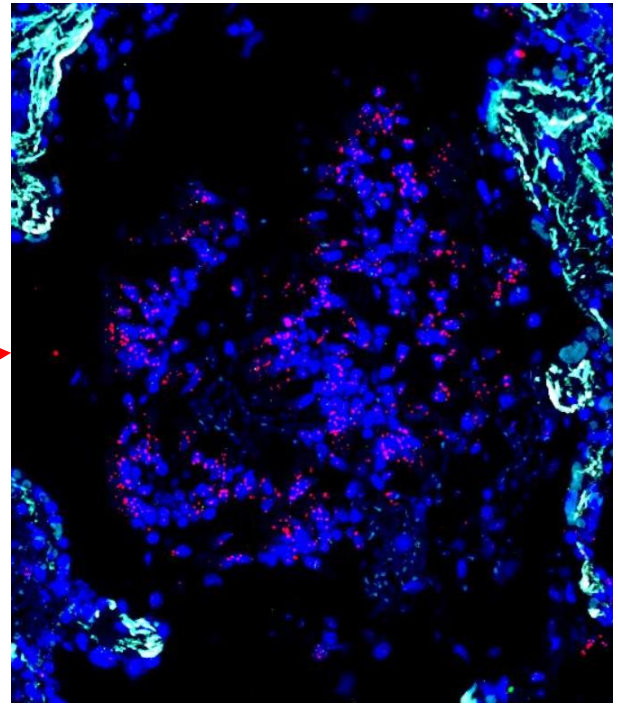


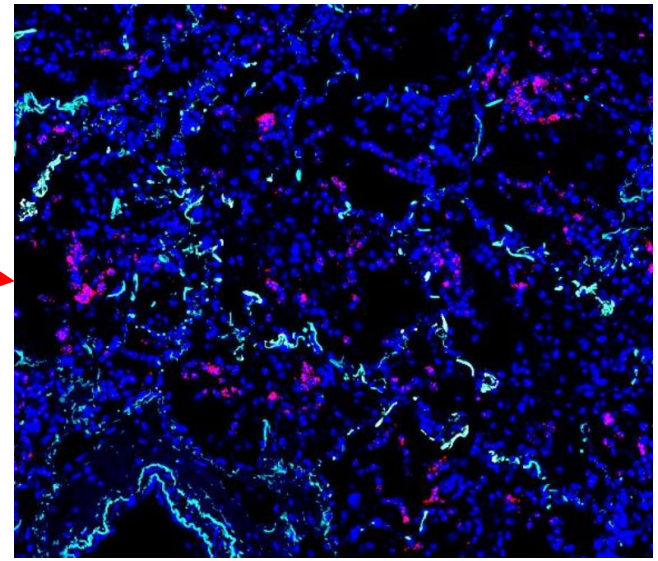
Illumina NGS  --or-- NanoString nCounter 

Covid21_ High TMPRSS2 in both large airway and Alveoli







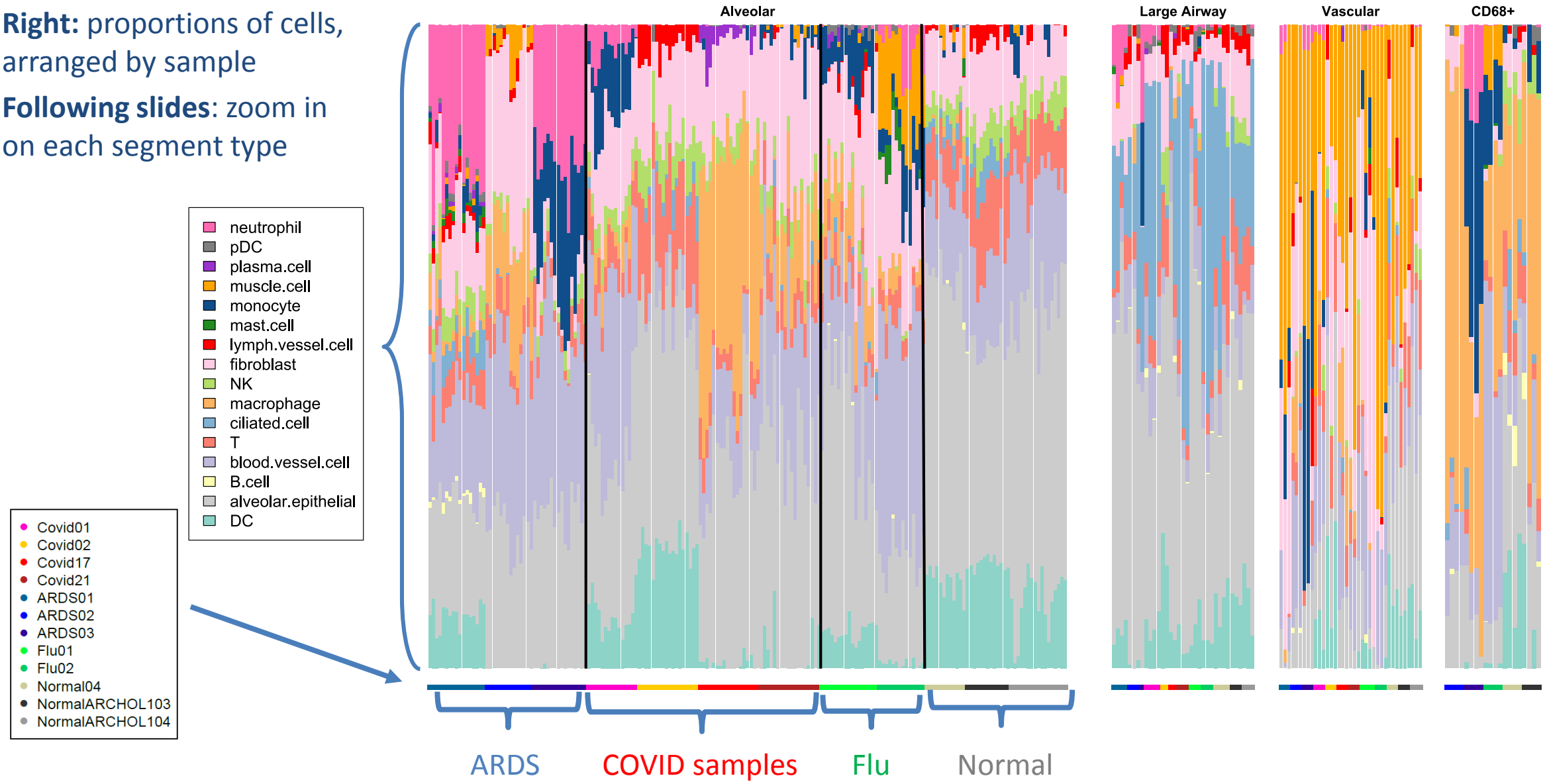


DNA
V-nCoV2019-S
Hs-ACE2
Hs-TMPRSS2

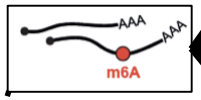
Cell abundances of all segments

Right: proportions of cells, arranged by sample

Following slides: zoom in on each segment type



Lysis / RNA extraction



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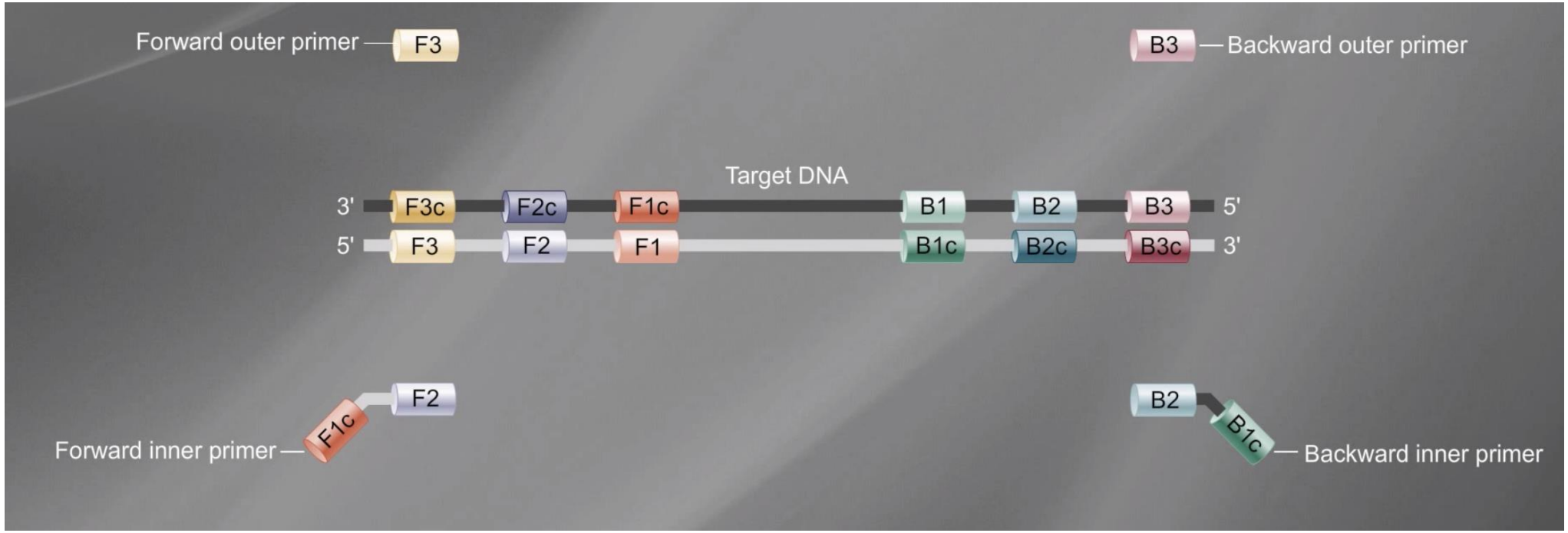
1ng
10ng
200ng
200ng
500ng
100ng

Loop-Mediated Isothermal Amplification (LAMP)



A fast (30-40min) and inexpensive (<$5) RT-PCR assay for targeted amplification of COVID-19 genes:

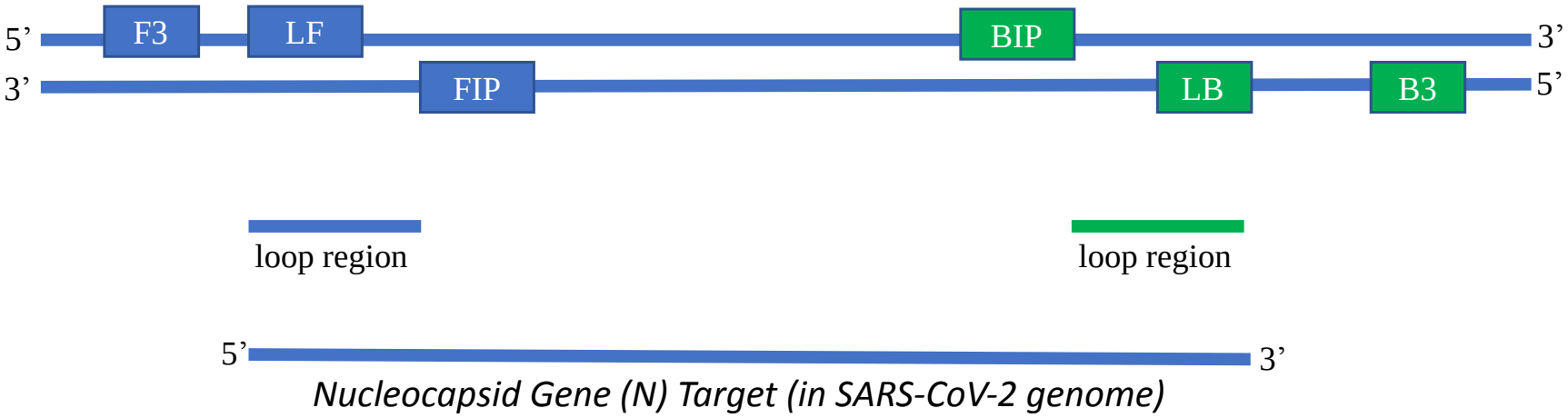
RNA→cDNA→dsDNA
with Bst strand-displacing polymerase

Loop Mediated Isothermal Amplification (LAMP) from New England BioLabs: https://www.youtube.com/watch?v=L5zi2P4lggw | https://www.neb.com/protocols/2014/06/17/loop-mediated-isothermal-amplification-lamp

COVID-19 Primers for Loop-Mediated Isothermal Amplification

(LAMP)

Loop-mediated Isothermal Amplification (LAMP) Primer Design



COVID-19 Primers: Gene N (Nucleocapsid gene)

GeneN-F3 TGGCTACTACCGAAGAGCT

GeneN-B3 TGCAGCATTGTTAGCAGGAT

GeneN-FIP TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG

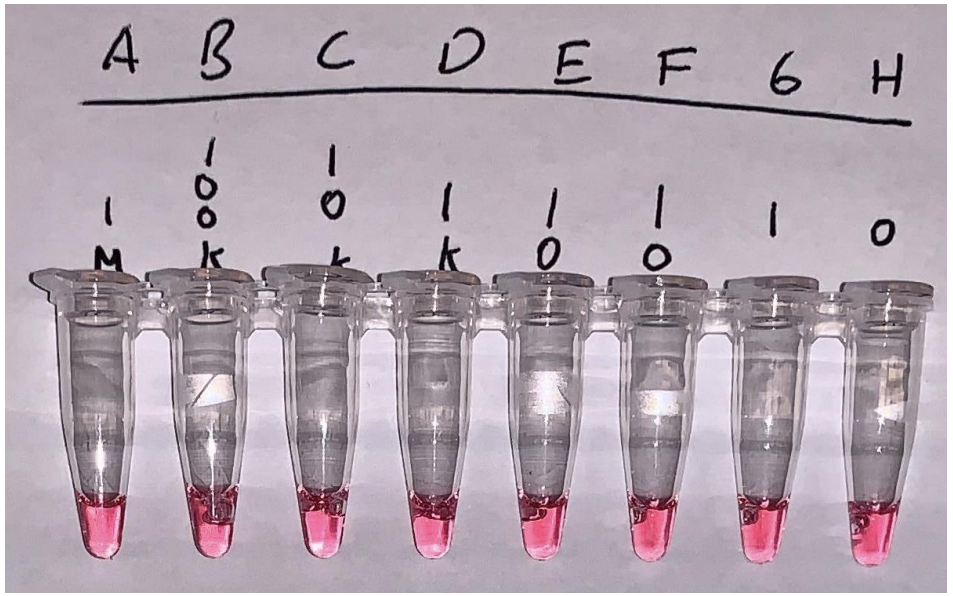
GeneN-BIP AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT

GeneN-LF GGACTGAGATCTTTCATTTTACCGT

GeneN-LB ACTGAGGGAGCCTTGAATACA

The LAMP Reaction Starts All Pink

Reaction time:
0 minutes

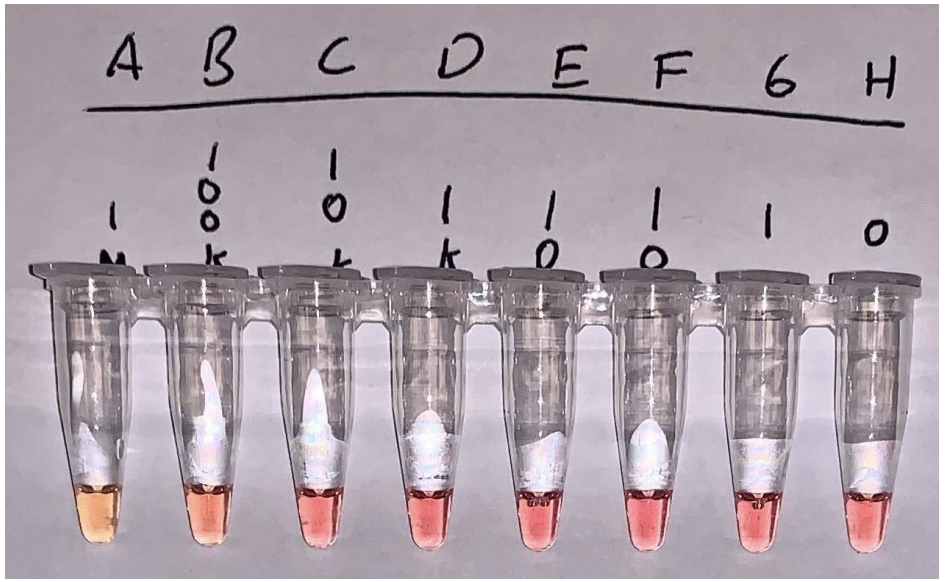


10^6 10^5 10^4 10^3 10^2 10^1 10^0 0

Copies of COVID-19 RNA

Then begins to show a colorimetric shift

Reaction time:
15 minutes

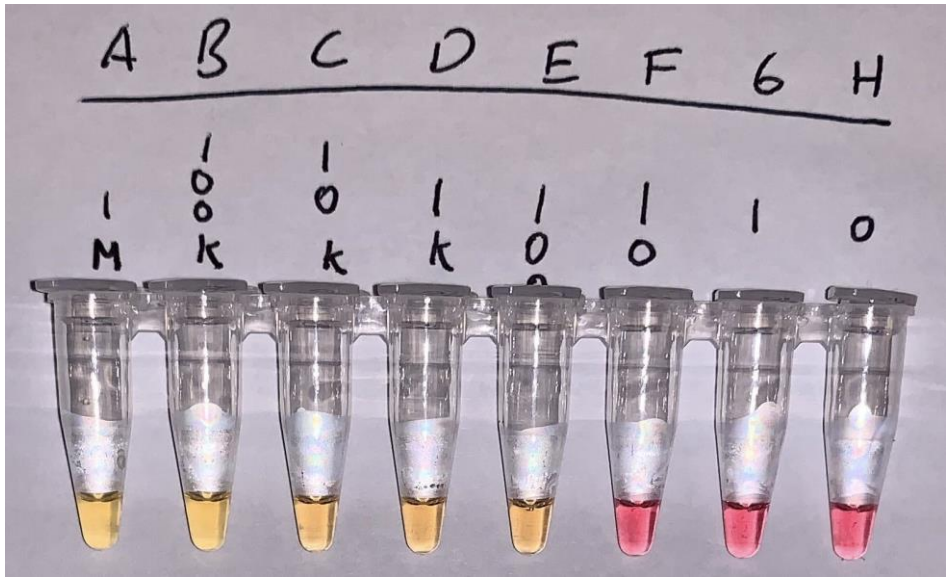


10^6 10^5 10^4 10^3 10^2 10^1 10^0 0

Copies of COVID-19 RNA

LAMP reaction is complete at 30 minutes

Reaction time:
30 minutes

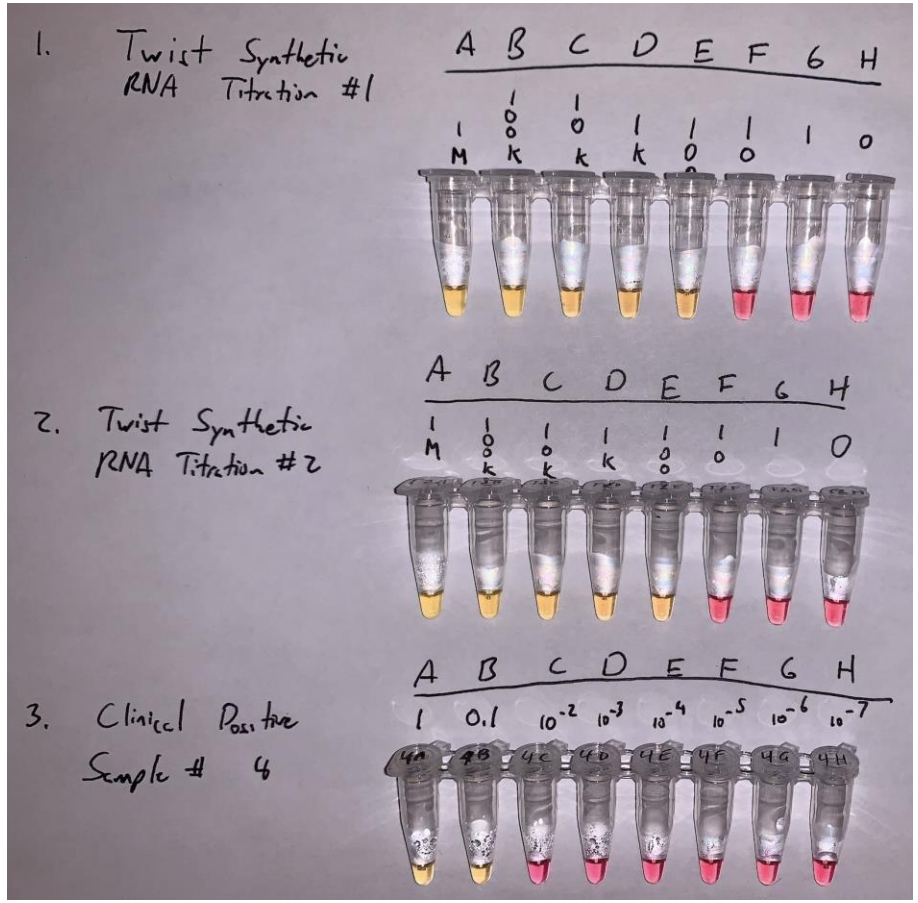


Reproducible across replicates and validated on clinical positives

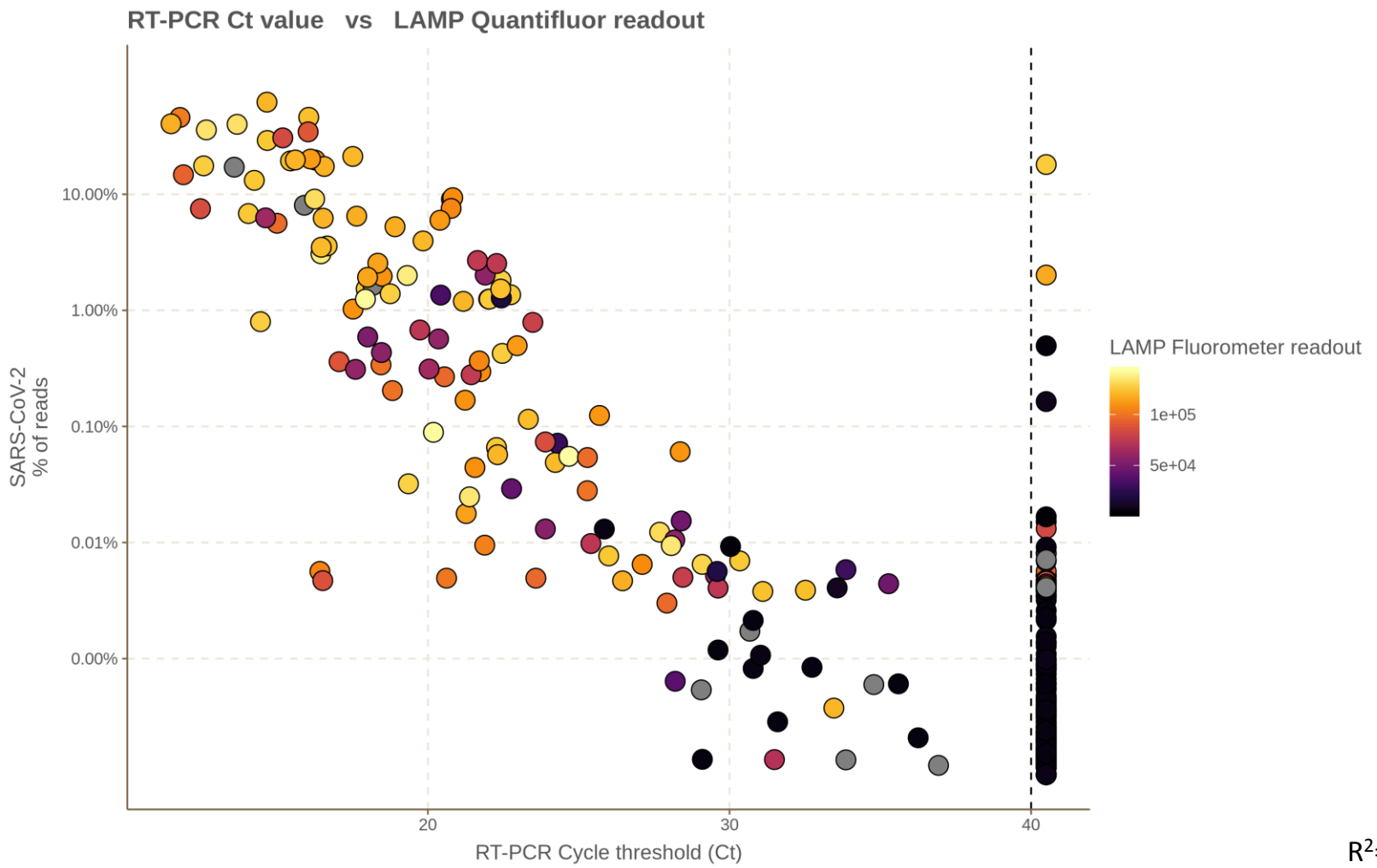
Twist Synthetic RNA #1
(MT007544.1)

Twist Synthetic RNA #2
(MN908947.3)

Clinical COVID-19+ Sample with Serial Log Dilution
(qRT-PCR+, <0.05ng/mL; below Qubit lower limit)

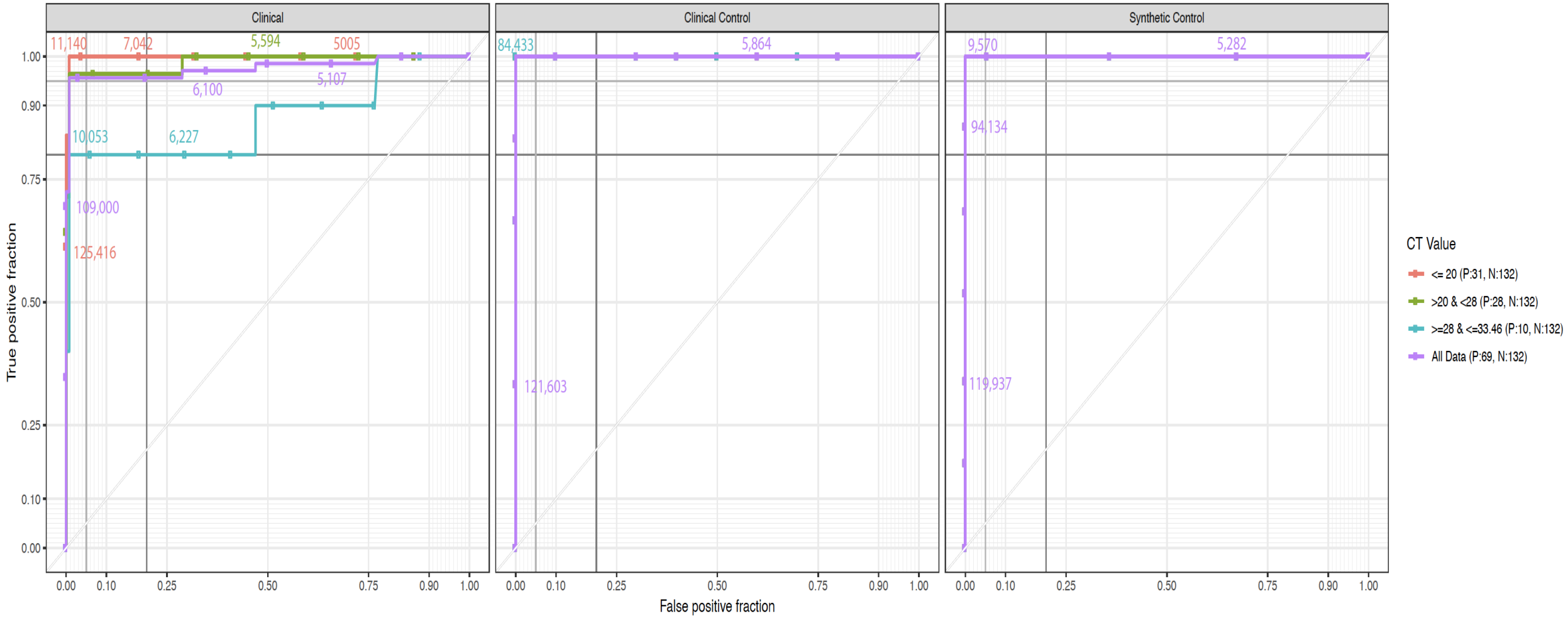


Overall high correlation with qRT-PCR and RNA-seq

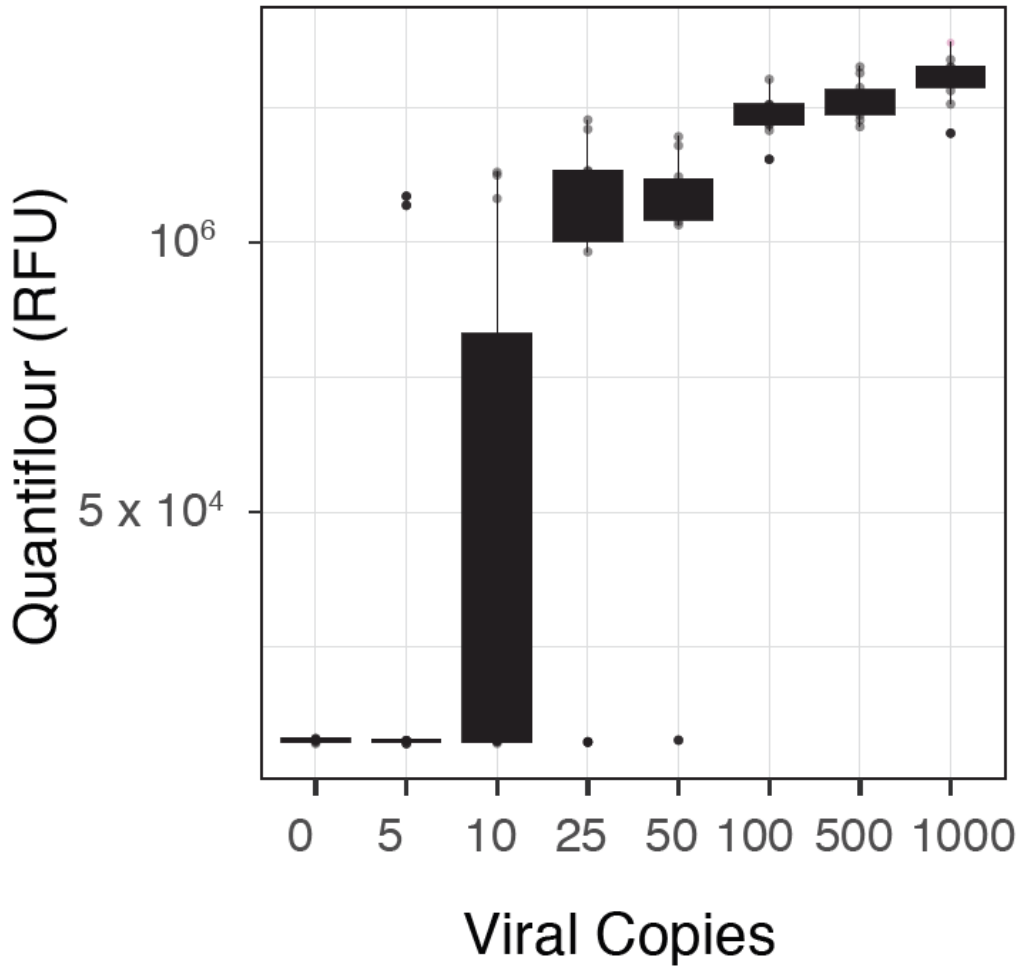


R^2=0.-78

96% Sensitivity and 99% Specificity



New, Dual SARS-CoV-2 Primers for LAMP (E and N genes)
in one reaction improves sensitivity 2-4 fold (LOD down to 5-25 copies vs. 10-100)



Colorimetric LAMP now FDA-approved in partnership with Color/Broad/Weill Cornell

FierceBiotech

Color gets green light for LAMP-based COVID-19 screening test

by Conor Hale | May 20, 2020 11:00am

To develop its tests for the pandemic, Color has been working with the Broad Institute of MIT and Harvard as well as Weill Cornell Medicine. The company is also publishing its LAMP **protocol** (PDF) for use by other laboratories.

https://www.fda.gov/media/138249/download

Also now a full kit:

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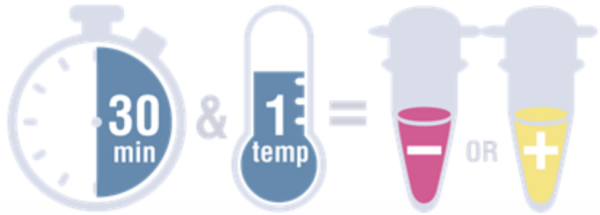
Home > PCR, qPCR & Amplification Technologies > Products > SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit

SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit

This product is intended for research purposes only.

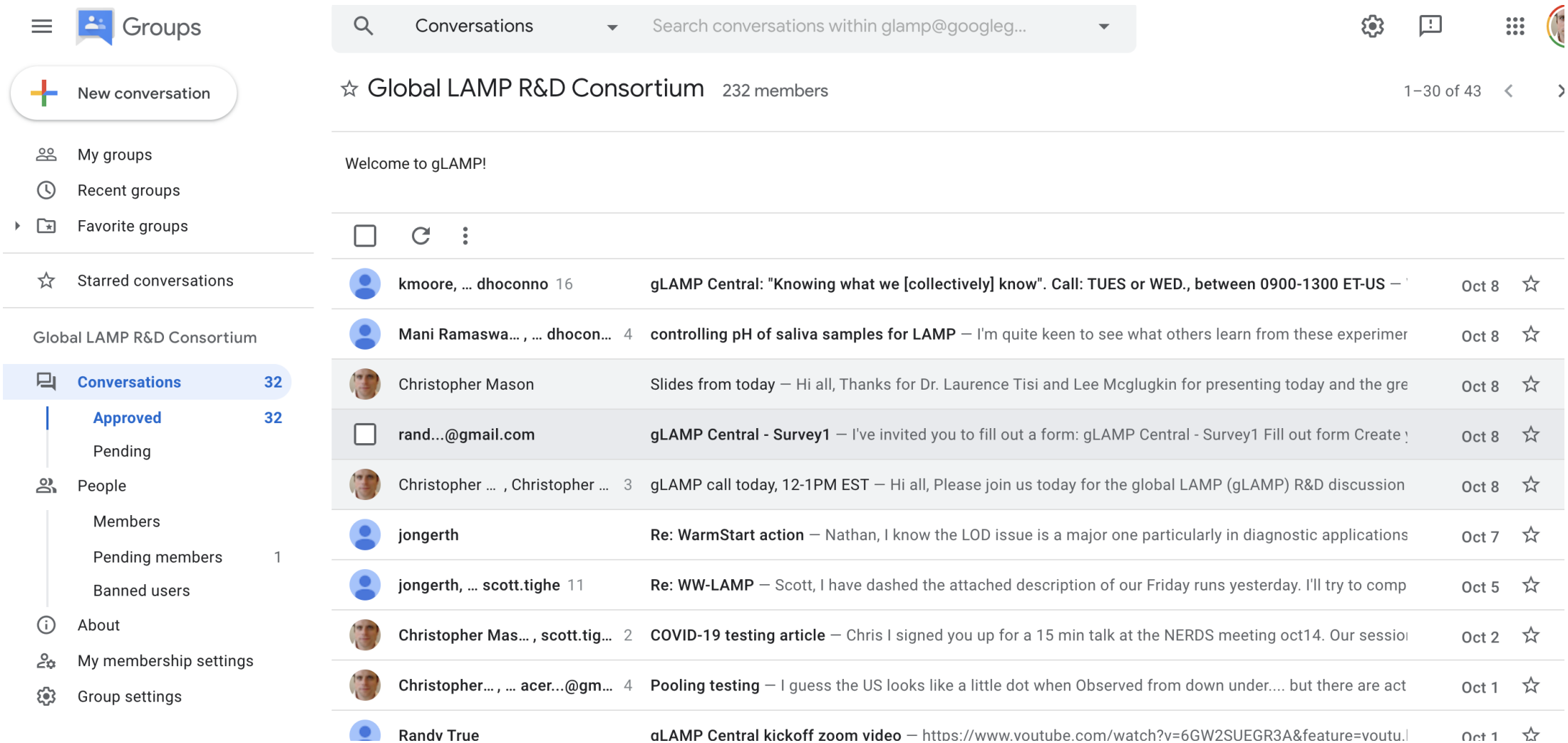
The SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit utilizes isothermal amplification for use in the analysis of SARS-CoV-2, the novel coronavirus that causes COVID-19.

- Colorimetric LAMP enables simple, visual detection (pink-to-yellow) of amplification of SARS-CoV-2 nucleic acid
- Set up reactions quickly and easily, using a simple heat source and unique WarmStart® technology
- Reduce risk of carryover contamination, with UDG and dUTP included in the master mix
- Assay targets N and E regions of the SARS-CoV-2 genome, for optimized sensitivity and specificity
- Bring confidence to your results using the provided controls
- Learn more about LAMP and other isothermal amplification methods
- Learn more about how NEB is supporting COVID-19 research



https://www.neb.com/products/e2019-sars-cov-2-rapid-colorimetric-lamp-assay-kit

And a global group!



https://groups.google.com/g/glamp/

Can we enable rapid testing in Cities with “Pop-Up Labs?”

Make it easy for ANYONE to get tested with
NYC as a model for others (SF, Seattle, Chicago).
About $15K/site set up and $1K/day reagents

1) Equipment:
- Staff/testers minimally trained in molecular biology;
- Small freezer or 4C for reagents $500-1000;
- 2 Basic laptops with barcode reader $2000;
- Heat blocks (96-well) - $400;
- Pipetteman (two per heatblock) ~$600 per two sets;
- Folding table - $75.

2) Consumables:
- Pipet tips - $300 per heat block;
- Gloves - $24/box;
- Waste bins - $100 ea;
- Tubes - $347/250;
- DNA/RNA-free Water- $50.

3) PPE:
- Personal Protection Equipment (masks, eye protection) - $500.

4) Decontamination supplies:
- Bleach and wipes - $200.

On-site swabbing at the Annual Association of Biomolecular Resource Facilities Meeting (2018)

Racine partnering on new, quicker COVID-19 test

Christina Lieffring 2 hrs ago 7

1 of 3

https://journaltimes.com/news/local/govt-and-politics/racine-partnering-on-new-quicker-covid-19-test/article_df721f98-9006-55f0-b8f1-a3e573d15cd5.html

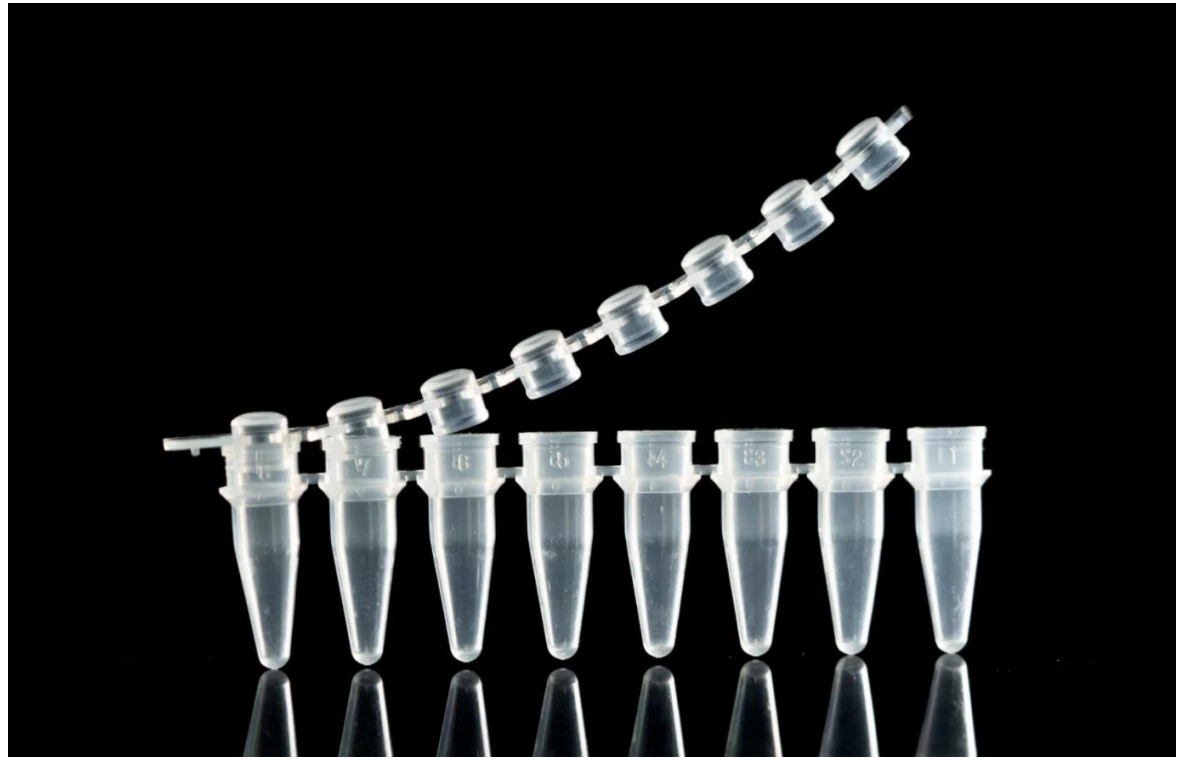
Racine City Hall On-site Testing


COMPLIAN
CENTER

GREGORY BARBER SCIENCE 07.23.2020 07:00 AM

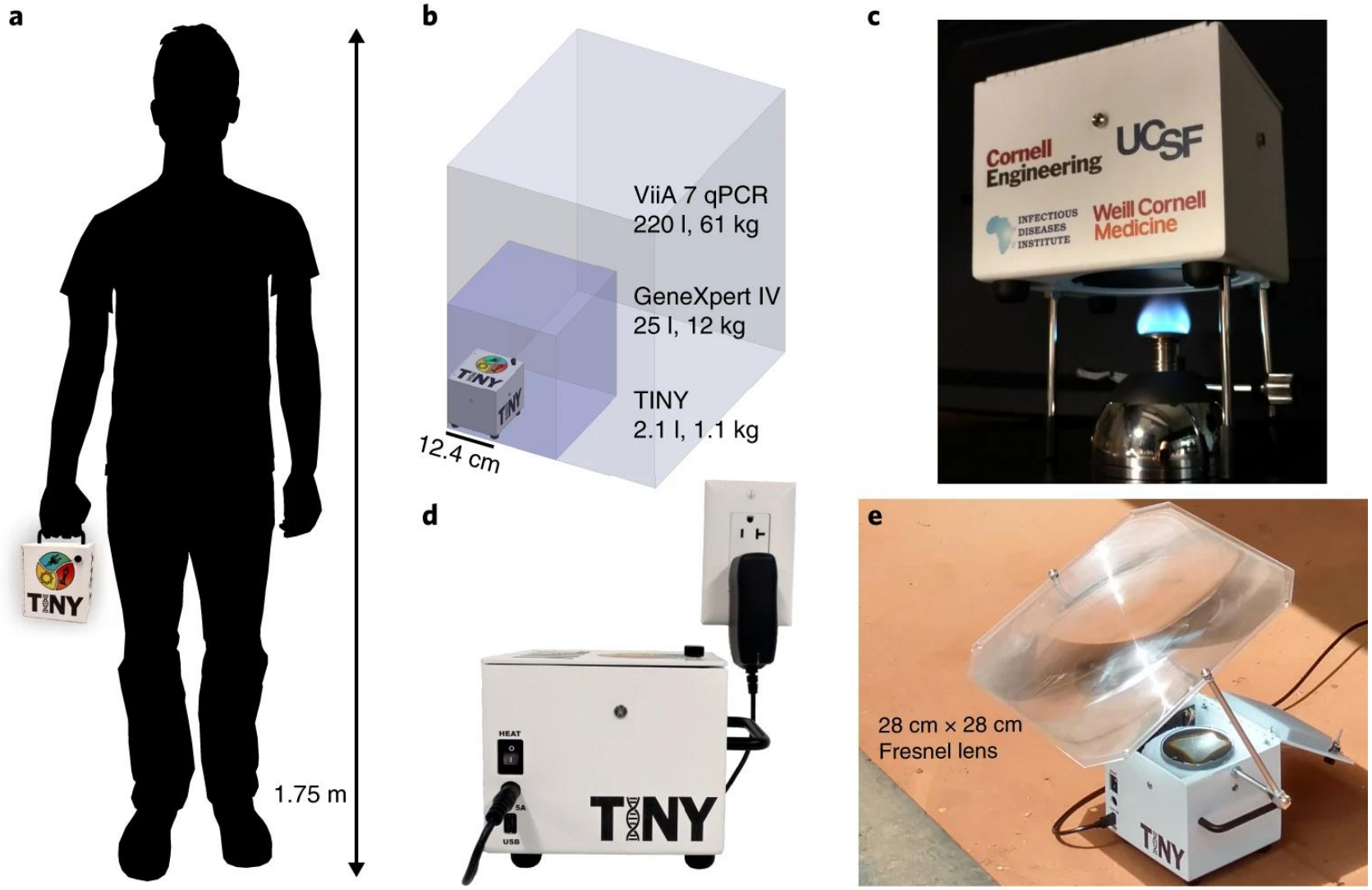
A Wisconsin City Experiments With a Faster, DIY Covid-19 Test

In a former boxing gym in Racine, firefighters are trying out a spit test that's simpler and cheaper than PCR. Could it change how we screen for the virus?



https://www.wired.com/story/a-wisconsin-city-experiments-with-a-faster-diy-covid-19-test/

Can it be easier?



nature biomedical engineering

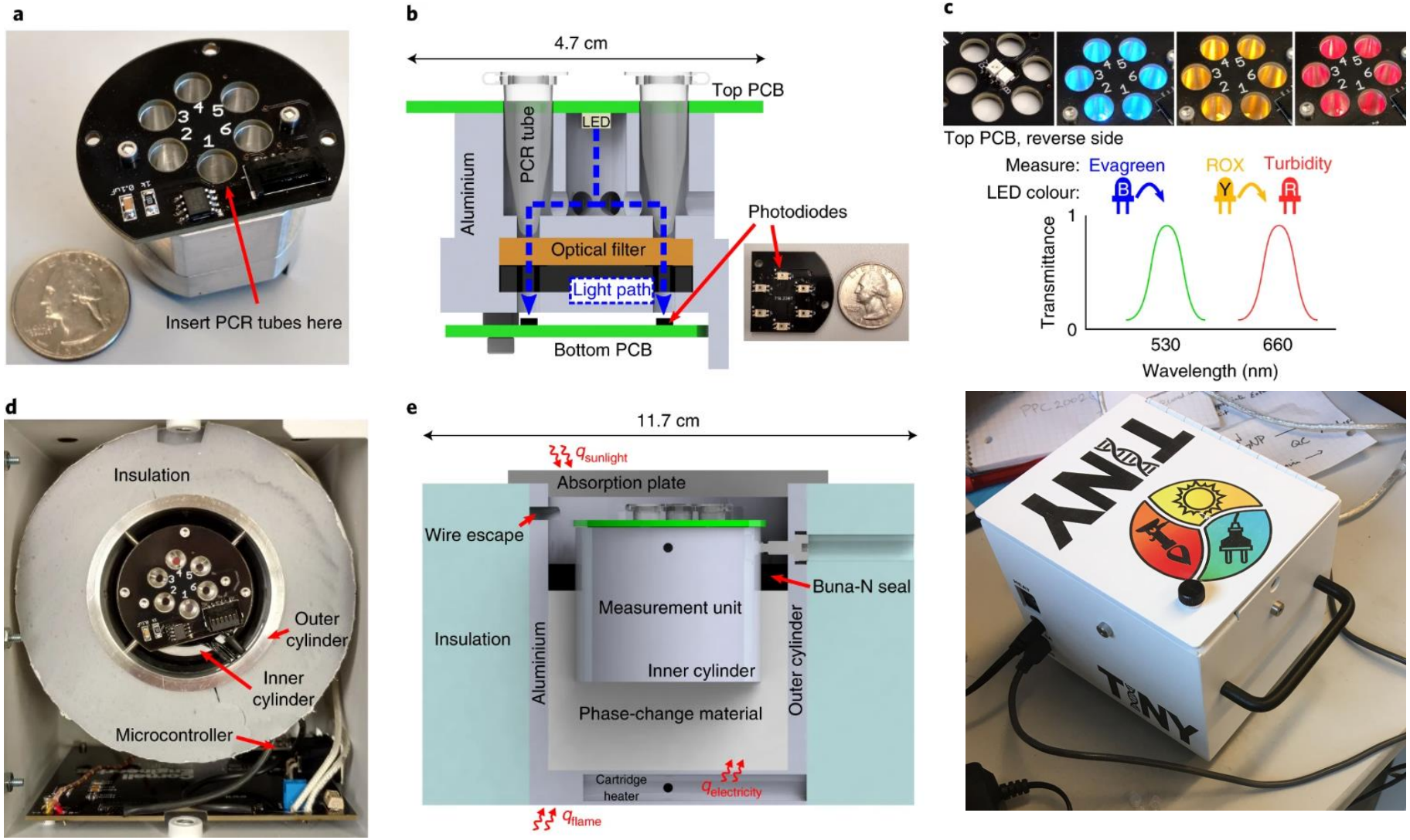
Article | Published: 11 September 2018

A portable device for nucleic acid quantification powered by sunlight, a flame or electricity

Ryan Snodgrass, Andrea Gardner, Aggrey Semeere, Varun Lingaiah Kopparthy, Jens Duru, Toby Maurer, Jeffrey Martin, Ethel Cesarman & David Erickson

Nature Biomedical Engineering **2**, 657–665(2018) | Cite this article

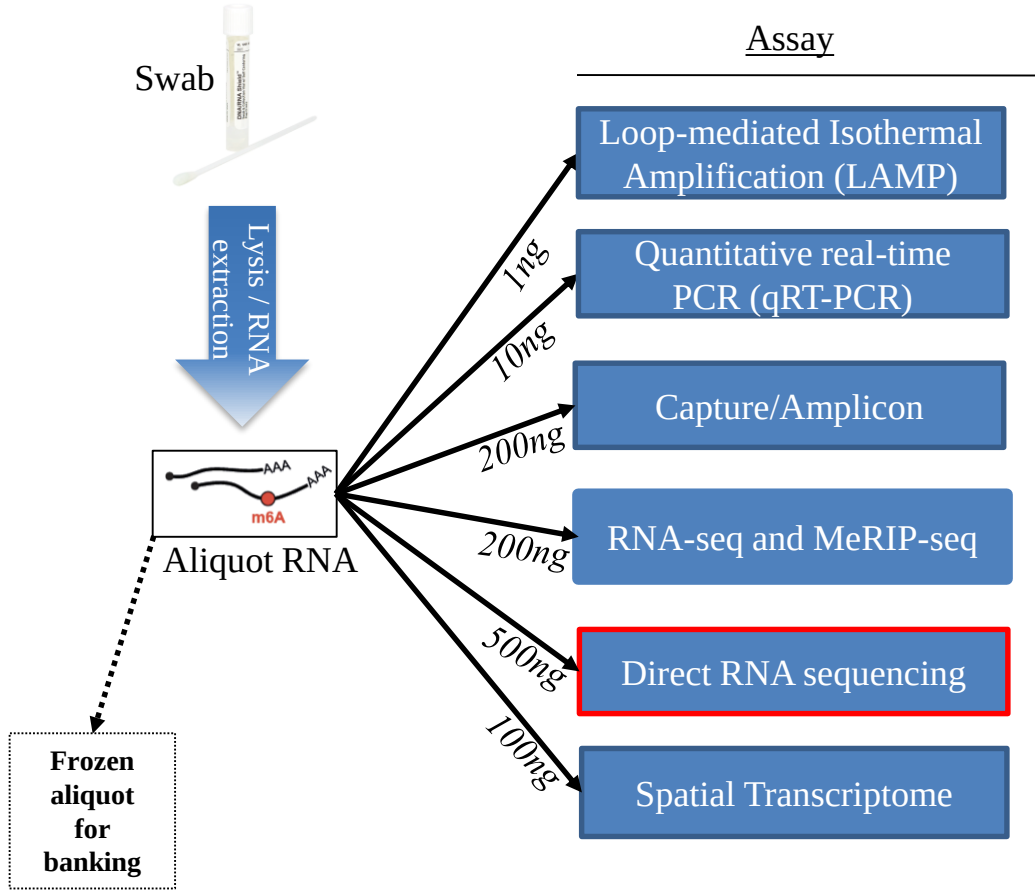
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6425734/


a
Insert PCR tubes here
b
4.7 cm
Top PCB
LED
PCR tube
Aluminium
Optical filter
Light path
Photodiodes
Bottom PCB
c
Top PCB, reverse side
Measure: Evagreen ROX Turbidity
LED colour:
Transmittance
1
0
530
660
Wavelength (nm)
d
Insulation
Outer cylinder
Inner cylinder
Microcontroller
e
11.7 cm
$q_{sunlight}$
Absorption plate
Wire escape
Buna-N seal
Measurement unit
Insulation
Aluminium
Inner cylinder
Outer cylinder
Phase-change material
Cartridge heater
$q_{electricity}$
q_{flame}

TINY Device and Methods now being used in the Racine and the ER at NYP

These methods and new SARS-CoV-2 protocols are currently deployed in the emergency room (ER) at New York Presbyterian (NYP) Hospital for nasopharyngeal (NP) swabs and direct from saliva.

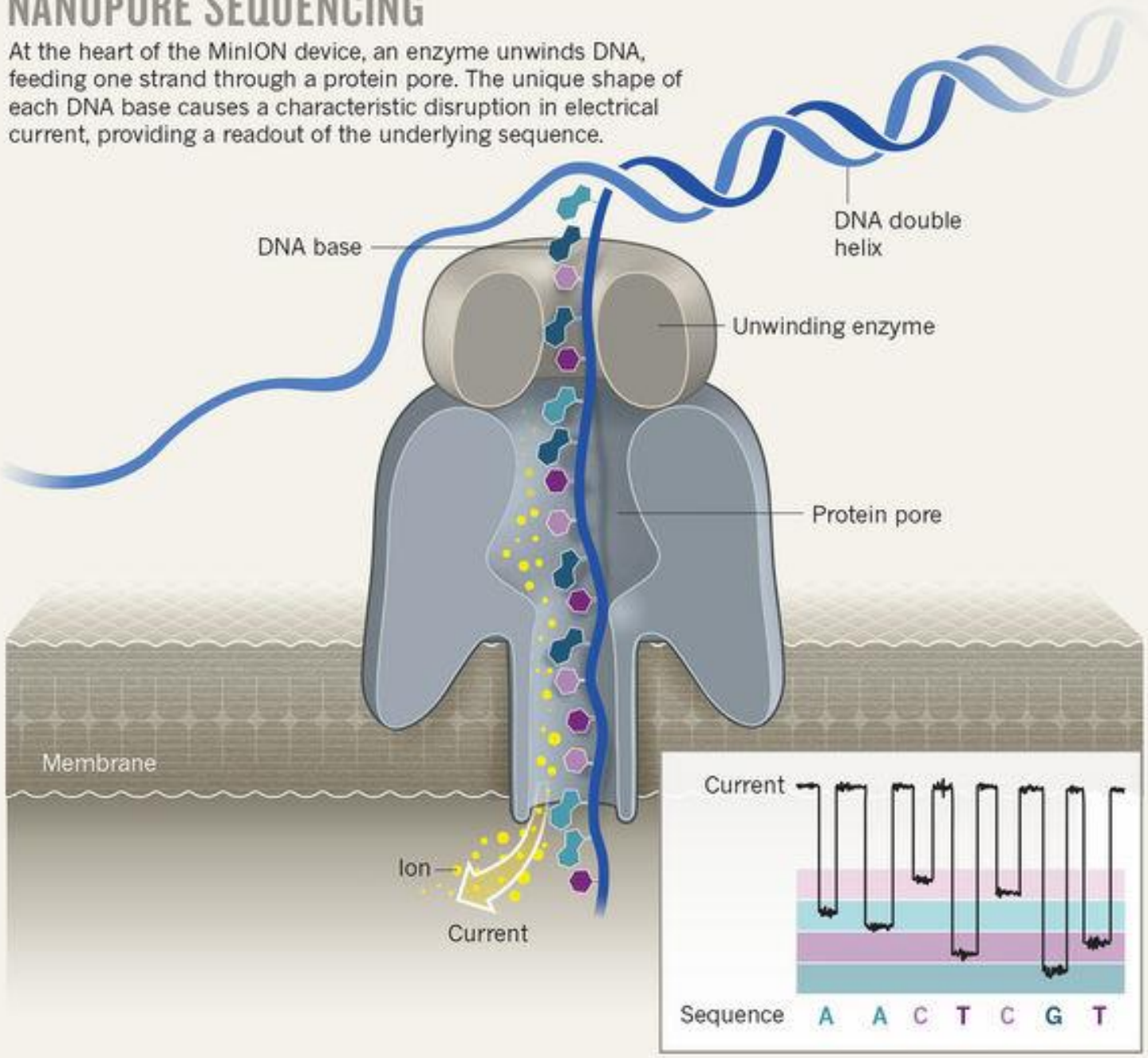
We now have an IRB protocol to help with screening and testing patients, health care workers, and staff.



Assay	TAT	Measurements and Metrics
Loop-mediated Isothermal Amplification (LAMP)	20-30 minutes	*Polymerase and pH colorimetric output:* COVID-19 presence/absence
Quantitative real-time PCR (qRT-PCR)	3-4 hours	*Two Primer Sets for COVID-19:* The E (envelope) and S (spike) genes.
Capture/Amplicon	1-3 days	*Next-Generation cDNA Sequencing:* Viral cDNAs, host cDNAs
RNA-seq and MeRIP-seq	1-3 days	*Next-Generation cDNA Sequencing:* Non-coding Viral and host cDNAs; m^6A
Direct RNA sequencing	0-2 days	*Next-Generation RNA Sequencing:* Viral and host polyA RNAs (MinION)
Spatial Transcriptome	1-3 days	*Bespoke Baits and Primers:* Virus, Host, and/or BCR/TCRs

NANOPORE SEQUENCING

At the heart of the MinION device, an enzyme unwinds DNA, feeding one strand through a protein pore. The unique shape of each DNA base causes a characteristic disruption in electrical current, providing a readout of the underlying sequence.



http://blogs.nature.com/naturejobs/2017/10/16/techblog-the-nanopore-toolbox/

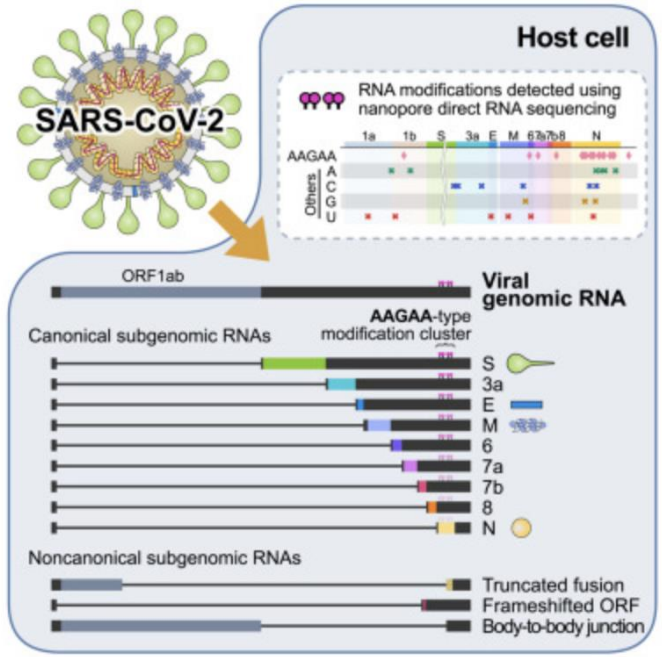
Cell

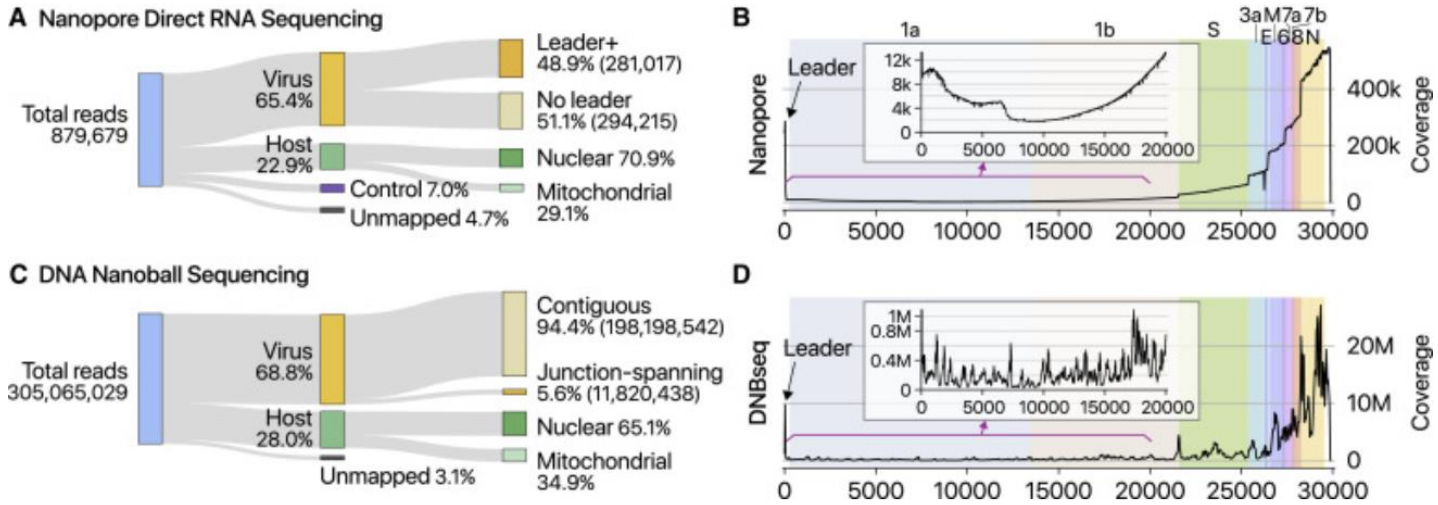
Volume 181, Issue 4, 14 May 2020, Pages 914-921.e10

Resource

The Architecture of SARS-CoV-2 Transcriptome

Dongwan Kim [1, 2], Joo-Yeon Lee [3], Jeong-Sun Yang [3], Jun Won Kim [3], V. Narry Kim [1, 2, 4] ✉, Hyeshik Chang [1, 2] ✉





New places

Can we just sequence in space?

OpNom: Biomolecule Sequencer

Principal Investigator(s)

Aaron Burton, Ph.D., NASA JSC, Houston, TX, United States

Co-Investigator(s)/Collaborator(s)

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Kristen John, Ph.D., NASA JSC, Houston, TX, United States

Sarah Stahl, M.S., NASA Johnson Space Center, Houston, TX, United States

Douglas Botkin, Ph.D., Johnson Space Center, Houston, TX, United States

Jason Dworkin, Ph.D., NASA GSFC, Greenbelt, MD, United States

Mark Lupisella, Ph.D., NASA GSFC, Greenbelt, MD, United States

David Smith, Ph.D., NASA Ames, Moffett Field, CA, United States

Christopher Mason, Ph.D., Weill Cornell Medical College, New York, NY, United States

James Brayer, Oxford Nanopore Technologies Inc., Cambridge, MA, United States

Developer(s)

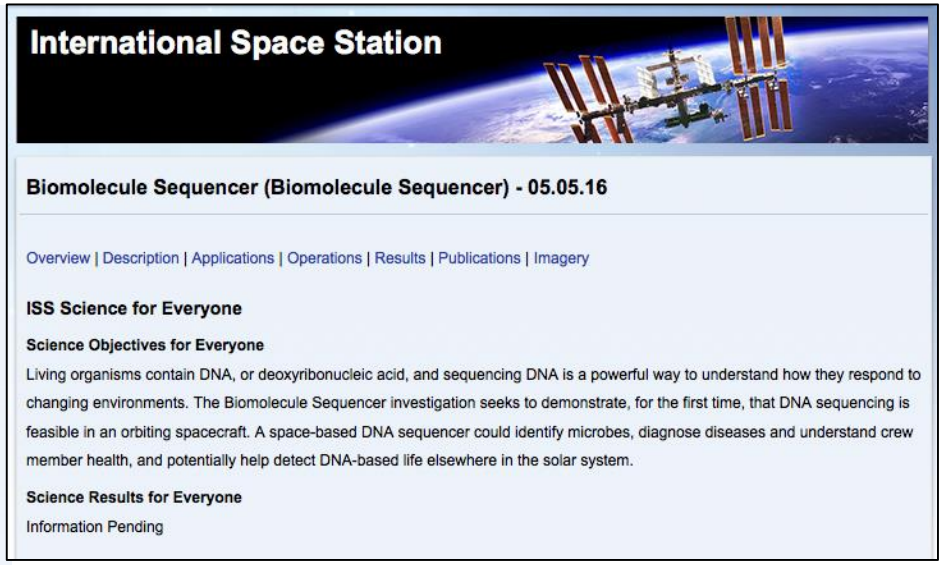
NASA Johnson Space Center, Houston, TX, United States

Sponsoring Space Agency

National Aeronautics and Space Administration (NASA)

Sponsoring Organization

Technology Demonstration Office (TDO)



- Operational environmental monitoring of microorganisms
  - Allow for in-flight identification of microbes, which is currently not possible but is essential for travel beyond our moon.
  - Inform real-time decisions and remediation strategies.
- Medical operations
  - Real-time analysis can impact medical intervention and define countermeasure efficacy.
- Research
  - DNA from any organism can be sequenced to assist any scientific investigation on the ISS.
- Astrobiology
  - ISS demonstration serves as functional testing for integration into robotics for Mars exploration missions.
  - This technology is superiorly suited for the detection of life based on DNA and DNA-like molecules.

http://www.nasa.gov/mission_pages/station/research/experiments/2181.html

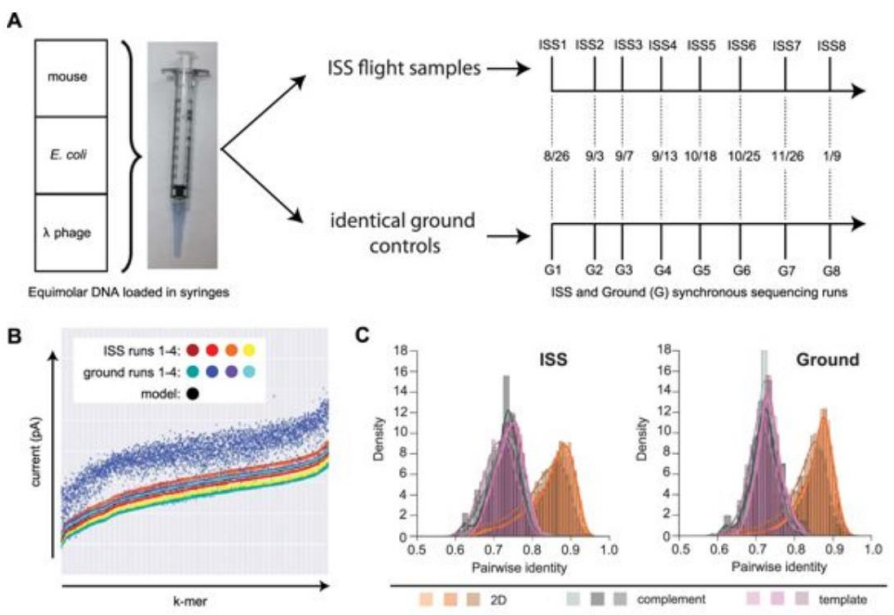

NASA
OXFORD NANOPORE
WEILL CORNELL
BIOMOLECULE SEQUENCER PROJECT

The first genome sequenced and assembled off Earth

SCIENTIFIC REPORTS

Article | OPEN | Published: 21 December 2017

Nanopore DNA Sequencing and Genome Assembly on the International Space Station



Castro Wallace et al., 2017

https://www.nature.com/articles/s41598-017-18364-0

The first space epigenome

nature COMMUNICATIONS

Article | OPEN | Published: 04 February 2019

Single-molecule sequencing detection of N6-methyladenine in microbial reference materials

Alexa B. R. McIntyre, Noah Alexander, Kirill Grigorev, Daniela Bezdan, Heike Sichtig, Charles Y. Chiu & Christopher E. Mason

https://www.nature.com/articles/s41467-019-08289-9 https://github.com/al-mcintyre/mCaller

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Weekly Recap From the Expedition Lead Scientist

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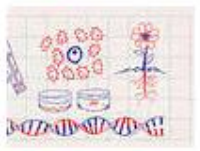

Biological Sciences on the International Space Station

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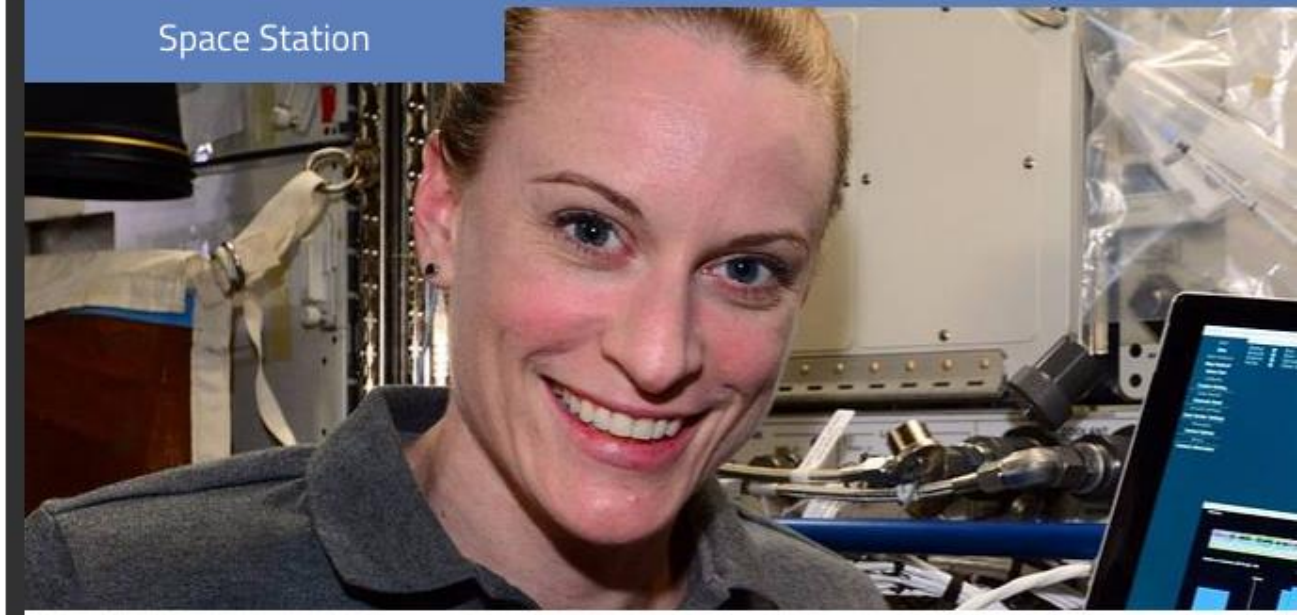
Space Station



Aug. 29, 2016

First DNA Sequencing in Space a Game Changer

For the first time ever, DNA was successfully sequenced in microgravity as part of the **Biomolecule Sequencer** experiment performed by NASA astronaut Kate Rubins this weekend aboard the **International Space Station**. The ability to sequence the DNA of living organisms in space opens a whole new world of scientific and medical possibilities. Scientists consider it a game changer.

DNA, or deoxyribonucleic acid, contains the instructions each cell in an organism on Earth needs to live. These instructions are represented by the letters A, G, C and T, which stand for the four chemical bases of DNA, adenine, guanine, cytosine, and thymine. Both the number and arrangement of these bases differ among organisms, so their order, or sequence, can be used to identify a specific organism.

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NASA Astronaut Kate Rubins, Crewmates Arrive Safely at Space Station
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NASA to Host Preview Briefings, Interviews for First Crew Rotation Mission with SpaceX
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NASA Seeks Next Class of Flight Directors for Human Spaceflight Missions
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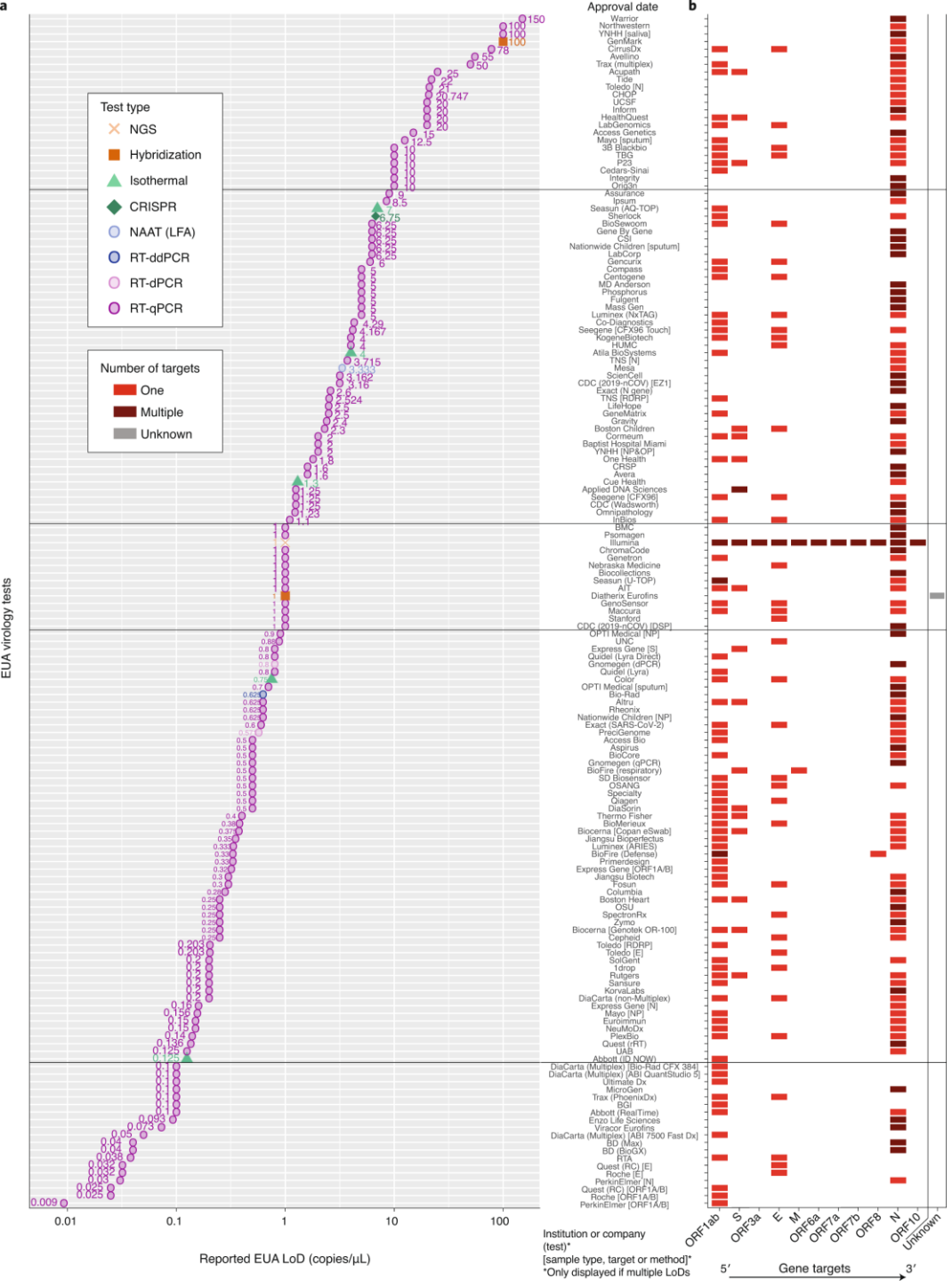
Oct. 14, 2020
RELEASE 20-099

NASA Astronaut Kate Rubins, Crewmates Arrive Safely at Space Station

New methods



Also pushing to get more and more kinds of tests

Correspondence | Published: 20 August 2020

The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing

Matthew J. MacKay, Anna C. Hooker, Ebrahim Afshinnekoo, Marc Salit, Jason Kelly, Jonathan V. Feldstein, Nick Haft, Doug Schenkel, Subhalaxmi Nambi, Yizhi Cai, Feng Zhang, George Church, Junbiao Dai, Cliff L. Wang, Shawn Levy, Jeff Huber, Hanlee P. Ji, Alison Kriegel, Anne L. Wyllie & Christopher E. Mason

Nature Biotechnology (2020) | Cite this article

https://xprize.org/prizes/covidtesting

https://www.nature.com/articles/s41587-020-0655-4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Deep Gratitude to Many People:

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Maria Sierra
Delia Tomoiaga
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Craig Westover
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Shawn Levy

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Iwijn De Vlaminck
Olivier Elemento
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Iman Hajirasouliha
Ari Melnick (Melnick Lab)
Margaret Ross
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Biotia
Niamh O'Hara (Biotia)
Rachid Ounit (Biotia)

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Jane Carlton
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Rockefeller
Jeanne Garbarino
Charles Rice

UVA
Francine Garrett-Bakelman

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Charles Chiu

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