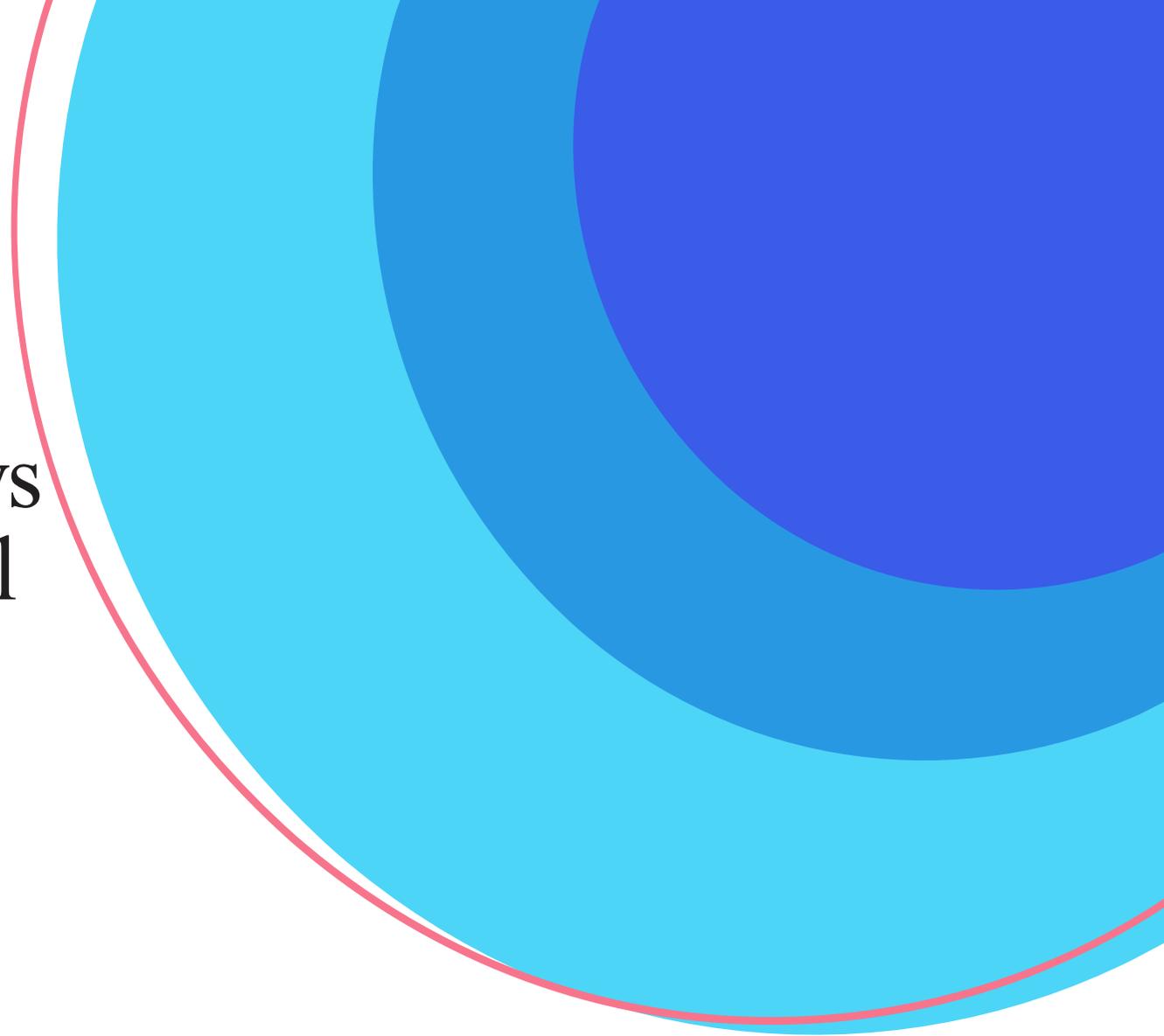


# How RT-PCR Testing Allows Labcorp to Perform National SARS-CoV-2 Pandemic Surveillance

Brian Krueger, PhD  
Associate Vice President, Research and Development  
Center for Esoteric Testing / Center for Molecular Biology and Pathology

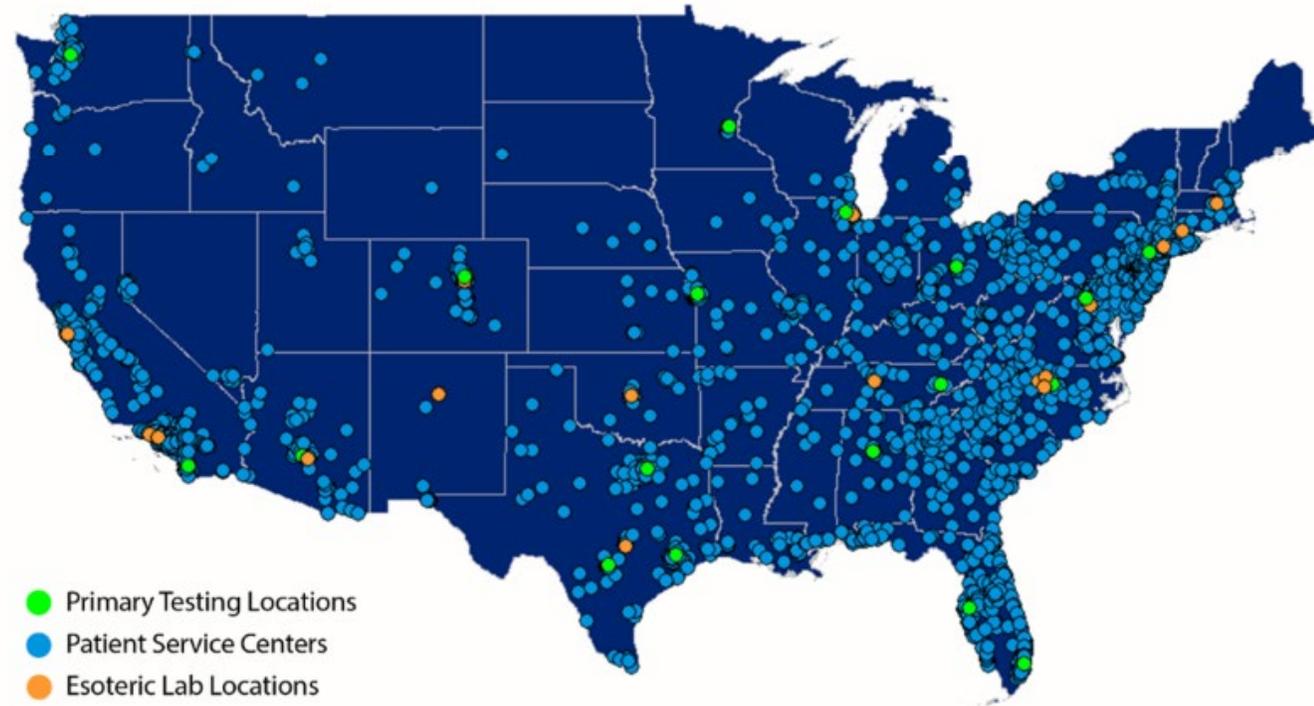
4/23/2021

**labcorp**

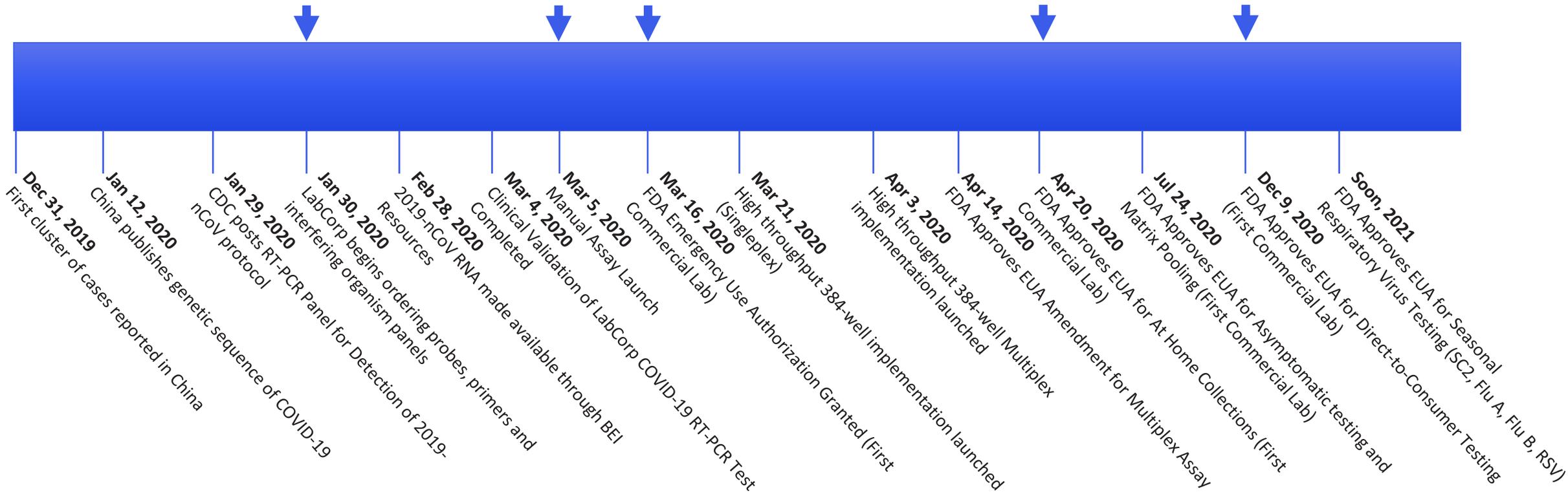


# Labcorp: Leading Laboratory Service Provider

- Large scale national molecular testing
- PIXEL at home collection kits expand reach
- National laboratory information system
- Specimen tracking
- Sample meta data attached to each sample



# SARS-CoV-2 RT-PCR Test Development Timeline



# LabCorp COVID-19 RT-PCR Test

- A high throughput implementation of the CDC RT-PCR Test
- Development
  - Extraction:
    - Roche MagnaPure – 96 samples per hour
    - ThermoFisher KingFisher MagMax– 96 samples per hour
    - Hamilton MagEx – 192 samples per hour
  - Detection
    - QuantStudio 7 384-well
      - Multiplex Assay (384 samples, 1 rxn per sample) ~ 6,000 samples/instrument/day



# LabCorp COVID-19 RT-PCR Test

- Assay Multiplex Development began in February
  - Launched on manual SinglePlex because of quick access to reagents
- Assay Multiplexed by changing Fluorescent dyes
  - N1 – FAM
  - N2 – Yakima Yellow
  - RP – Cy5
- Automated on Hamilton Star Liquid Handlers
  - 1,152 rxns/instrument/hr
- More than half of Labcorp SARS-CoV-2 testing is performed on the Labcorp COVID-19 RT-PCR Test
  - >40M tests performed since March 2020

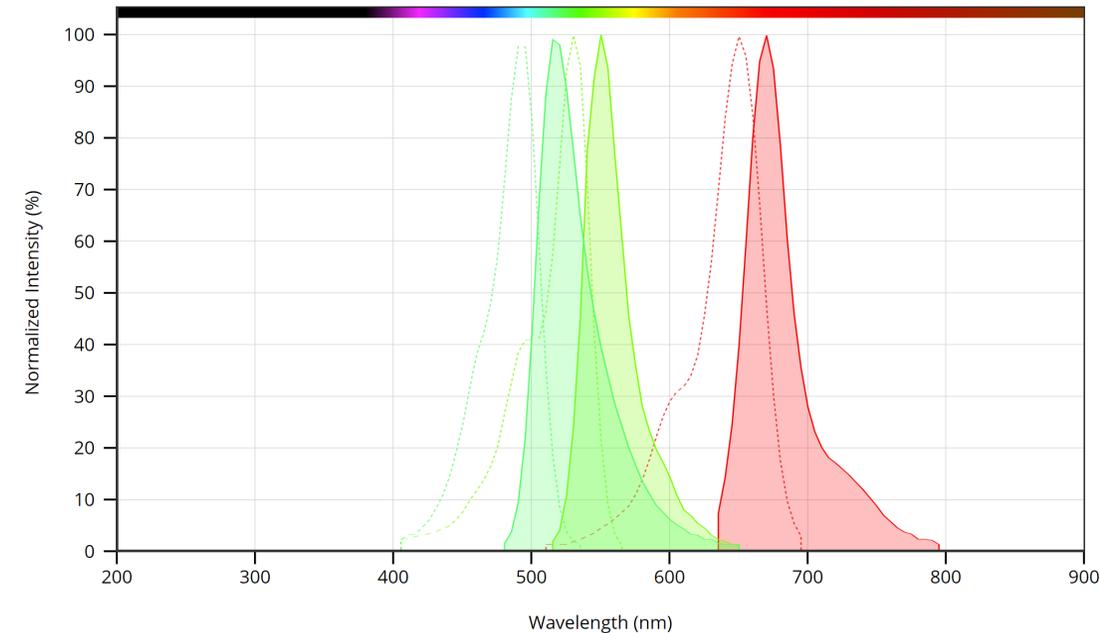


Image: <https://www.aatbio.com/fluorescence-excitation-emission-spectrum-graph-viewer/>

# Supply Chain Constraints DEFINED the Pandemic

- Swabs
  - Cotton Swabs (qTips)
  - Foam Swabs
  - 3D Printed Swabs
- Transport Media
  - UTM
  - Saline
- Extraction Methods
- 1-Step RT Enzymes
- Heat/Quick Extraction
- Saliva Collection



# LabCorp Total Unique RT-PCR Positives

Cumulative

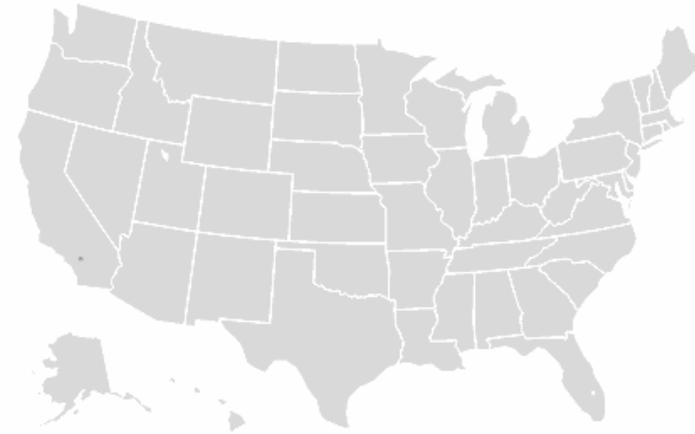
2020-02-02  
Legend=Cases by zip  
Total=1



Weekly

Legend=Cases by zip  
Between:  
2020-02-02 and 2020-01-19

0 - 10



0 - 10

# PCR Testing vs Genome Sequencing

- Our PCR testing detects 2 locations in the SARS-CoV-2 Genome
- Both are in the N protein, or Nucleocapsid Protein
  - Resides inside of the viral particle and protects the Viral genome
- Genome Sequencing determines the genetic code of the entire virus

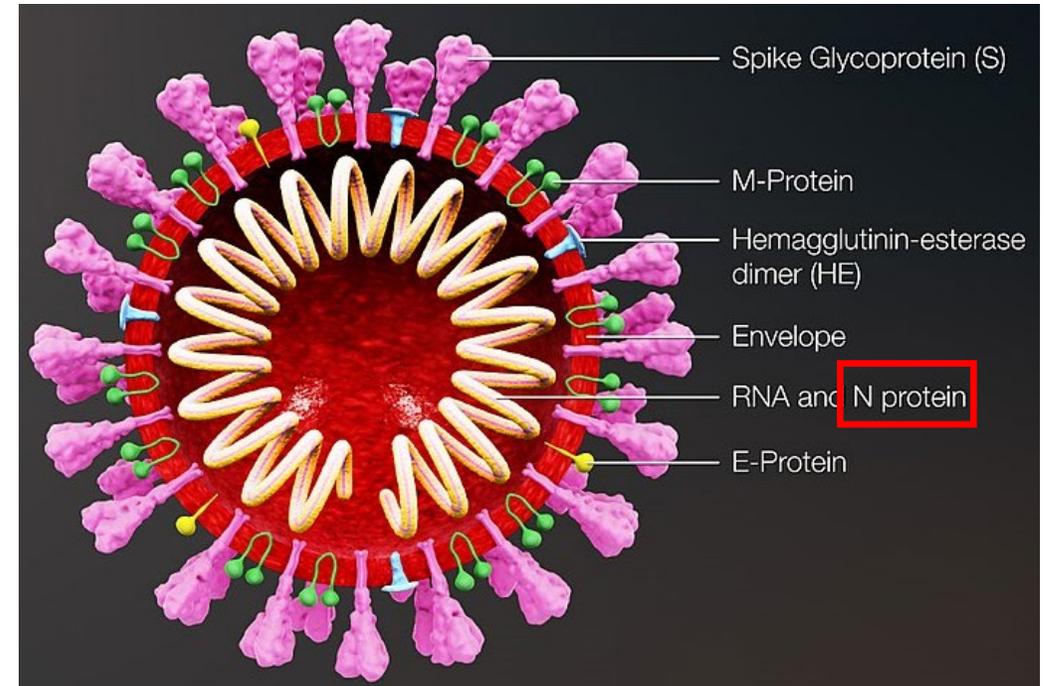
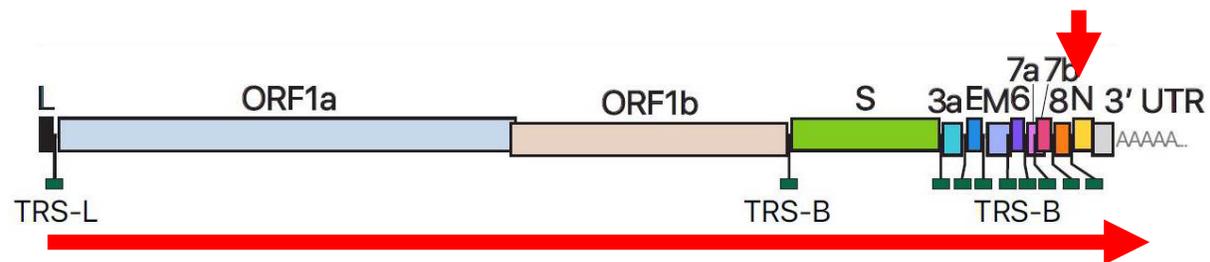
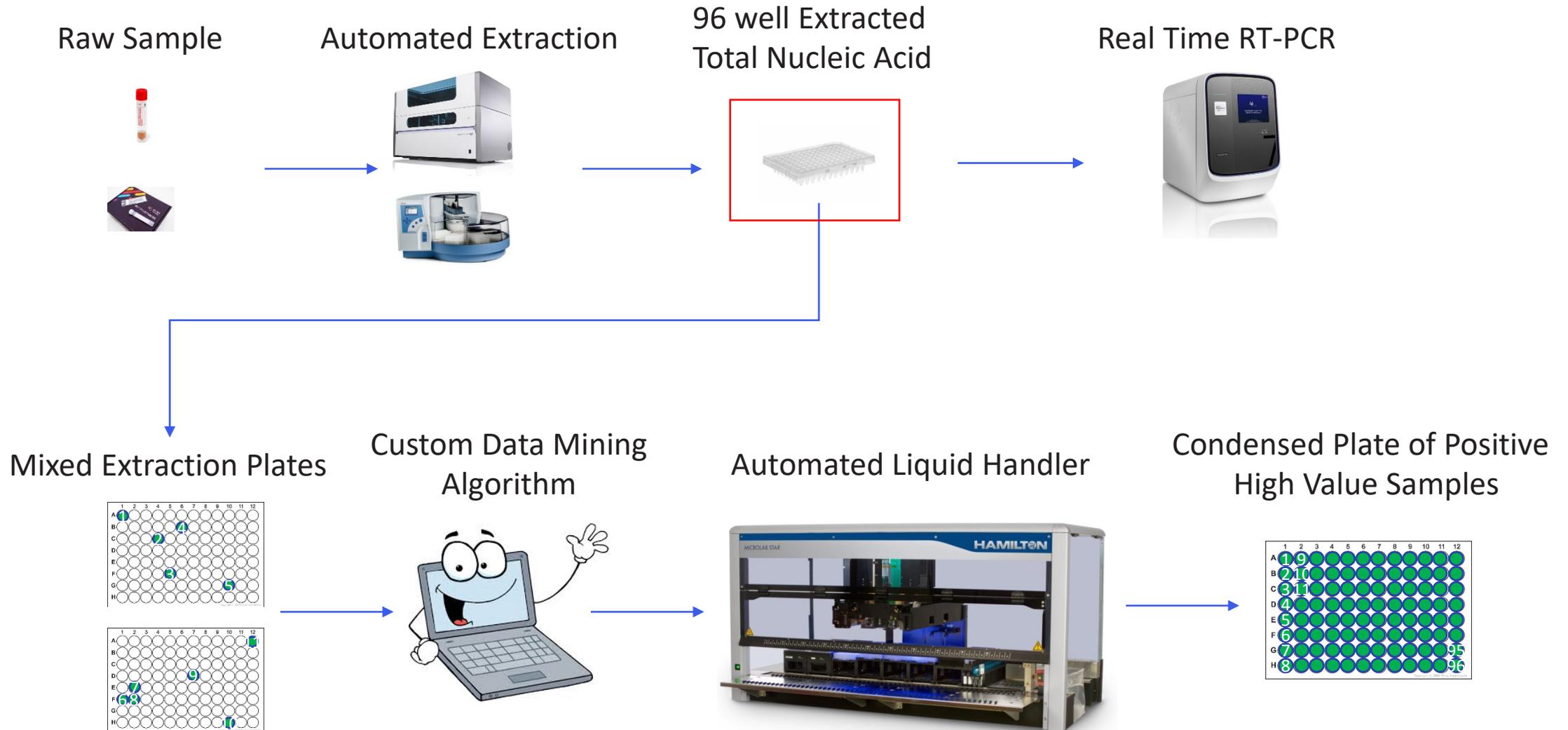


Image: <https://www.scientificanimations.com>, CC BY-SA 4.0

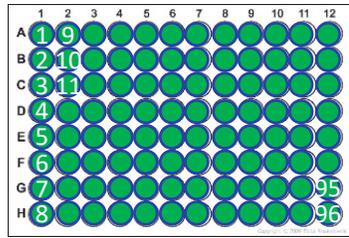


# Molecular RT-PCR Testing Workflow Enables Viral Genome Surveillance



# Sequencing and Analysis Pipeline

Condensed Plate of Positive High Value Samples



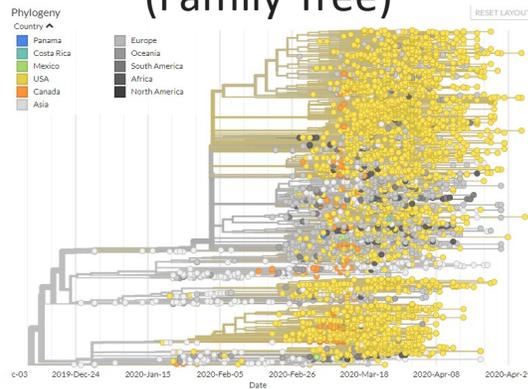
Whole Genome Tiled Amplification  
(2 Pools of Overlapping 1.2kb Amplicons)



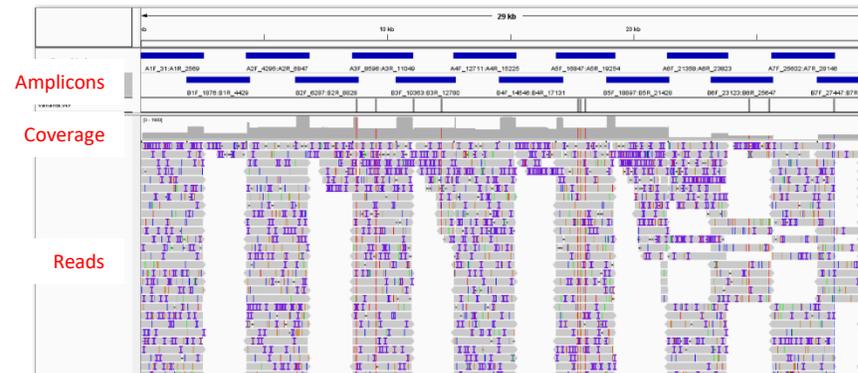
PacBio Sequel II



Phylogenetic Assignment  
(Family Tree)



Alignment and Variant Calling



# What's a viral genome?

## Genome Medicine

Home About

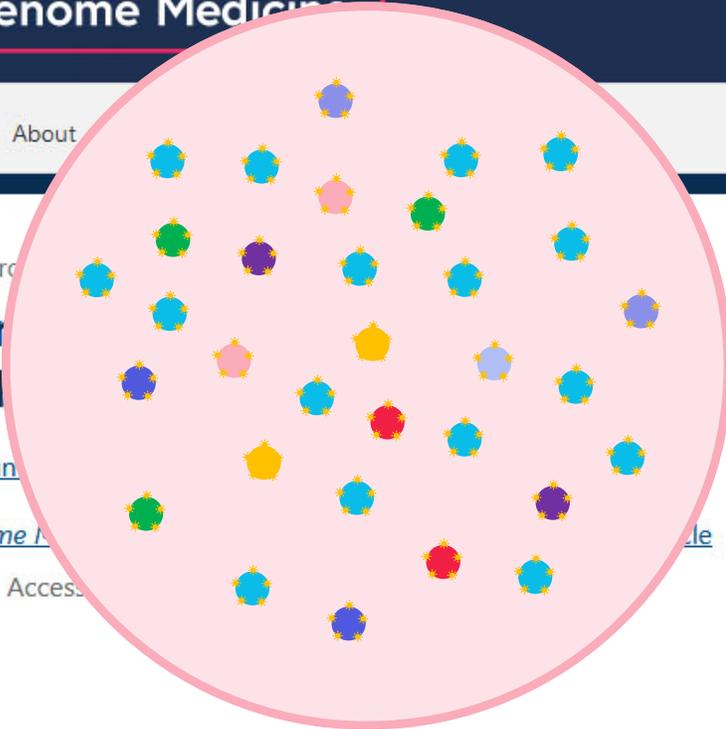
Research

Intro  
SAI

Yanqun

Genome

1236 Access

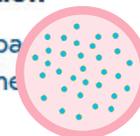


### ry dynamics of -19 patients

Download PDF

#### Collection

The impact of genomics on precision  
public health



Sections

Figures

References

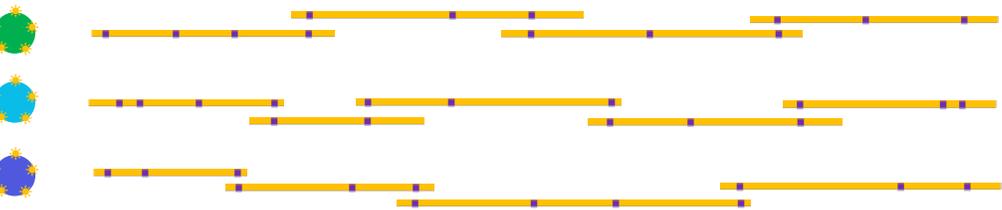
Diaphragm

# What's a viral genome?

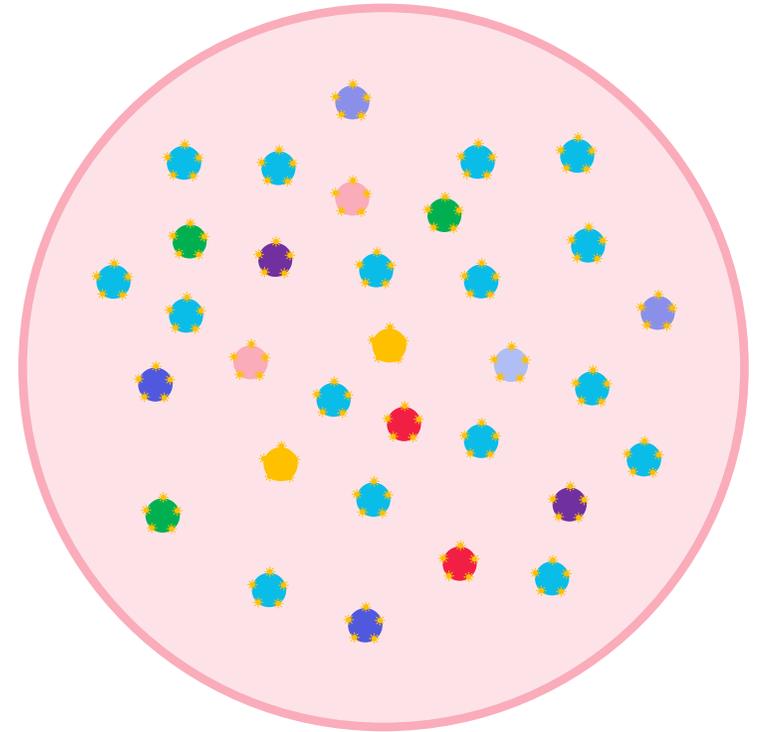
- Viruses evolve within the host
- New mutations arising continuously
- A host has a population of viruses, many of which can have distinct genetic differences



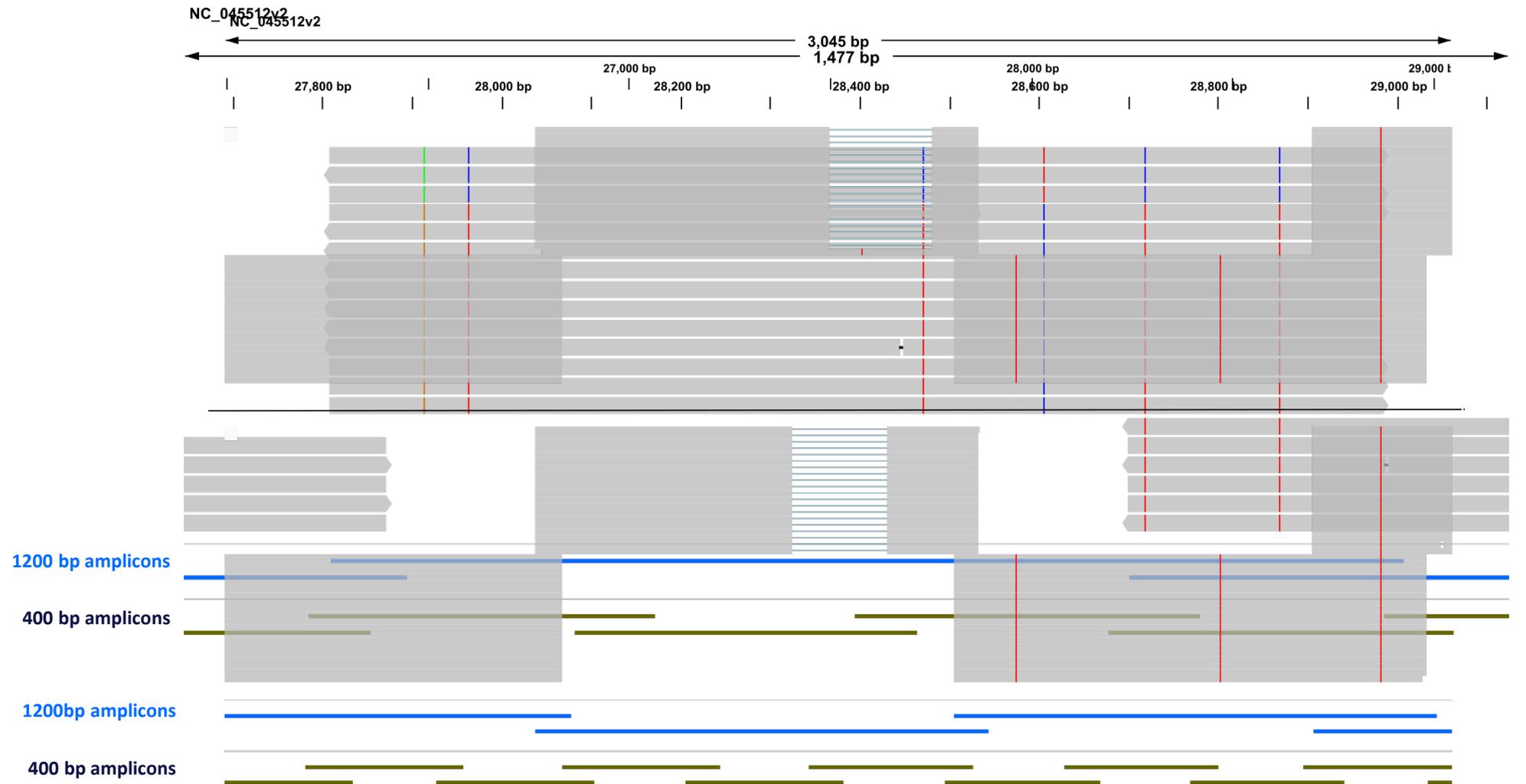
Long Read Sequencing (PacBio)



Short Read Sequencing (Illumina)

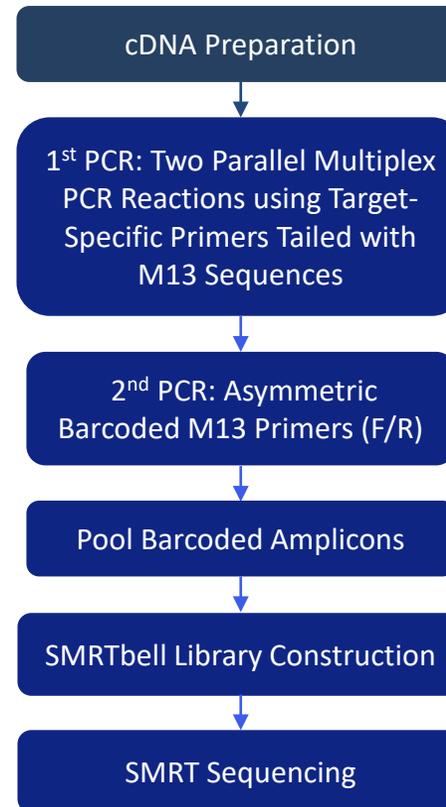


# Large Variant Detection and Phasing



Images: Liz Tseng, PacBio

# Sequencing Library Protocol



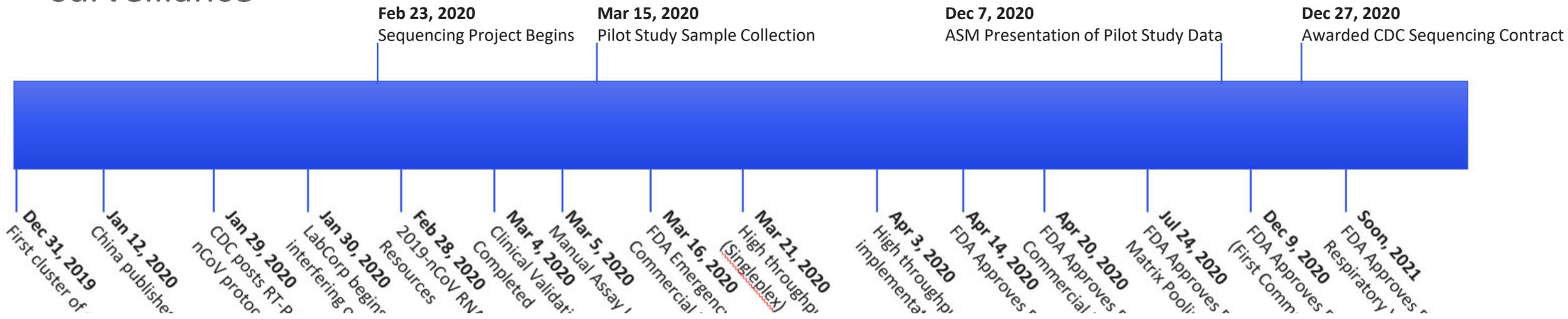
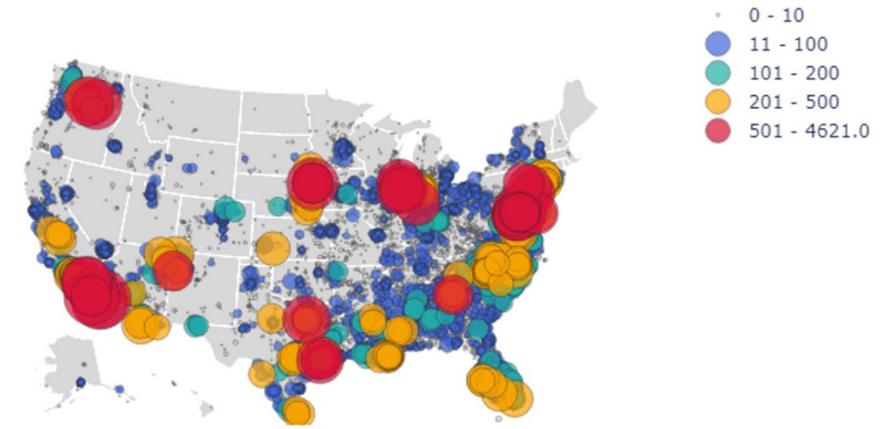
Freed, et al, 2020 – DOI: [10.1093/biomethods/bpaa014](https://doi.org/10.1093/biomethods/bpaa014)  
Labcorp optimized protocol is available on the PacBio website

# Analysis Pipeline

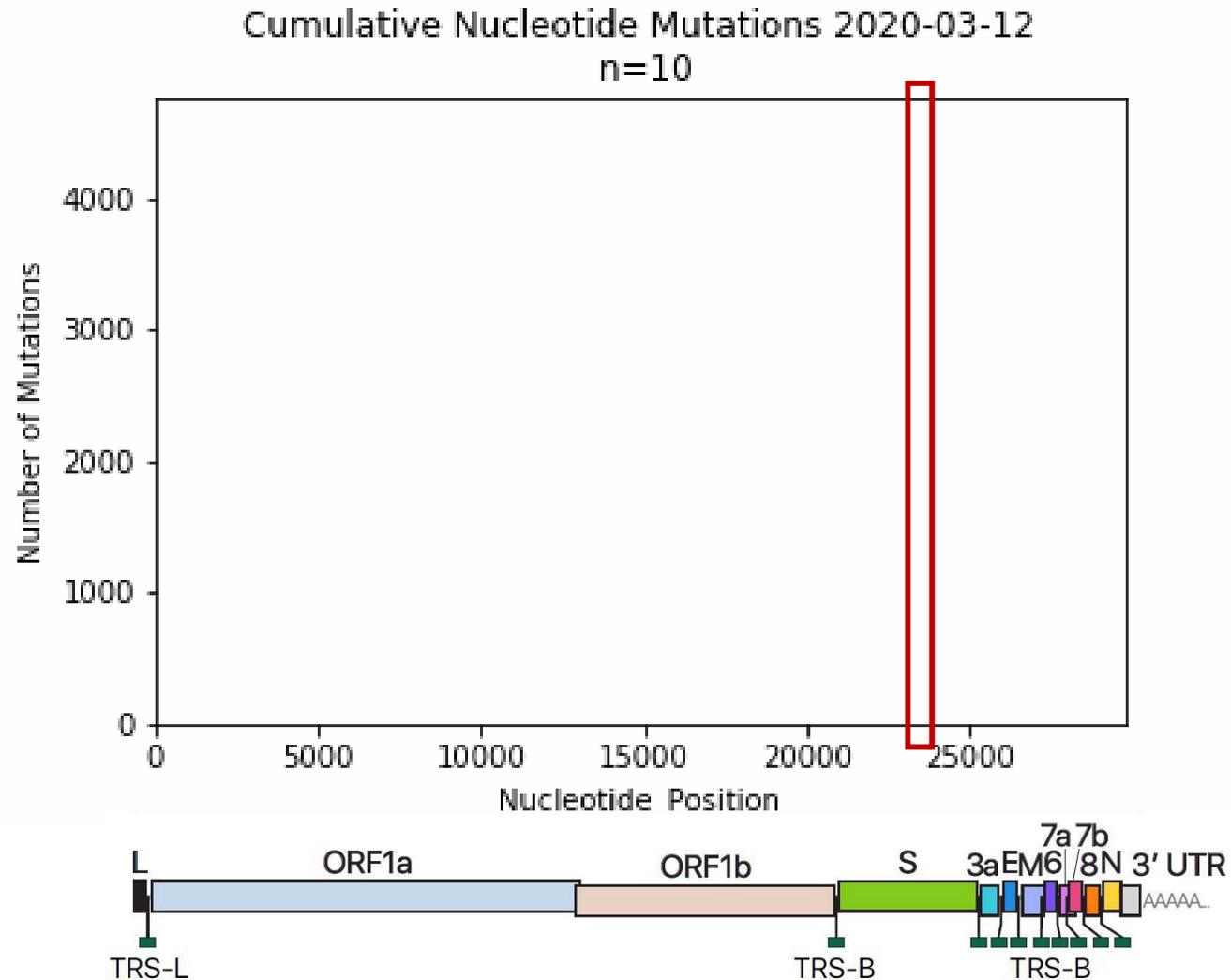
The screenshot shows the bioRxiv preprint server interface. At the top left is the Cold Spring Harbor Laboratory (CSH) logo and the bioRxiv logo with the tagline 'THE PREPRINT SERVER FOR BIOLOGY'. A navigation menu includes 'HOME | ABOUT | SUBMIT | NEWS & NOTES | ALERTS / RSS | CHANNELS'. A search bar is present with a magnifying glass icon and a link to 'Advanced Search'. A yellow banner contains a warning: 'bioRxiv is receiving many new papers on coronavirus SARS-CoV-2. A reminder: these are preliminary reports that have not been peer-reviewed. They should not be regarded as conclusive, guide clinical practice/health-related behavior, or be reported in news media as established information.' The main article title is 'VCFCons: a versatile VCF-based consensus sequence generator for small genomes' by Elizabeth Tseng, Qiandong Zeng, and Lax Iyer. The article is dated February 27, 2021. It includes options to 'Download PDF', 'Email', 'Share', and 'Citation Tools'. There are also 'Tweet' and 'Like 0' buttons. A 'Subject Area' section highlights 'Bioinformatics'. On the right side, there are three vertical boxes labeled 'LC BAM', 'Primers', and 'ERRORS'. At the bottom left, a vertical box is partially labeled 'Acc'.

# Pilot Surveillance Study

- 70,000 SARS-CoV-2 positive samples banked from March, 2020 – Dec, 2020
- Initial pilot study of 10,000 early pandemic samples (March 15, 2020 - May 21, 2020 )
- Developed a model for national viral genome surveillance



# SARS-CoV-2 Mutations Over Time



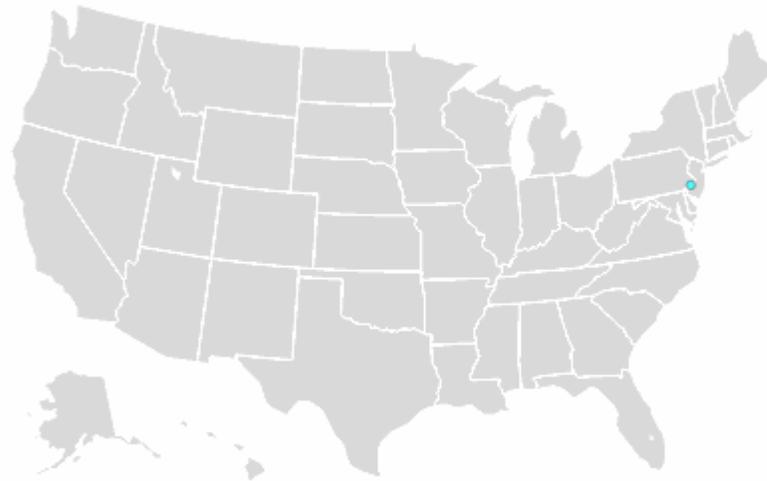
# S:D614G Spread During the Early Pandemic When Few Were Looking

2020-03-12

Legend=Cases by zip

Total=1

● D614G Neg



# New Variants of Concern

- ***B.1.1.7 lineage (20I/501Y.V1)*** ←

- Emerged in the UK September 2020
- Likely has increased transmissibility
- Detected in the US Dec 29, 2020

- ***P.1 lineage (20J/501Y.V3)*** ←

- Brazilian origin
- Likely increased transmissibility and antibody resistance
- Detected in the US Jan 25, 2020

- ***B.1.351 lineage (20H/501Y.V2)*** ←

- South African origin
- Likely increased transmissibility and antibody resistance
- Detected in the US Jan 28, 2020

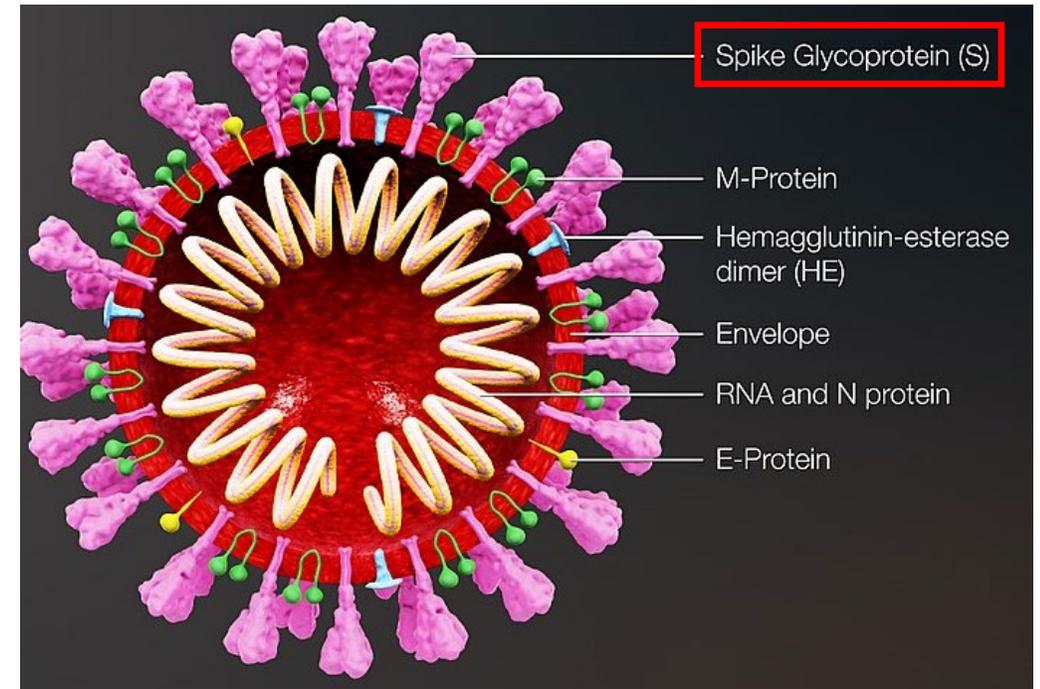
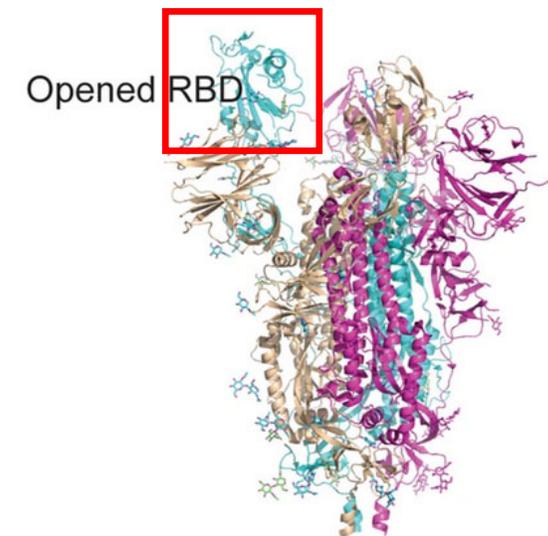
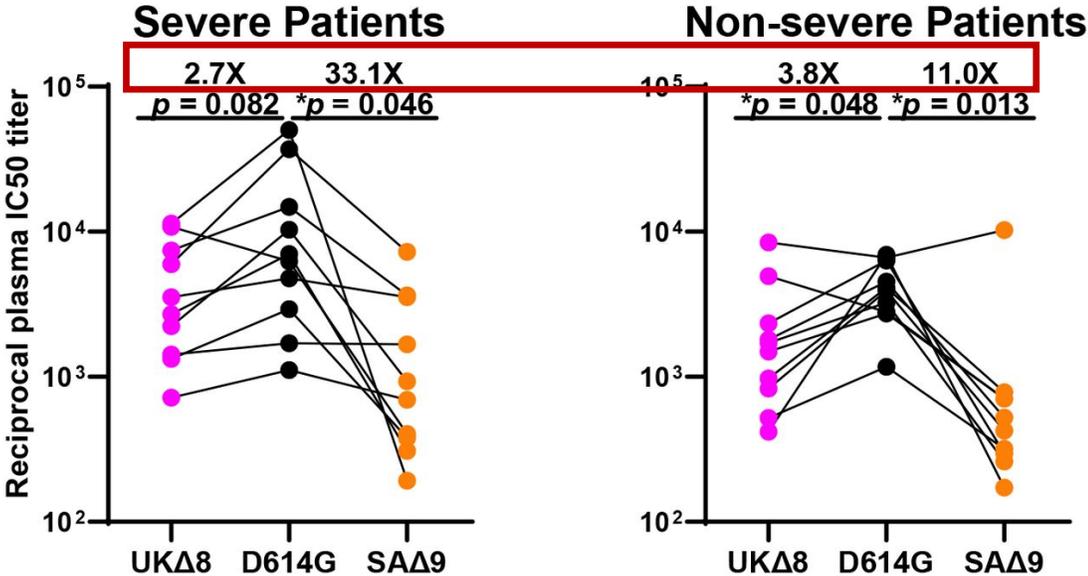


Image: <https://www.scientificanimations.com>, CC BY-SA 4.0

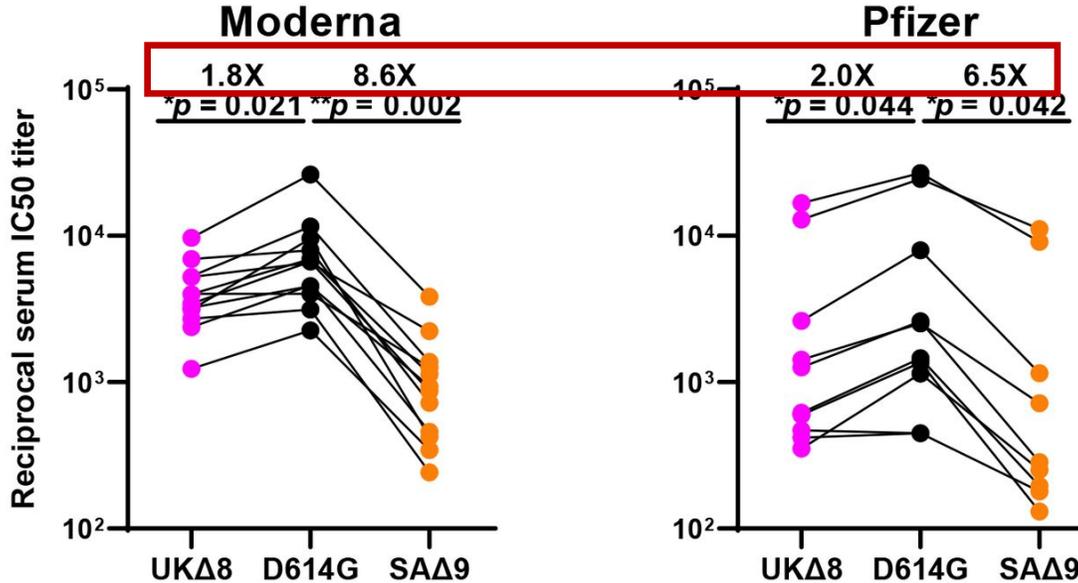


# Spike Mutations Can Reduce Immunity to the Virus

## Convalescent Plasma (Immunity from viral infection)



## Vaccine Plasma (Immunity from vaccination)



Wang et al, 2021, <https://doi.org/10.1101/2021.01.25.428137>

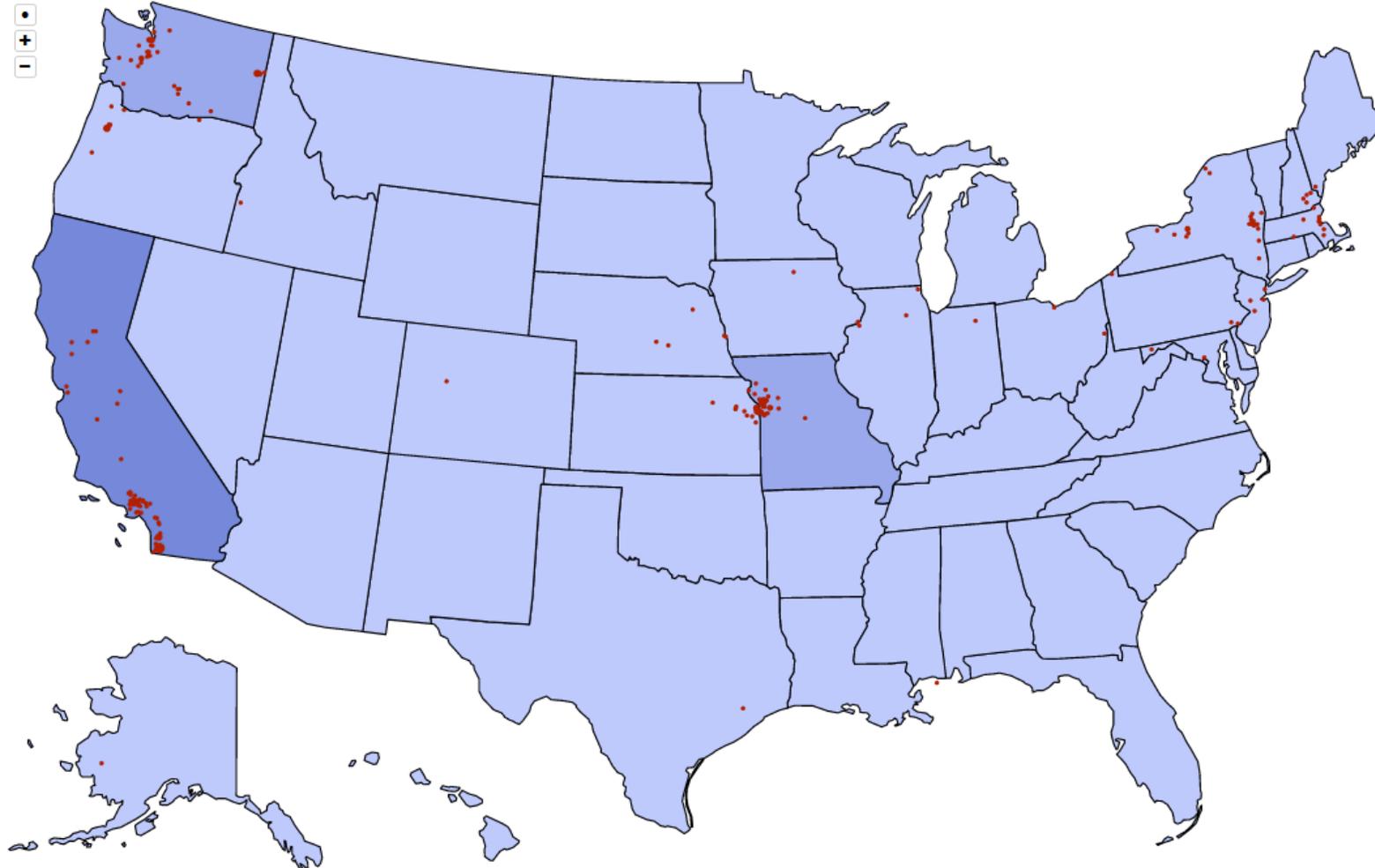
# Overview of Current CDC Sequencing Results

- Sampling from ~3,500 samples every week
  - CDC Baseline Study
  - Minimum of 10 per state
    - ~60% Full genomes, +~30% Lineage typed
  - Results reported weekly on CDC SPHERES calls
- Share Summary statistics, CCS BAMs and FASTA consensus
- Tracking individual mutations and viral lineages
- Aiding in CDC response and national action planning for containment of new mutants

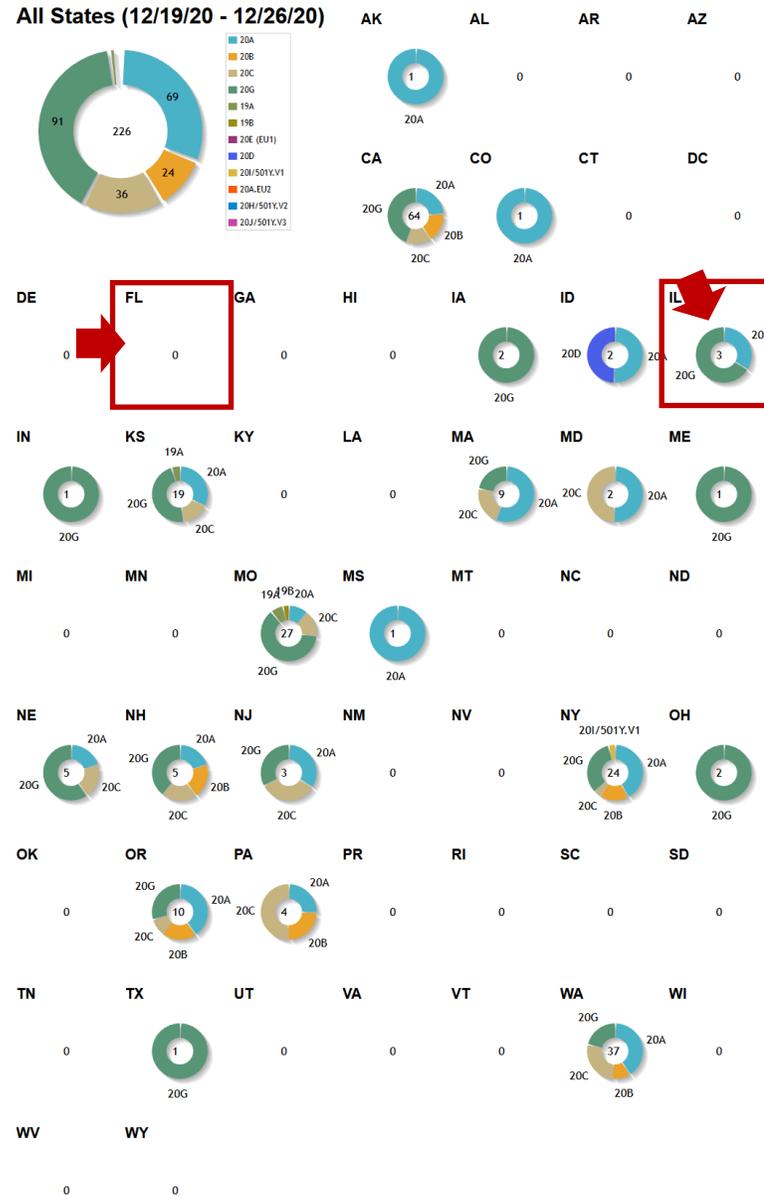


# CDC Sequencing by Week

**Samples Sequenced (12/19/20 - 12/26/20)<sup>+/-</sup>**



# CDC Clade Distribution



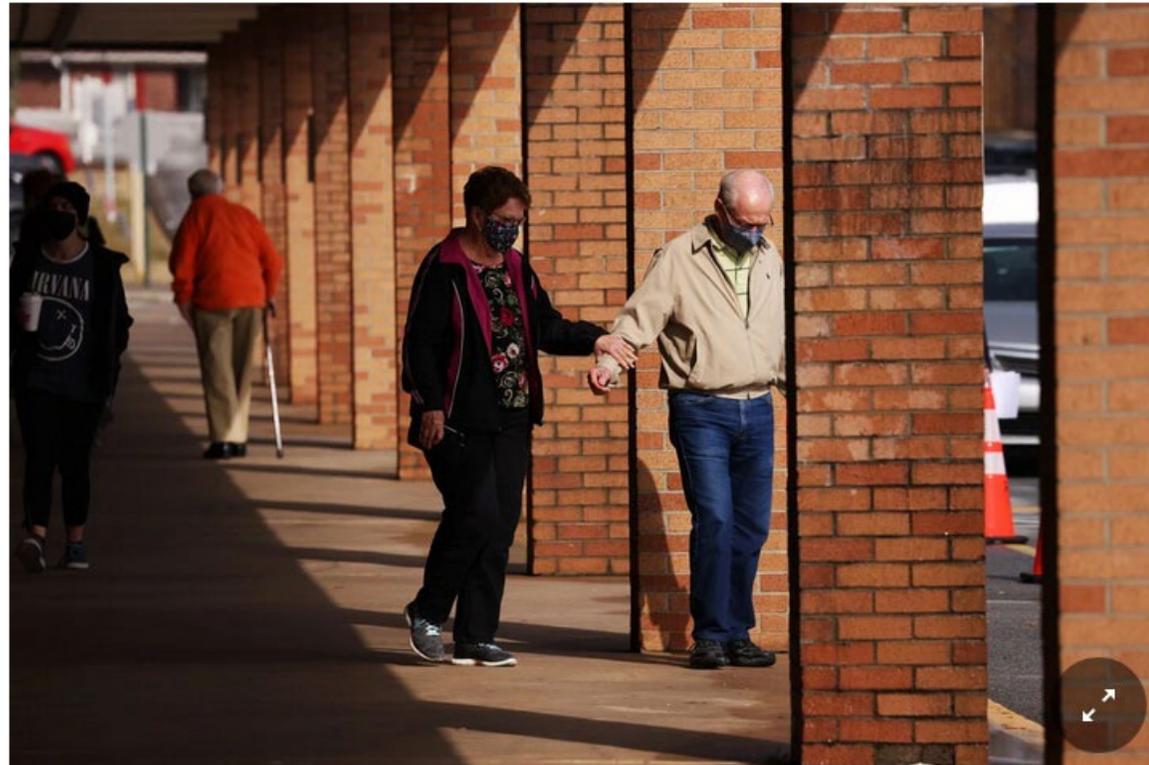
# Overview of Cur

- Sampling from ~100  
- CDC Baseline Study  
- Minimum of 10 p  
• ~60% Full genome  
- Results reported
- Share Summary sta
- Tracking individual
- Aiding in CDC resp

< Covid-19 Live Updates: Variant Found in South Africa Now Detected in U.S.

## The variant that emerged in South Africa is reported in the U.S. for the first time.

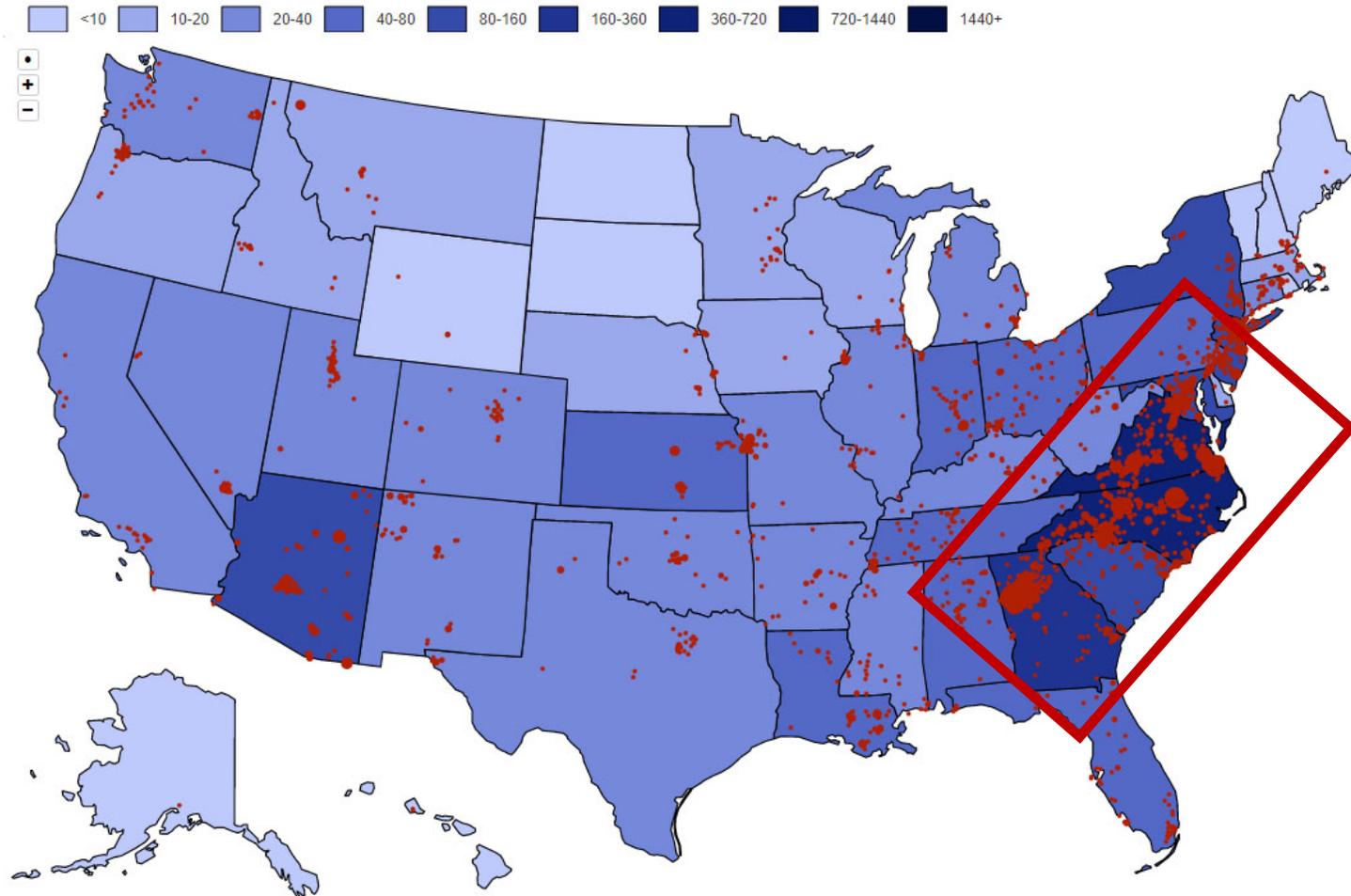
 2 hours ago  
By Karen Zaick



ew variants

# Geographic Focused Surveillance

**Week 5 (PBT1049, PBT1050, PBT1051, PBT1052)**

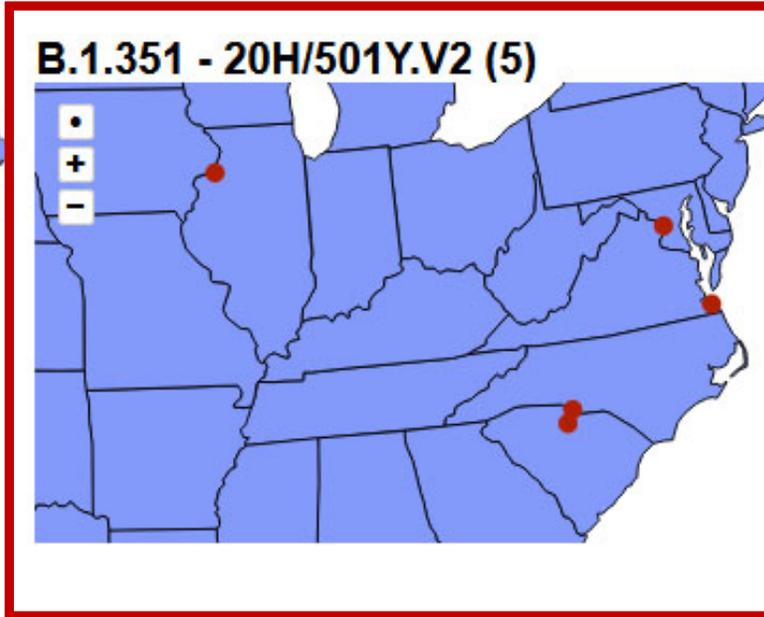


# Geographic Focused Surveillance

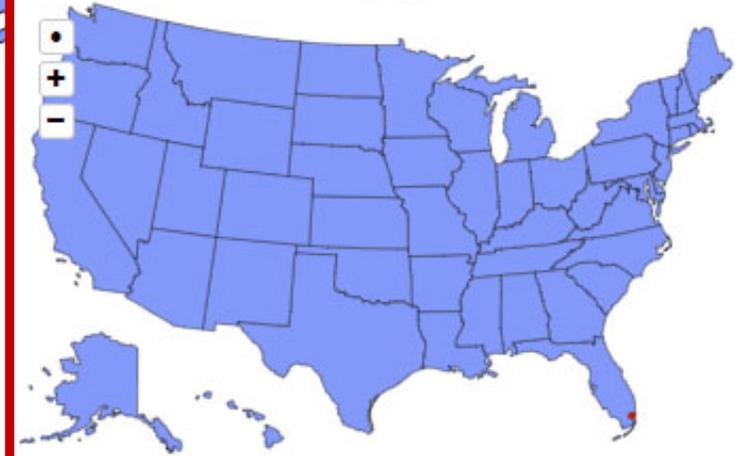
**B.1.1.7 - 20I/501Y.V1 (17)**



**B.1.351 - 20H/501Y.V2 (5)**



**P.1 - 20J/501Y.V3 (1)**



# Overview of C

- Sampling from ~
  - CDC Baseline St
  - Minimum of 10
    - ~60% Full gen
  - Results reporte
- Share Summary
- Tracking individu
- Aiding in CDC res

## CORONAVIRUS ILLINOIS

# Illinois Reports First Case of South African COVID-19 Variant

The B.1.351 variant was detected in Rock Island

Enter

Published February 11, 2021 • Updated on February 11, 2021 at 4:01 pm



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 Chicago Snowstorm Timeline: How Much Snow Your Area Could See and...

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 Chicago Snowstorm Updates: Winter Storm Warning in Effect, Dangerous Travel...

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 How Much Snow Has Fallen in Chicago – And How it Compares to Past Winters

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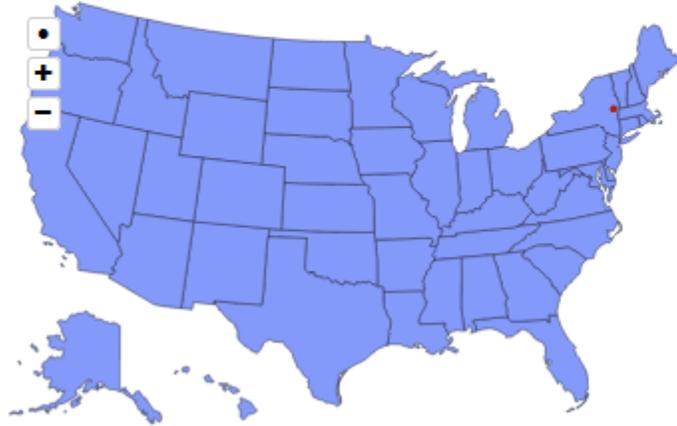
A

Illinois reported the first case of the South African COVID-19 variant Thursday, health officials

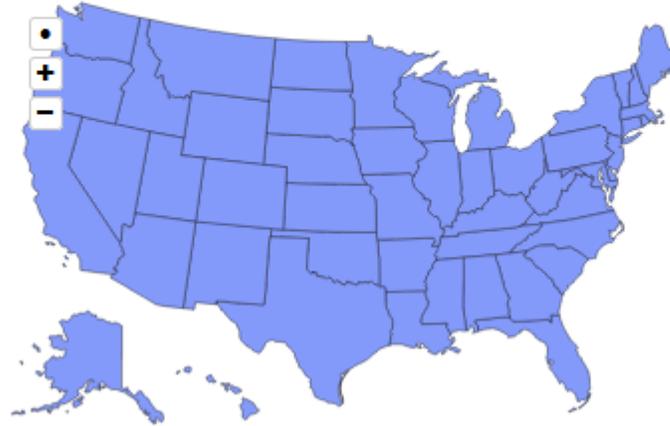
# Variants of Concern

## Variants of Concern (12/19/20 - 12/26/20)<sub>+/-</sub>

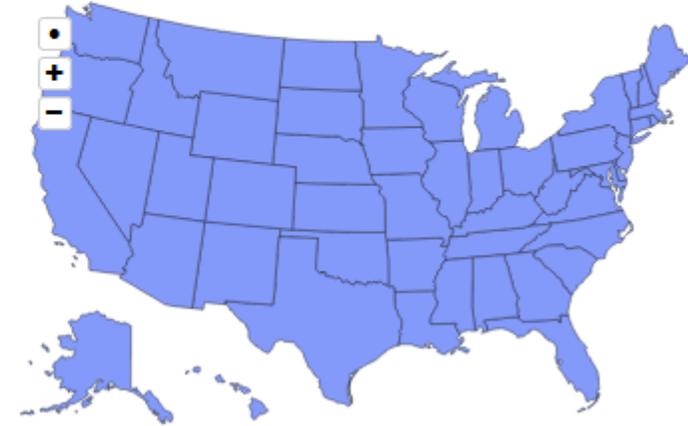
**B.1.1.7 - 20I/501Y.V1 (1)**



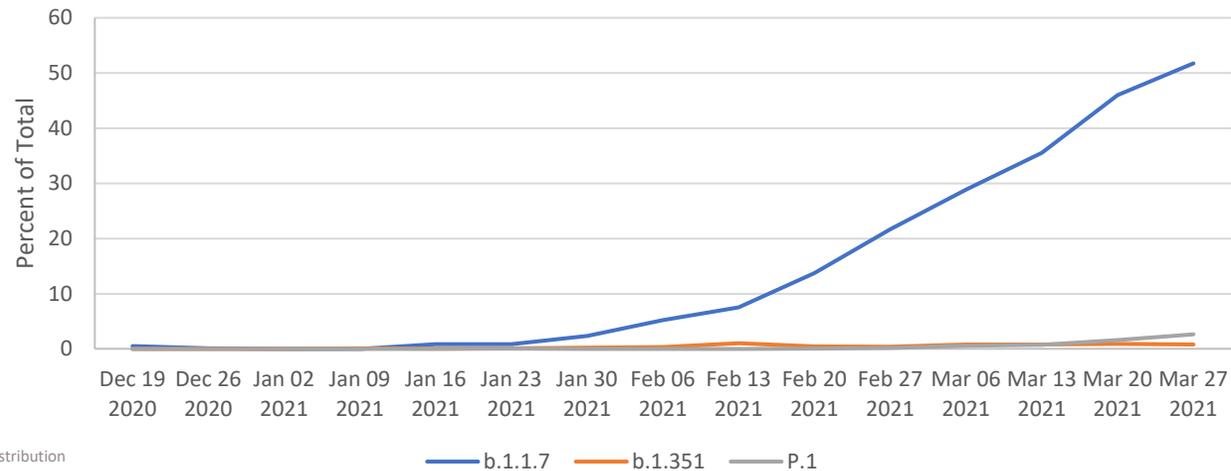
**B.1.351 - 20H/501Y.V2 (0)**



**P.1 - 20J/501Y.V3 (0)**

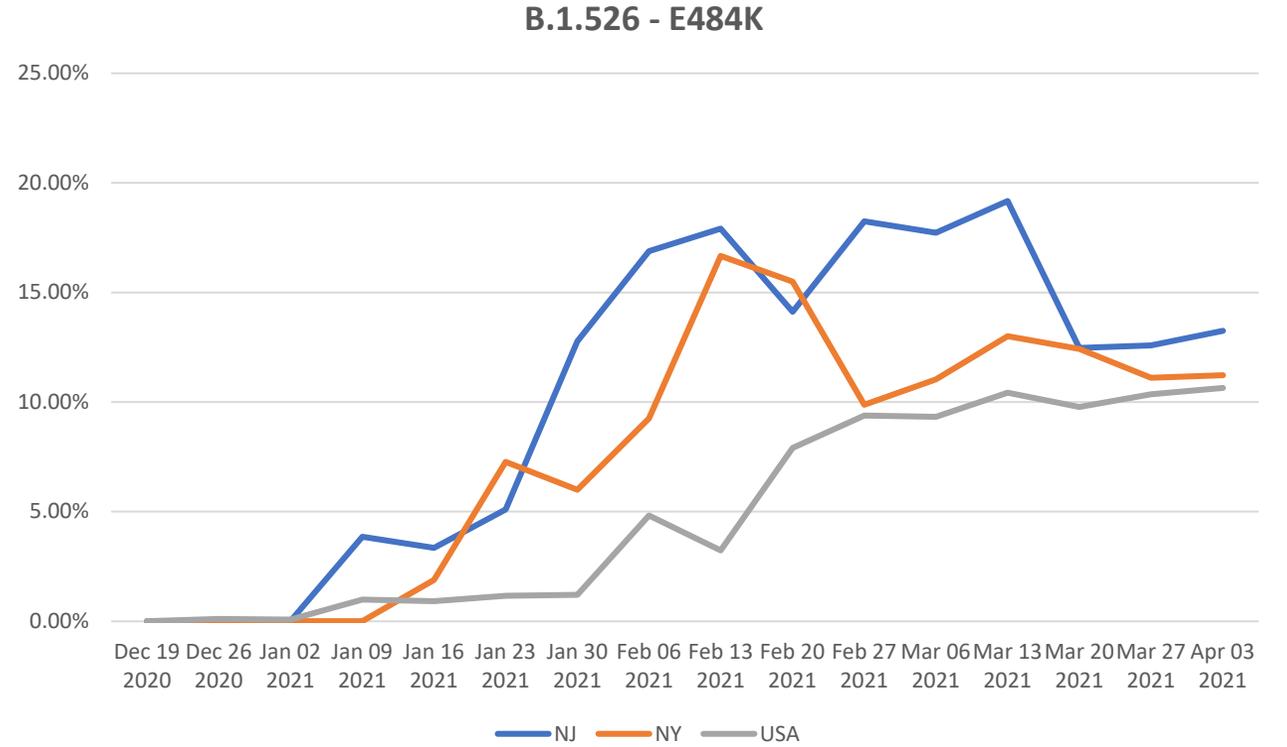
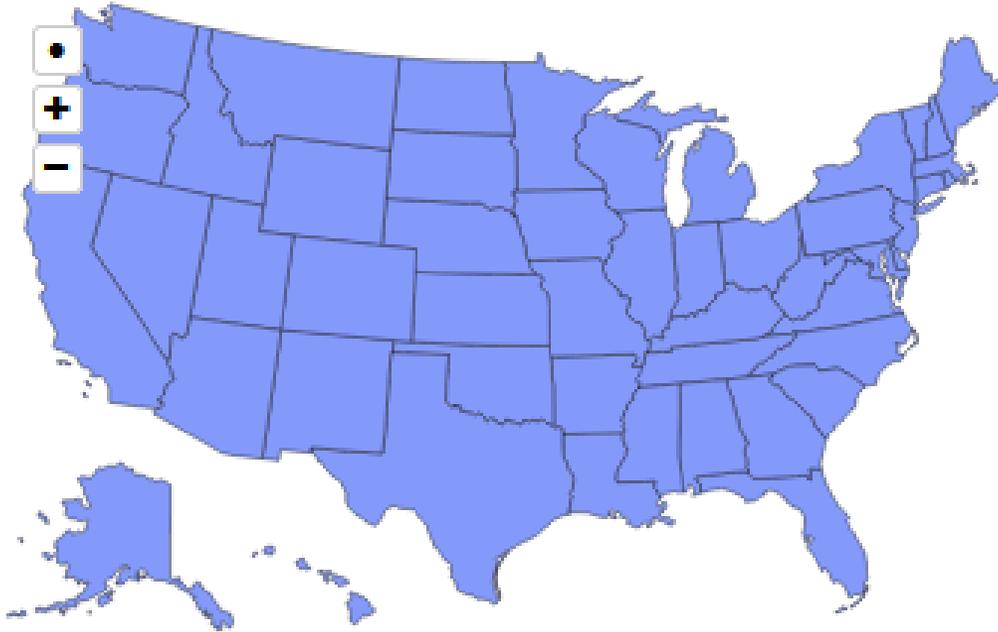


VOC

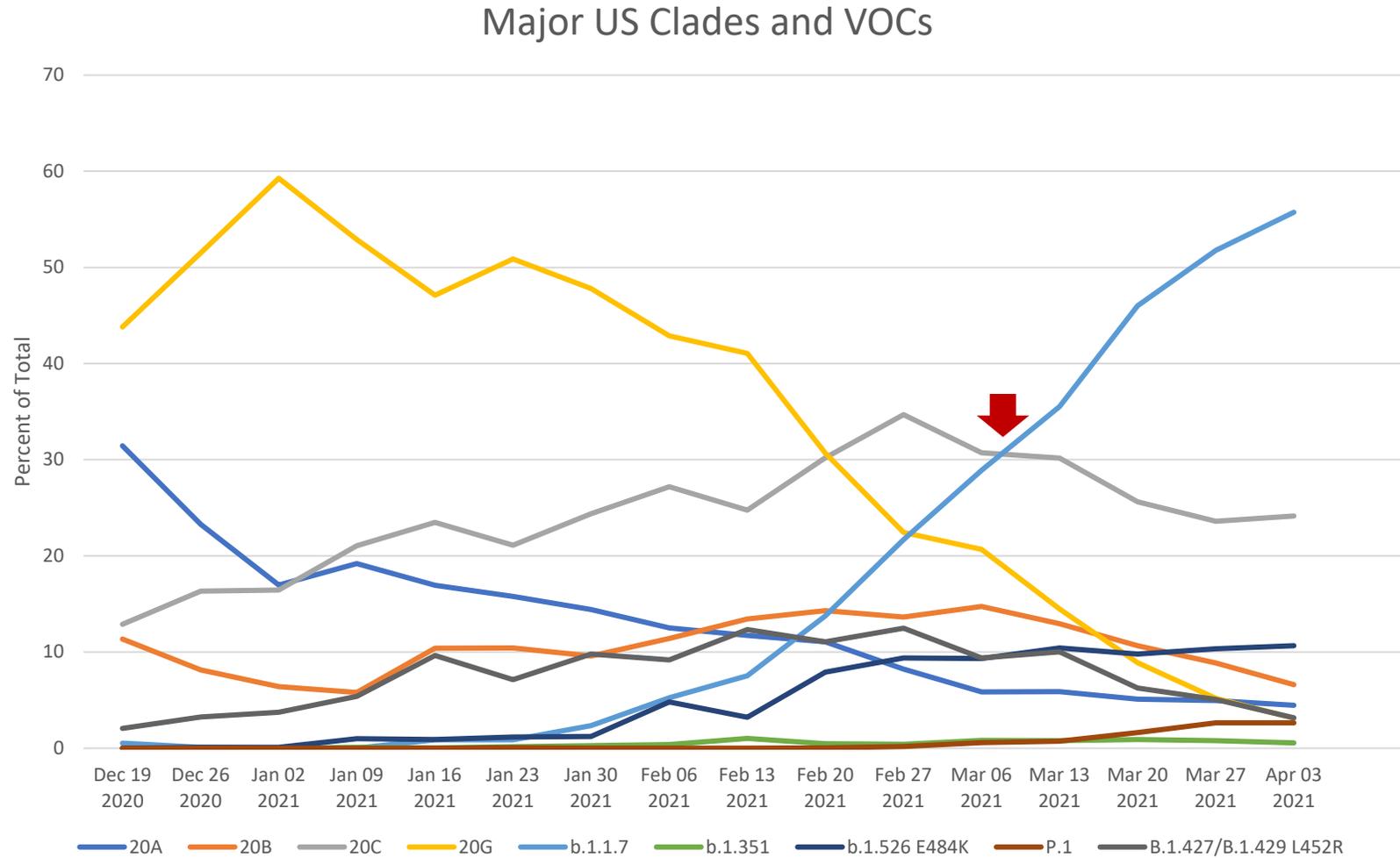


# Variants of Concern – B.1.526 E484K

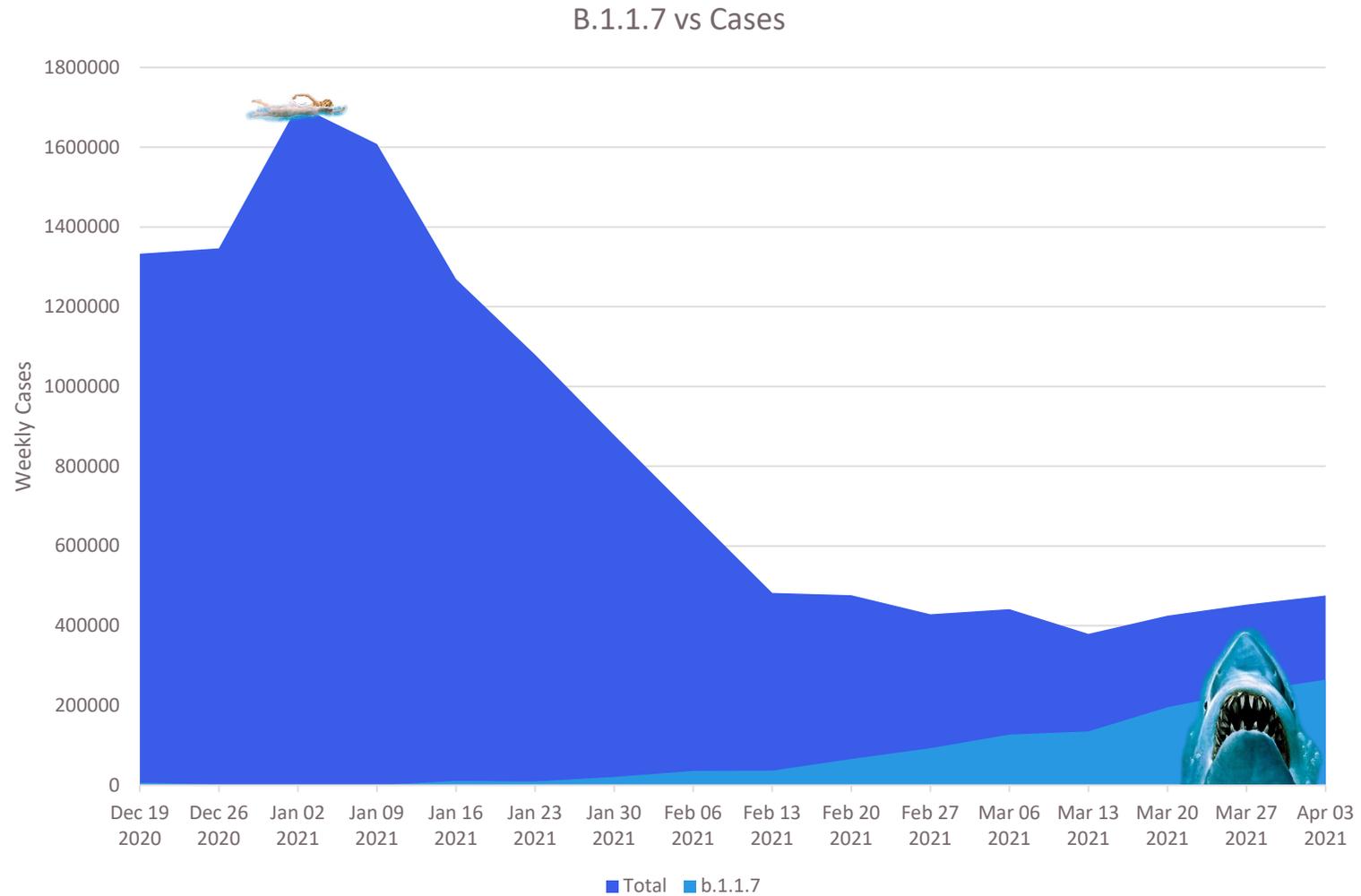
## B.1.526 - E484K (0)



# A Change in the Clade Distribution?



# B.1.1.7 Cases Are Increasing, Total Cases Have Plateaued



# Pandemic C



- Labcorp has SARS-CoV-2
- Scalable to
- Proven success
- Pacific Biosciences genotypes
- Labcorp and beginning c

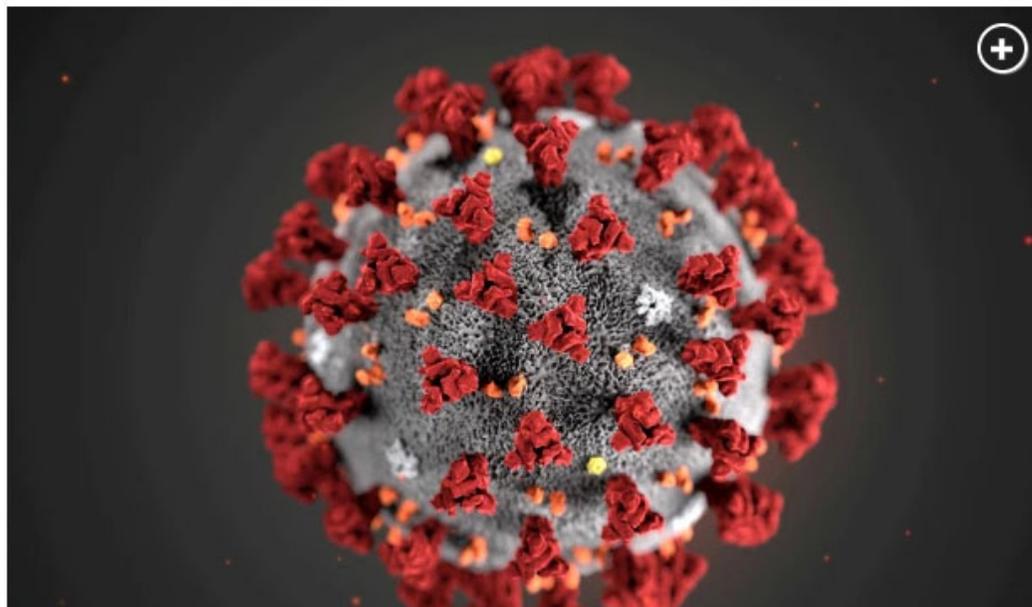
itive

NEWS

Seven new highly contagious COVID-19 variants found across US

By Aaron Feis February 15, 2021 | 1:16pm | Updated

f t r e



**TRENDING NOW**

151,366

Tekashi 6ix9ine doc director calls rapper a 'horrible human being'

---

126,912

Elon Musk wants major cryptocurrency holders to sell

---

97,511

'Gorilla Glue girl' returns home wearing sweatshirt of herself

---

72,749

Toddler drowned in pool as mom and boyfriend dozed: cops

# Acknowledgements



## Molecular Micro and Genetics R&D

Mike Levandoski PhD  
Scott Parker  
John Pruitt  
Kim Wagner  
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Thomas Urban PhD  
Amanda Suchanek PhD

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Lax Iyer PhD  
Qian Zeng PhD  
Stan Letovsky PhD

## DNA Identity

Brian Norvell  
Jon Williams PhD

**Marcia Eisenberg, PhD**  
**Chief Scientific Officer**



Elizabeth Tseng PhD  
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Jason Underwood PhD  
Andy Larrea PhD  
Jonas Korlach PhD



Duncan MacCannell PhD  
Dhwani Batra PhD  
Scott Sammons PhD

# How RT-PCR Testing Allows Labcorp to Perform National SARS-CoV-2 Pandemic Surveillance

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[h2so4hurts](#)



[/in/BrianKruegerPhD](#)

**labcorp**