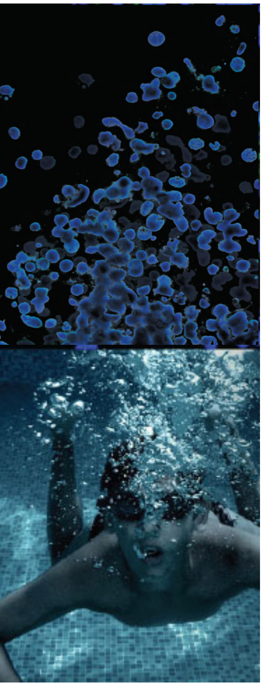

SARS-CoV-2 Reference Standards and Materials - Industry Perspectives from ATCC and BEI Resources

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Credible Leads to Incredible™

Agenda

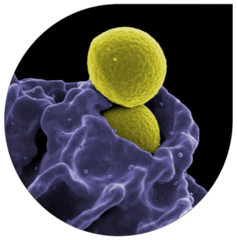
Industry Perspectives from ATCC and BEI Resources

- **History of ATCC, BEI Resources, and our respective missions**
- **Provide an overview of the available SARS-CoV-2 standards and reference materials in the ATCC and BEI catalogs**
- **Provide an overview of the NGS assembly pipeline and subsequent analysis**
- **Discuss the benefits of using NGS to authenticate our reference material**
  - Led to a discovery of a rare ORF6 deletion in the *passaged* Hong Kong isolate
- **Discuss our efforts to reduce genomic variability in cultured SARS-CoV-2**
  - Variability detected with multiplicity of infection (MOI), days post infection (dpi) and plaque picking techniques
- **The ATCC Genome Portal**
  - ATCC's enhanced authentication initiative

About ATCC

- Founded in 1925, ATCC is a not-for-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World’s largest, most diverse biological materials and information resource for microbes – the “*gold standard*”
- Innovative R&D company featuring gene editing, microbiome, NGS, and advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, microorganisms, and molecular standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

ATCC Portfolio



Credible Collections

The ATCC collection of cell and microbial reference materials remain at the heart of incredible breakthroughs in scientific exploration. ATCC is dedicated to providing biological standards backed by cutting-edge authentication techniques and essential resources that accelerate innovative research and ensure scientific reproducibility within the life sciences.

Authentication Resources

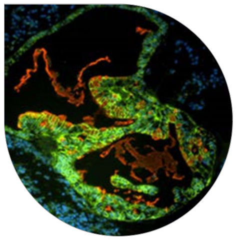
Advanced techniques in authentication allow you to test for contamination and track phenotypic or genotypic changes before they become a problem. Researchers look to ATCC for a wide range of authentication resources to safeguard reproducibility and meet requirements for funding, publication, and quality control.

Custom Solutions

Each scientific project is unique, which is why ATCC tailors custom services to the individual needs of our partners. With an unmatched combination of extensive expertise, cutting edge technologies, best practices, and a world-renowned collection of cells and microbes, ATCC is your ideal solutions partner to guide you through your next project.

Better Models

Advanced biological models enable greater specificity and functionality to the researcher's toolkit. ATCC is committed to bringing cutting-edge biological models to researchers at the forefront of extraordinary innovation and scientific progress.



Quality Standards

Your research depends on protocols and tools you can trust. As part of our mission, ATCC is a leader in the creation and maintenance of biological and published laboratory standards that protect public interests and provide quality reference material, education, accreditation, and certification services to the industry.

Biorepository Services

With nearly a century of experience in biomaterial management, ATCC continues to support global health by offering biorepository services for worldwide storage and distribution of biological materials. Whether you need a cGMP-compliant facility, small- or large-scale storage, or flexible storage choices, we can manage biomaterials to your specifications.

BEI Resources

Biodefense and Emerging Infections Resources

History

The National Institute of Allergy and Infectious Diseases (NIAID) awarded the BEI Resources contract to ATCC in 2003.

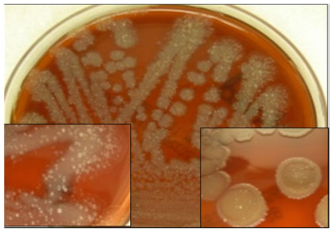
Mission of BEI Resources

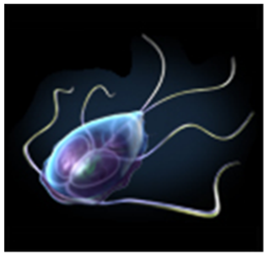
Provide NIAID with a central repository for the acquisition, authentication, production, preservation, storage, and distribution of a broad range of unique and quality assured research materials for the infectious disease research community that will aid in the development and evaluation of vaccines, therapeutics, and diagnostics.

Scope

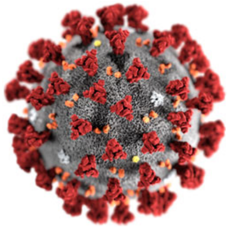
Reagents, tools and information covering NIAID’s Category A, B, and C priority pathogens, emerging infectious disease agents, non-pathogenic microbes and other microbiological materials of relevance to the research community.

Biomaterials Available Through BEI Resources

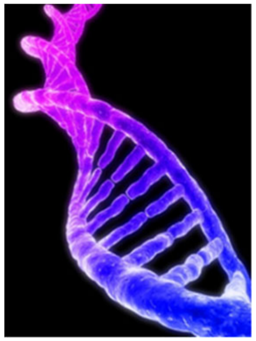


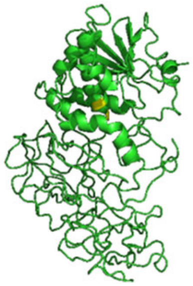


- Bacteria
- Viruses
  - **SARS-CoV-2**
- Toxins
- Pathogenic fungi
- Parasitic protists
- Parasitic worms
- Host and vector cell lines
- Arthropod vectors



- Nucleic acids
  - Genomic DNA & RNA
  - Expression vectors
  - Sequenced clones
  - Libraries and arrays
- Monoclonal antibodies
- Polyclonal antisera
- Recombinant proteins
- Synthetic peptides and arrays
- Assays & panels



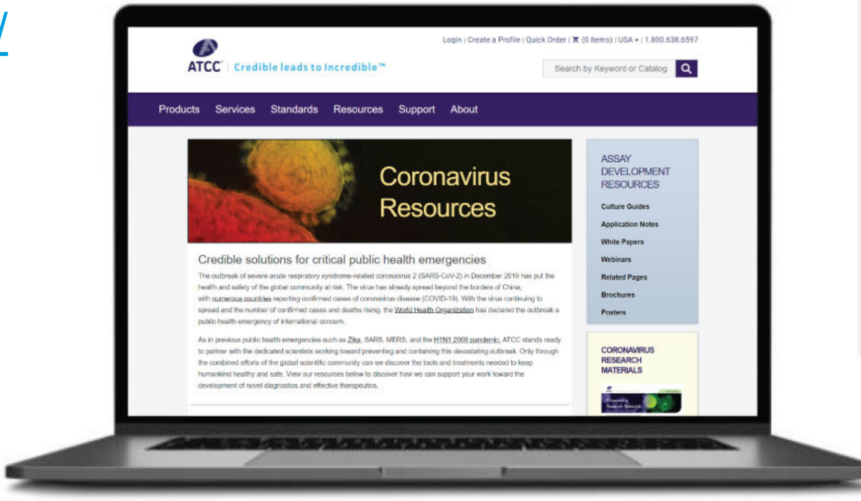


ATCC and BEI Resources SARS-CoV-2 Materials

Virus isolates, nucleic acids, synthetic molecular standards, and more

ATCC’s SARS-CoV-2 Resources

- Available SARS-CoV-2 products include:
  - Genomic RNA
  - Heat inactivated virus
  - Synthetic molecular standards
  - Cell lines, etc.
- For a comprehensive list, please visit www.atcc.org/coronavirus



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ATCC | Credible leads to Incredible™

Search by Keyword or Catalog

Products Services Standards Resources Support About

Coronavirus Resources

Credible solutions for critical public health emergencies

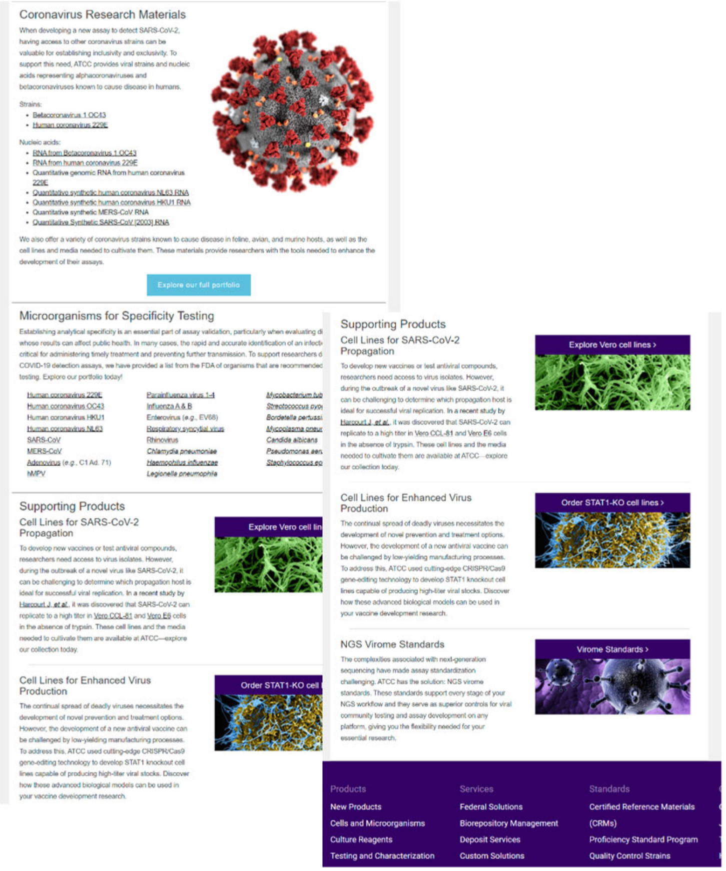
The outbreak of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) in December 2019 has put the health and safety of the global community at risk. The virus has already spread beyond the borders of China, with numerous countries reporting confirmed cases of coronavirus disease (COVID-19). With the virus continuing to spread and the number of confirmed cases and deaths rising, the World Health Organization has declared the outbreak a public health emergency of international concern.

As in previous public health emergencies such as Zika, SARS, MERS, and the H1N1 2009 pandemic, ATCC stands ready to partner with the dedicated scientists working toward preventing and containing this devastating outbreak. Only through the combined efforts of the global scientific community can we discover the tools and treatments needed to keep humankind healthy and safe. View our resources below to discover how we can support your work toward the development of novel diagnostics and effective therapeutics.

ASSAY DEVELOPMENT RESOURCES

- Culture Guides
- Application Notes
- White Papers
- Webinars
- Related Pages
- Brochures
- Posters

CORONAVIRUS RESEARCH MATERIALS



Coronavirus Research Materials

When developing a new assay to detect SARS-CoV-2, having access to other coronavirus strains can be valuable for establishing inclusivity and exclusivity. To support this need, ATCC provides viral strains and nucleic acids representing alphacoronaviruses and betacoronaviruses known to cause disease in humans.

Strains:
- Betacoronavirus 1 OC43
- Human coronavirus 229E

Nucleic acids:
- RNA from Betacoronavirus 1 OC43
- RNA from human coronavirus 229E
- Quantitative genomic RNA from human coronavirus 229E
- Quantitative synthetic human coronavirus NL63 RNA
- Quantitative synthetic human coronavirus HKU1 RNA
- Quantitative synthetic MERS-CoV RNA
- Quantitative Synthetic SARS-CoV [2003] RNA

We also offer a variety of coronavirus strains known to cause disease in feline, avian, and murine hosts, as well as the cell lines and media needed to cultivate them. These materials provide researchers with the tools needed to enhance the development of their assays.

Explore our full portfolio

Microorganisms for Specificity Testing

Establishing analytical specificity is an essential part of assay validation, particularly when evaluating di whose results can affect public health. In many cases, the rapid and accurate identification of an infecti critical for administering timely treatment and preventing further transmission. To support researchers d COVID-19 detection assays, we have provided a list from the FDA of organisms that are recommended testing. Explore our portfolio today!

Human coronavirus 229E
Human coronavirus OC43
Human coronavirus HKU1
Human coronavirus NL63
SARS-CoV
MERS-CoV
Adenovirus (*e.g.*, C1 Ad. 71)
hMPV

Parainfluenza virus 1-4
Influenza A & B
Enterovirus (*e.g.*, EV68)
Respiratory syncytial virus
Rhinovirus
Chlamydia pneumoniae
Haemophilus influenzae
Legionella pneumophila

Mycobacterium tub
Streptococcus pyo
Bordetella pertussi
Mycoplasma pneu
Candida albicans
Pseudomonas aer
Staphylococcus ep

Supporting Products

Cell Lines for SARS-CoV-2 Propagation

To develop new vaccines or test antiviral compounds, researchers need access to virus isolates. However, during the outbreak of a novel virus like SARS-CoV-2, it can be challenging to determine which propagation host is ideal for successful viral replication. In a recent study by Harcourt J, *et al.*, it was discovered that SARS-CoV-2 can replicate to a high titer in Vero CCL-81 and Vero E6 cells in the absence of trypsin. These cell lines and the media needed to cultivate them are available at ATCC—explore our collection today.

Explore Vero cell lin

Cell Lines for Enhanced Virus Production

The continual spread of deadly viruses necessitates the development of novel prevention and treatment options. However, the development of a new antiviral vaccine can be challenged by low-yielding manufacturing processes. To address this, ATCC used cutting-edge CRISPR/Cas9 gene-editing technology to develop STAT1 knockout cell lines capable of producing high-titer viral stocks. Discover how these advanced biological models can be used in your vaccine development research.

Order STAT1-KO cell

Supporting Products

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Explore Vero cell lines ›

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Order STAT1-KO cell lines ›

NGS Virome Standards

The complexities associated with next-generation sequencing have made assay standardization challenging. ATCC has the solution: NGS virome standards. These standards support every stage of your NGS workflow and they serve as superior controls for viral community testing and assay development on any platform, giving you the flexibility needed for your essential research.

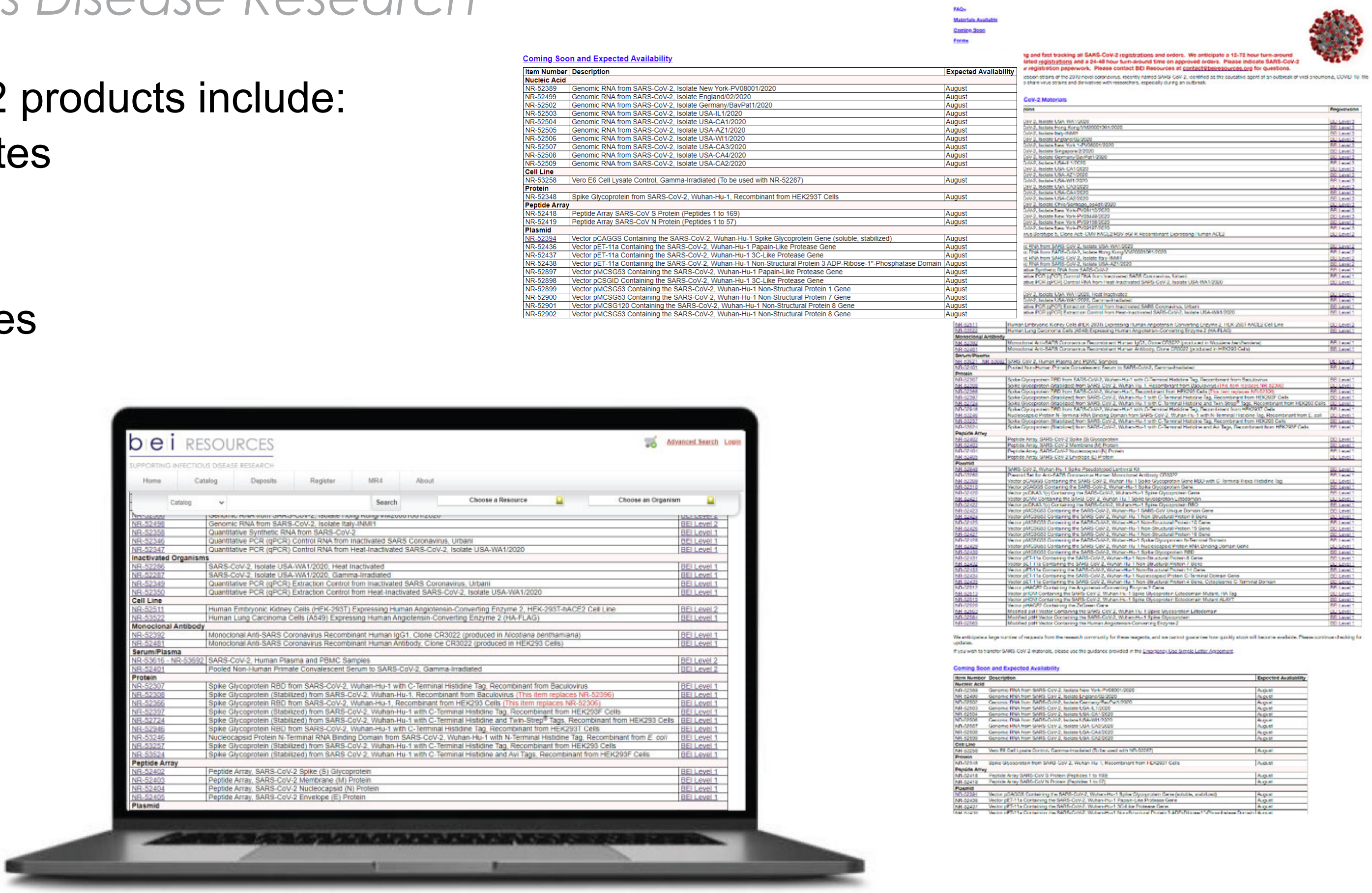
Virome Standards ›

Products	Services	Standards
New Products	Federal Solutions	Certified Reference Materials (CRMs)
Cells and Microorganisms	Biorepository Management	Proficiency Standard Program
Culture Reagents	Deposit Services	Quality Control Strains
Testing and Characterization	Custom Solutions	

BEI Resources’ SARS-CoV-2 Resources

Supporting Infectious Disease Research

- Available SARS-CoV-2 products include:
  - –19 distinct viral isolates
  - –Nucleic acids
  - –Inactivated virus
  - –Monoclonal antibodies
  - –Serum/plasma
  - –Proteins, peptide arrays, plasmids, cell lines, etc.
- For a comprehensive list, please visit www.beiresources.org



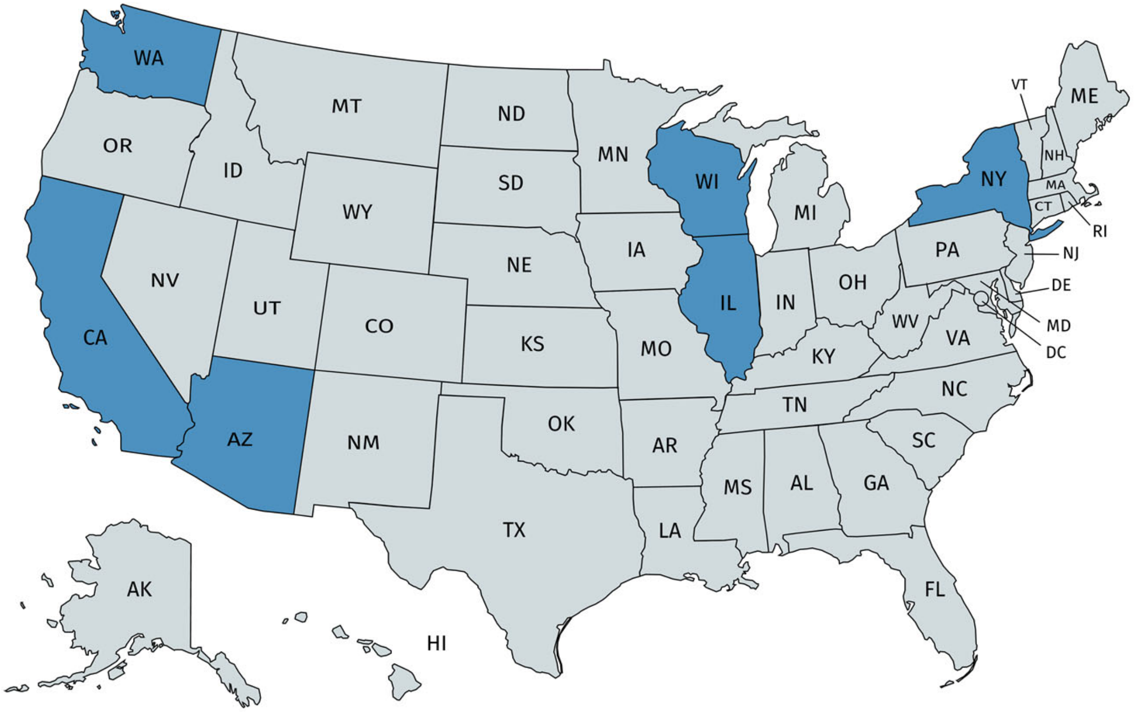
NR-52498	Genomic RNA from SARS-CoV-2, Isolate Italy-INMI1	BEI Level 2
NR-52358	Quantitative Synthetic RNA from SARS-CoV-2	BEI Level 1
NR-52346	Quantitative PCR (qPCR) Control RNA from Inactivated SARS Coronavirus, Urbani	BEI Level 1
NR-52347	Quantitative PCR (qPCR) Control RNA from Heat-Inactivated SARS-CoV-2, Isolate USA-WA1/2020	BEI Level 1
Inactivated Organisms		
NR-52286	SARS-CoV-2, Isolate USA-WA1/2020, Heat Inactivated	BEI Level 1
NR-52287	SARS-CoV-2, Isolate USA-WA1/2020, Gamma-Irradiated	BEI Level 1
NR-52349	Quantitative PCR (qPCR) Extraction Control from Inactivated SARS Coronavirus, Urbani	BEI Level 1
NR-52350	Quantitative PCR (qPCR) Extraction Control from Heat-Inactivated SARS-CoV-2, Isolate USA-WA1/2020	BEI Level 1
Cell Line		
NR-52511	Human Embryonic Kidney Cells (HEK-293T) Expressing Human Angiotensin-Converting Enzyme 2, HEK-293T-hACE2 Cell Line	BEI Level 2
NR-53522	Human Lung Carcinoma Cells (A549) Expressing Human Angiotensin-Converting Enzyme 2 (HA-FLAG)	BEI Level 1
Monoclonal Antibody		
NR-52392	Monoclonal Anti-SARS Coronavirus Recombinant Human IgG1, Clone CR3022 (produced in *Nicotiana benthamiana*)	BEI Level 1
NR-52481	Monoclonal Anti-SARS Coronavirus Recombinant Human Antibody, Clone CR3022 (produced in HEK293 Cells)	BEI Level 1
Serum/Plasma		
NR-53616 - NR-53692	SARS-CoV-2, Human Plasma and PBMC Samples	BEI Level 2
NR-52401	Pooled Non-Human Primate Convalescent Serum to SARS-CoV-2, Gamma-Irradiated	BEI Level 2
Protein		
NR-52307	Spike Glycoprotein RBD from SARS-CoV-2, Wuhan-Hu-1 with C-Terminal Histidine Tag, Recombinant from Baculovirus	BEI Level 1
NR-52308	Spike Glycoprotein (Stabilized) from SARS-CoV-2, Wuhan-Hu-1, Recombinant from Baculovirus (This item replaces NR-52396)	BEI Level 1
NR-52366	Spike Glycoprotein RBD from SARS-CoV-2, Wuhan-Hu-1, Recombinant from HEK293 Cells (This item replaces NR-52306)	BEI Level 1
NR-52397	Spike Glycoprotein (Stabilized) from SARS-CoV-2, Wuhan-Hu-1 with C-Terminal Histidine Tag, Recombinant from HEK293F Cells	BEI Level 1
NR-52724	Spike Glycoprotein (Stabilized) from SARS-CoV-2, Wuhan-Hu-1 with C-Terminal Histidine and Twin-Strep® Tags, Recombinant from HEK293 Cells	BEI Level 1
NR-52946	Spike Glycoprotein RBD from SARS-CoV-2, Wuhan-Hu-1 with C-Terminal Histidine Tag, Recombinant from HEK293T Cells	BEI Level 1
NR-53246	Nucleocapsid Protein N-Terminal RNA Binding Domain from SARS-CoV-2, Wuhan-Hu-1 with N-Terminal Histidine Tag, Recombinant from *E. coli*	BEI Level 1
NR-53257	Spike Glycoprotein (Stabilized) from SARS-CoV-2, Wuhan-Hu-1 with C-Terminal Histidine Tag, Recombinant from HEK293 Cells	BEI Level 1
NR-53524	Spike Glycoprotein (Stabilized) from SARS-CoV-2, Wuhan-Hu-1 with C-Terminal Histidine and Avi Tags, Recombinant from HEK293F Cells	BEI Level 1
Peptide Array		
NR-52402	Peptide Array, SARS-CoV-2 Spike (S) Glycoprotein	BEI Level 1
NR-52403	Peptide Array, SARS-CoV-2 Membrane (M) Protein	BEI Level 1
NR-52404	Peptide Array, SARS-CoV-2 Nucleocapsid (N) Protein	BEI Level 1
NR-52405	Peptide Array, SARS-CoV-2 Envelope (E) Protein	BEI Level 1
Plasmid		

Coming Soon and Expected Availability

Item Number	Description	Expected Availability
Nucleic Acid		
NR-52389	Genomic RNA from SARS-CoV-2, Isolate New York-PV08001/2020	August
NR-52499	Genomic RNA from SARS-CoV-2, Isolate England/02/2020	August
NR-52502	Genomic RNA from SARS-CoV-2, Isolate Germany/BavPat1/2020	August
NR-52503	Genomic RNA from SARS-CoV-2, Isolate USA-IL1/2020	August
NR-52504	Genomic RNA from SARS-CoV-2, Isolate USA-CA1/2020	August
NR-52505	Genomic RNA from SARS-CoV-2, Isolate USA-AZ1/2020	August
NR-52506	Genomic RNA from SARS-CoV-2, Isolate USA-WI1/2020	August
NR-52507	Genomic RNA from SARS-CoV-2, Isolate USA-CA3/2020	August
NR-52508	Genomic RNA from SARS-CoV-2, Isolate USA-CA4/2020	August
NR-52509	Genomic RNA from SARS-CoV-2, Isolate USA-CA2/2020	August
Cell Line		
NR-53258	Vero E6 Cell Lysate Control, Gamma-Irradiated (To be used with NR-52287)	August
Protein		
NR-52348	Spike Glycoprotein from SARS-CoV-2, Wuhan-Hu-1, Recombinant from HEK293T Cells	August
Peptide Array		
NR-52418	Peptide Array SARS-CoV S Protein (Peptides 1 to 169)	August
NR-52419	Peptide Array SARS-CoV N Protein (Peptides 1 to 57)	August
Plasmid		
NR-52394	Vector pCAGGS Containing the SARS-CoV-2, Wuhan-Hu-1 Spike Glycoprotein Gene (soluble, stabilized)	August
NR-52436	Vector pET-11a Containing the SARS-CoV-2, Wuhan-Hu-1 Papain-Like Protease Gene	August
NR-52437	Vector pET-11a Containing the SARS-CoV-2, Wuhan-Hu-1 3C-Like Protease Gene	August
NR-52438	Vector pET-11a Containing the SARS-CoV-2, Wuhan-Hu-1 Non-Structural Protein 3 ADP-Ribose-1"-Phosphatase Domain	August
NR-52897	Vector pMCSG53 Containing the SARS-CoV-2, Wuhan-Hu-1 Papain-Like Protease Gene	August
NR-52898	Vector pCSGID Containing the SARS-CoV-2, Wuhan-Hu-1 3C-Like Protease Gene	August
NR-52899	Vector pMCSG53 Containing the SARS-CoV-2, Wuhan-Hu-1 Non-Structural Protein 1 Gene	August
NR-52900	Vector pMCSG53 Containing the SARS-CoV-2, Wuhan-Hu-1 Non-Structural Protein 7 Gene	August
NR-52901	Vector pMCSG120 Containing the SARS-CoV-2, Wuhan-Hu-1 Non-Structural Protein 8 Gene	August
NR-52902	Vector pMCSG53 Containing the SARS-CoV-2, Wuhan-Hu-1 Non-Structural Protein 8 Gene	August

Domestic SARS-CoV-2 Isolates Accessioned To-Date

Producing SARS-CoV-2 Isolates



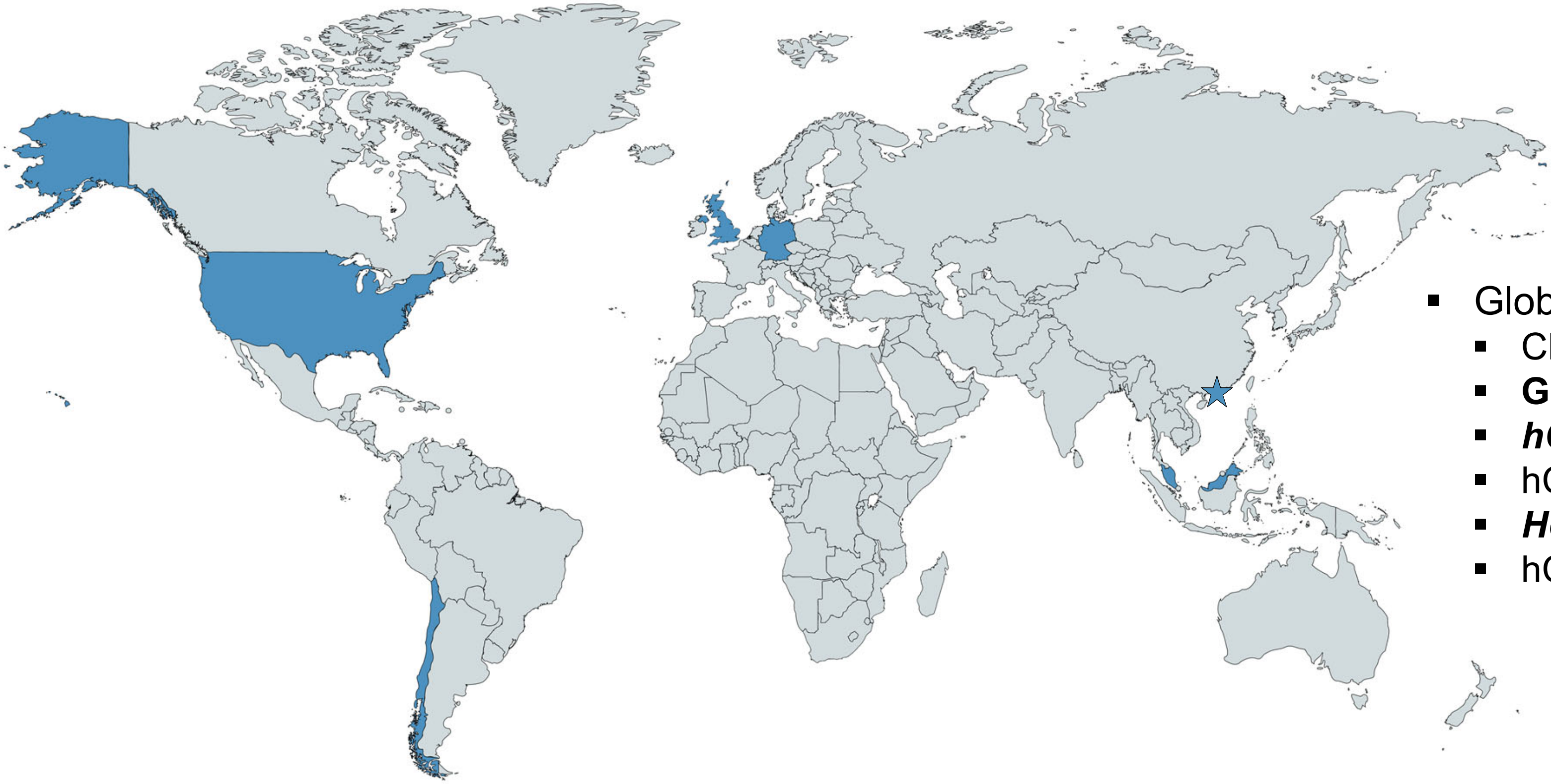
- United States isolates (12)
  - ***SARS-CoV-2/human/USA/USA-WA1/2020****
  - hCoV-19/USA/AZ1/2020
  - hCoV-19/USA/CA1/2020
  - hCoV-19/USA/CA2/2020
  - hCoV-19/USA/CA3/2020
  - hCoV-19/USA/CA4/2020
  - hCoV-19/USA/IL1/2020
  - hCoV-19/USA/NY-PV08410/2020
  - hCoV-19/USA/NY-PV08449/2020
  - hCoV-19/USA/NY-PV09158/2020
  - hCoV-19/USA/NY-PV09197/2020
  - hCoV-19/USA/WI1/2020

All available as isolates from BEI Resources
* = cultured genome sequence publicly available

Italicized = available as gRNA from BEI Resources
Bold = also available as gRNA from ATCC

Global SARS-CoV-2 Isolates Accessioned To-Date

Producing SARS-CoV-2 Isolate

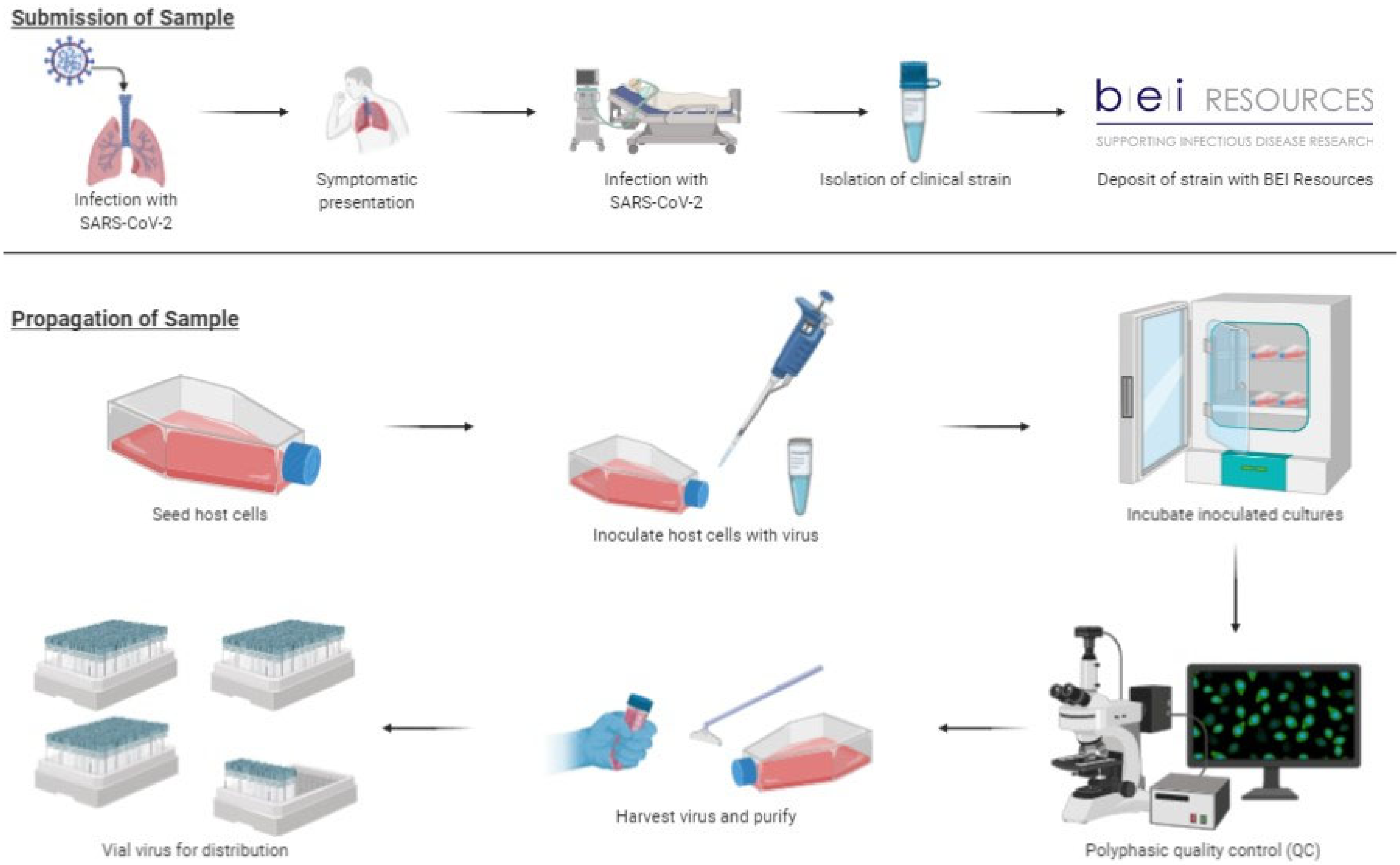


- Global isolates (6)
  - Chile/Santiago_op4d1/2020
  - **Germany/BavPat1/2020**
  - ***hCoV-19/Italy/INMI1/2020***
  - hCoV-19/England/02/2020
  - ***Hong Kong/VM20001061/2020****
  - hCoV-19/Singapore/2/2020

All available as isolates from BEI Resources
* = cultured genome sequence publicly available

Italicized = available as gRNA from BEI Resources
Bold = also available as gRNA from ATCC

The SARS-CoV-2 Production Process Summarized



Submission of Sample

Infection with SARS-CoV-2

Symptomatic presentation

Infection with SARS-CoV-2

Isolation of clinical strain

Deposit of strain with BEI Resources

Propagation of Sample

Seed host cells

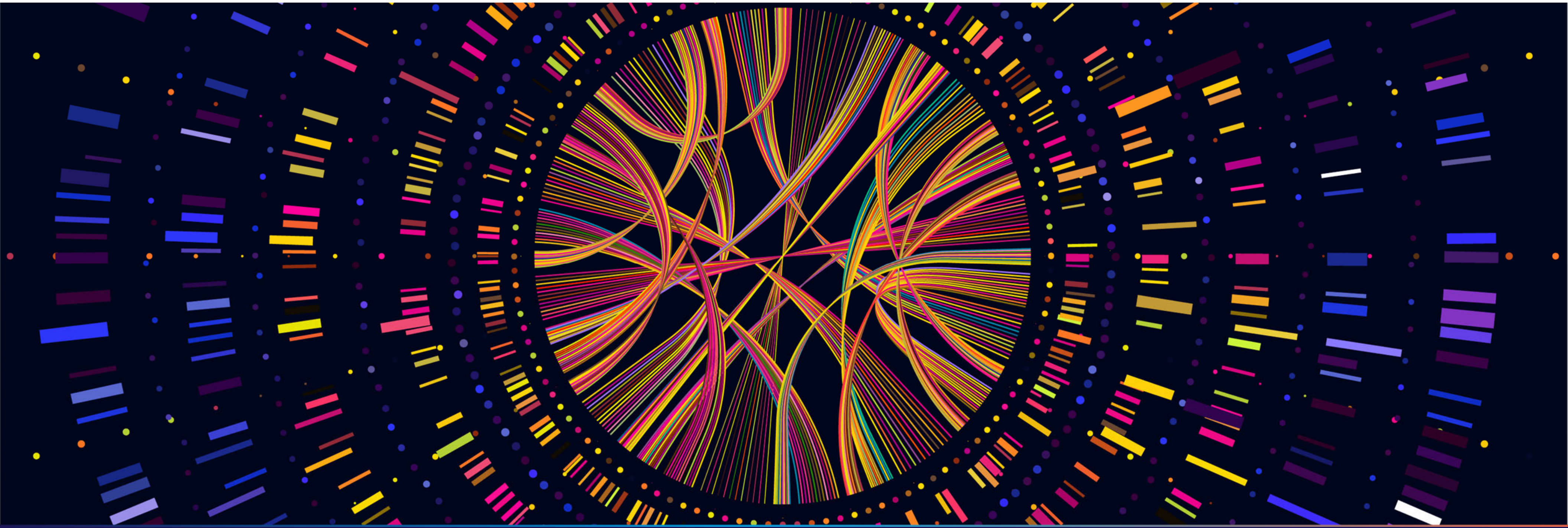
Inoculate host cells with virus

Incubate inoculated cultures

Polyphasic quality control (QC)

Harvest virus and purify

Vial virus for distribution

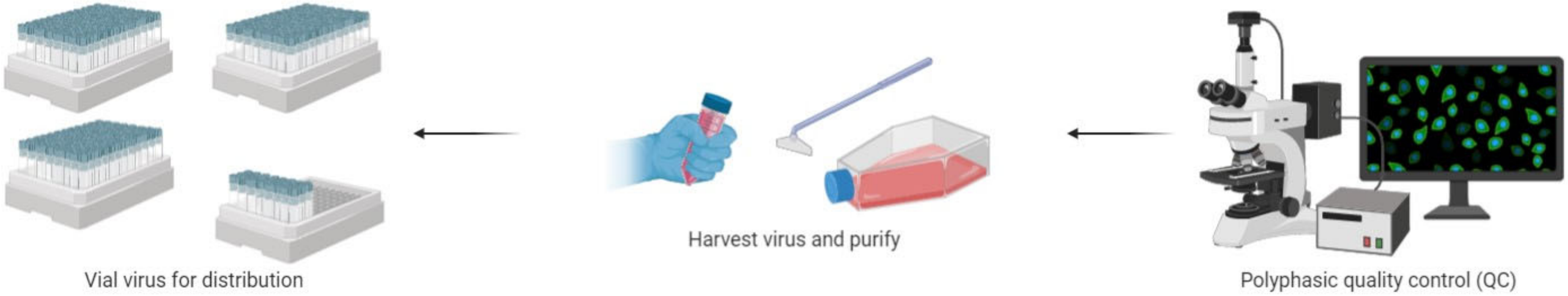


Sequencing SARS-CoV-2 Isolates

Insight from isolate sequencing, variant detection, and variant functional effects

NGS: Critical to SARS-CoV-2 Quality Control

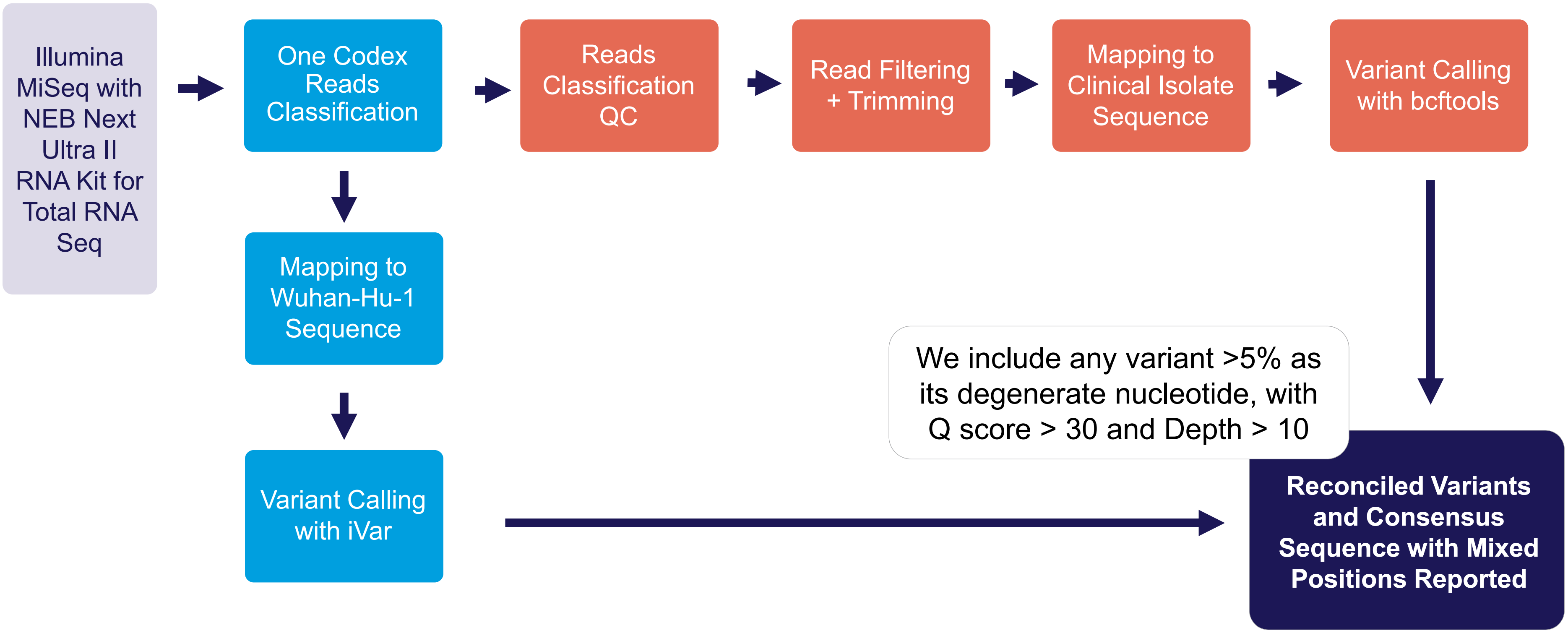
Sequencing SARS-CoV-2 Isolates



- **Maximizing genomic fidelity** to the clinical isolate is critical when producing SARS-CoV-2 research material and standards material
- Most effective manner to characterize genomic fidelity is next generation sequencing
- The COVID-19 pandemic has brought the importance of viral sequencing and genome variant detection to the forefront of microbiology

Reference-Based RNA Virus Pipeline

Sequencing SARS-CoV-2 Isolates



Illumina MiSeq with NEB Next Ultra II RNA Kit for Total RNA Seq

One Codex Reads Classification

Mapping to Wuhan-Hu-1 Sequence

Variant Calling with iVar

Reads Classification QC

Read Filtering + Trimming

Mapping to Clinical Isolate Sequence

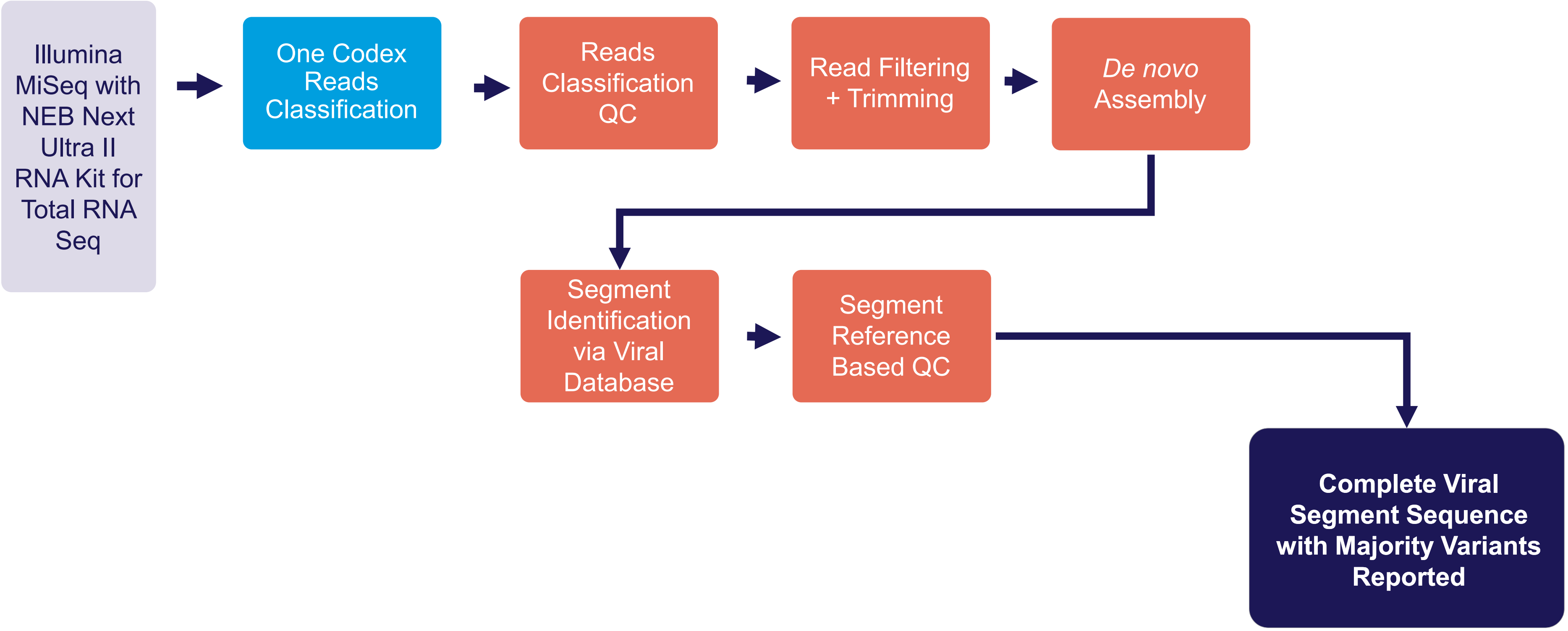
Variant Calling with bcftools

We include any variant >5% as its degenerate nucleotide, with Q score > 30 and Depth > 10

Reconciled Variants and Consensus Sequence with Mixed Positions Reported

De novo-Based RNA Virus Pipeline

Sequencing SARS-CoV-2 Isolates



Illumina MiSeq with NEB Next Ultra II RNA Kit for Total RNA Seq

One Codex Reads Classification

Reads Classification QC

Read Filtering + Trimming

De novo Assembly

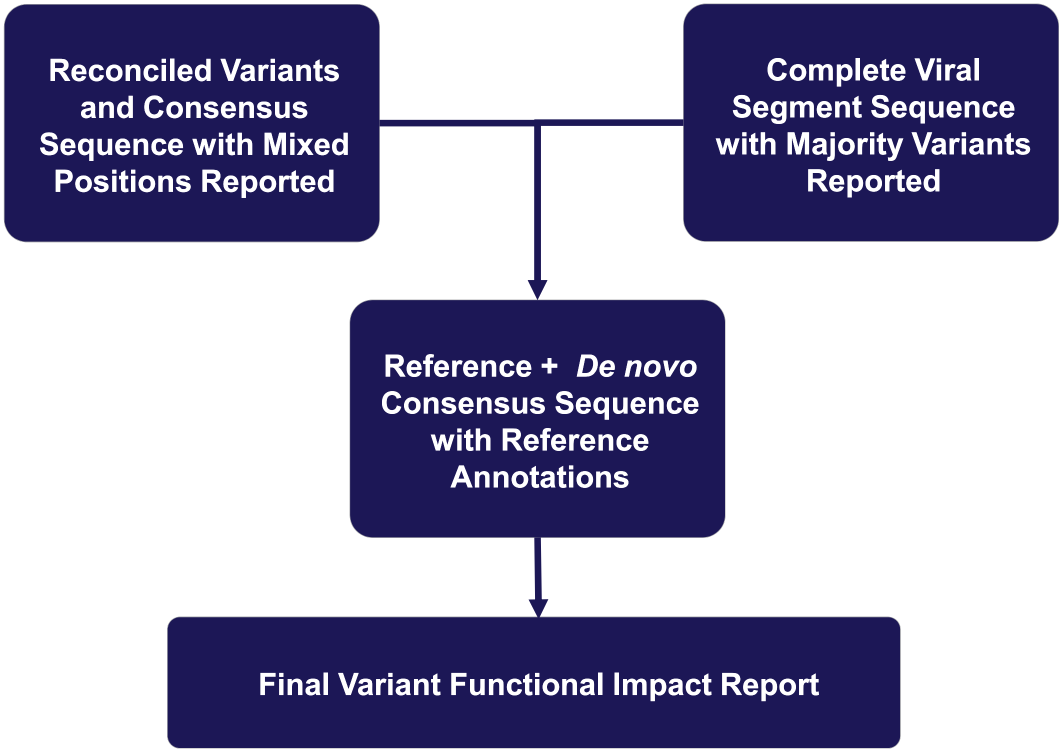
Segment Identification via Viral Database

Segment Reference Based QC

Complete Viral Segment Sequence with Majority Variants Reported

Cross Comparison, Annotation, and Functional Assessment

Sequencing SARS-CoV-2 Isolates



Reconciled Variants and Consensus Sequence with Mixed Positions Reported

Complete Viral Segment Sequence with Majority Variants Reported

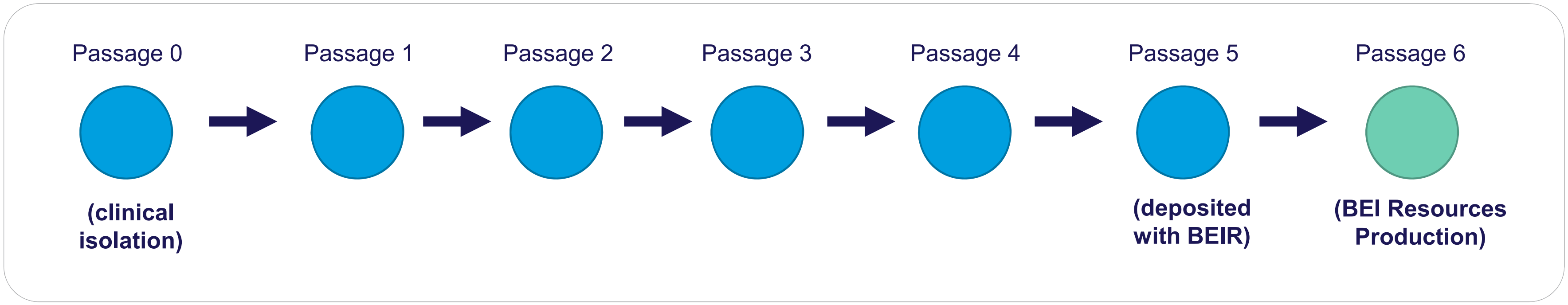
Reference + *De novo* Consensus Sequence with Reference Annotations

Final Variant Functional Impact Report

Hong Kong Isolate (BEI Resources NR-52282 + ATCC® VR-1991D™)

Sequencing SARS-CoV-2 Isolates

- Originally isolated from a nasopharyngeal aspirate and throat swab from an adult male patient in Hong Kong on January 22, 2020
- Passaged 5 times at the Hong Kong University in Vero E6 cells prior to its deposit with BEI Resources
- Complete genome of the SARS-CoV-2 Hong Kong/VM20001061/2020 clinical isolate (Passage 0) was previously sequenced (GISAID: EPI_ISL_412028) and served as the reference genome



Passage 0 **(clinical isolation)** → Passage 1 → Passage 2 → Passage 3 → Passage 4 → Passage 5 **(deposited with BEIR)** → Passage 6 **(BEI Resources Production)**

Hong Kong Isolate (BEI Resources NR-52282 + ATCC® VR-1991D™)

Sequencing SARS-CoV-2 Isolates

- Passage 6 was produced by BEI Resources then sequenced by the ATCC Sequencing and Bioinformatics Center (SBC)
- We noted an unexpected 27 nucleotide deletion in the ORF6 region
  - Confirmed with Sanger sequencing

- Was this present before p6 production?
- How common is this mutation?
- What are the structural/functional implications?

Variants to p0	Frequency	Gene	Mutation
c12919t	0.62	ORF1ab (nsp9)	Silent
c21636t	0.87	S	P25L
g23607a	0.96	S	R682Q
y24034t	1.00	S	--
c24566g	0.73	S	Q1002E (Conservative)
Δ27264-27290	**0.71**	**ORF6**	**Δ22-30 (ΔFKVSIWNLD)**
t29862g	1.00	3' UTR	Untranslated

Hong Kong Isolate (BEI Resources NR-52282 + ATCC® VR-1991D™)

Sequencing SARS-CoV-2 Isolates

- Was this present before p6 production? **Yes, the deposited material possessed the deletion**

Variant	p5 Frequency	p6 Frequency	Δ Frequency
c12919t	0.27	0.62	▲ 0.35
c21636t	0.79	0.87	▲ 0.08
g23607a	0.81	0.96	▲ 0.15
y24034t	1.00	1.00	0
c24566g	0.28	0.73	▲ 0.45
Δ27264-27290	**0.51**	**0.71**	▲ **0.20**
t29862g	1.00	1.00	0

- How common is this mutation? **Rare in GISAID + NCBI**
- Of 24,456 strains in GISAID + NCBI (at time of access: May 13, 2020) only **two** strains contained this mutation
  - hCoV-19/England/CAMB-722A9/2020 (EPI_ISL_439593)
  - hCoV-19/England/CAMB-77F07/2020 (EPI_ISL_441819)

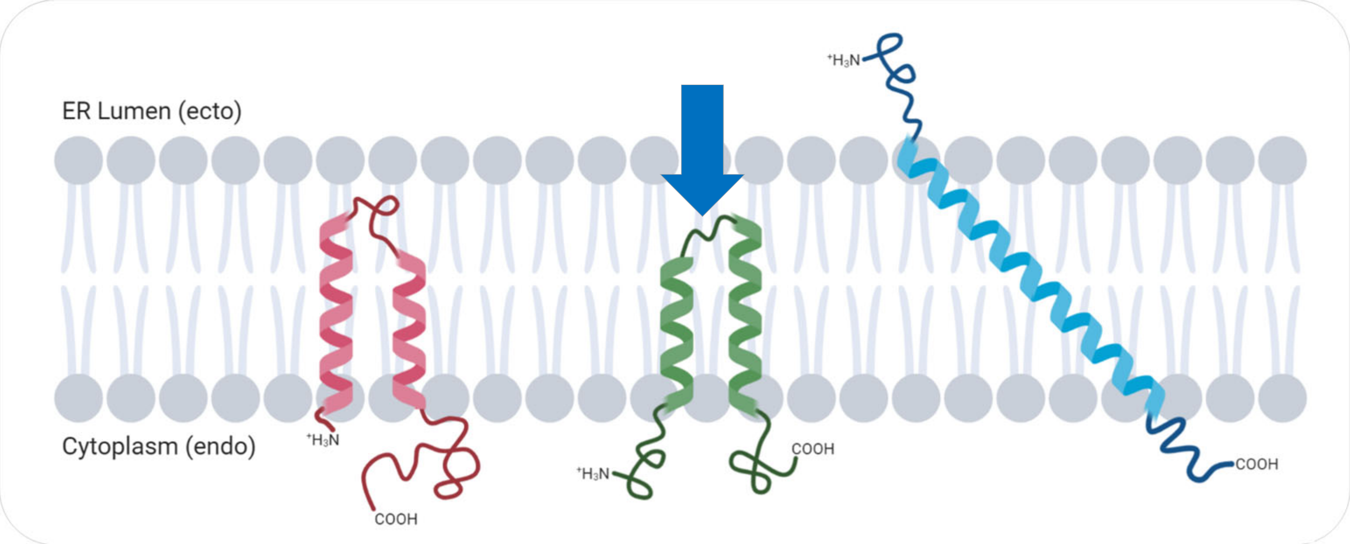
Hong Kong Isolate (BEI Resources NR-52282 + ATCC® VR-1991D™)

Sequencing SARS-CoV-2 Isolates

- Structural implications
  - Protein structure predicted with I-TASSER
    - 2 alpha helices → 1 alpha helix
  - Transmembrane localization predicted with TMHMM2.0
    - N-endo C-endo (embed) → N-ecto C-ecto (trans)

- Functional implications
  - ORF6 plays a role in interferon (IFN) response in SARS-CoV
  - C-terminus critical to interacting with IFN signaling pathway-mediating protein Nmi
  - N-terminus has unknown function

Potential transmembrane localizations of the ORF6 protein from SARS-CoV Tor2 (left), SARS-CoV-2 Wuhan-Hu-1 (middle), and NR-52282 (right)



Riojas & Frank *et al.* 2020 (*bioRxiv*) 10.1101/2020.06.09.134460

Hong Kong Isolate (BEI Resources NR-52282 + ATCC® VR-1991D™)

Sequencing SARS-CoV-2 Isolates

- Our hypothesis: Vero E6 cells are deficient in interferon response → **removal of selective pressure led to deletion?**

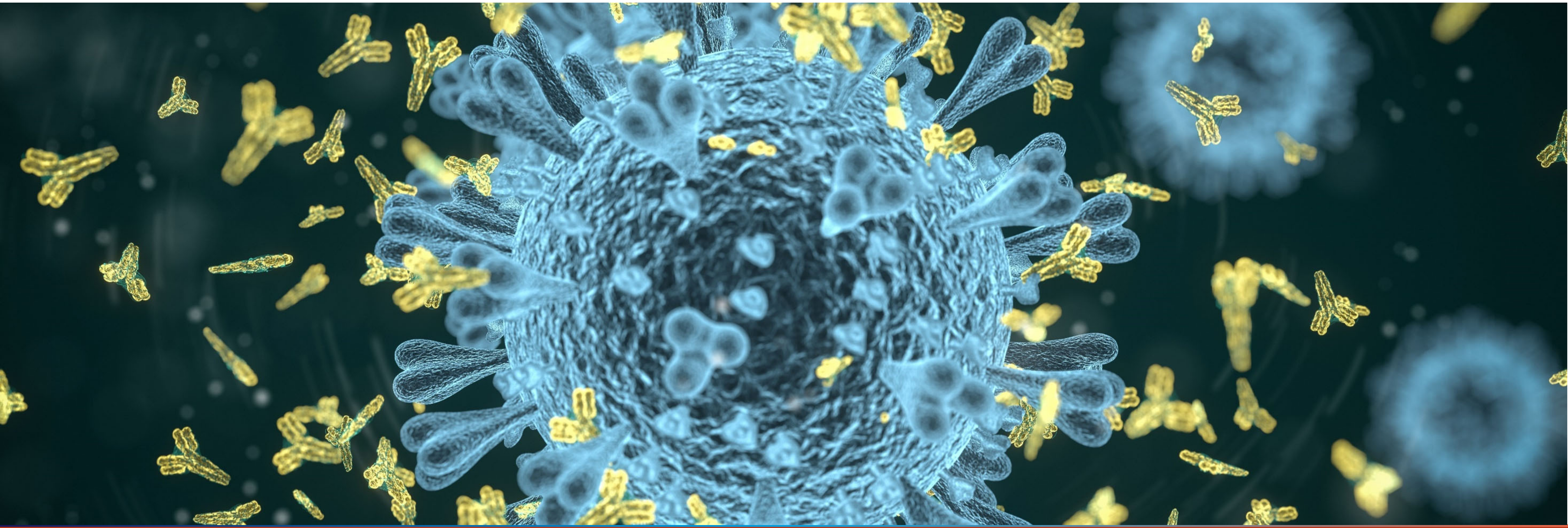
bioRxiv THE PREPRINT SERVER FOR BIOLOGY

A Rare Deletion in SARS-CoV-2 ORF6 Dramatically Alters the Predicted Three-Dimensional Structure of the Resultant Protein
Riojas & Frank *et al.* 2020
doi: https://doi.org/10.1101/2020.06.09.134460

- Passage 6 genome available on GenBank as MT547814.1

- Further supporting evidence of this mutation arising independently was recently published in Addetia *et al.* (*bioRxiv*)
  - 5 additional clinical isolates with the ORF6 Δ22-30 deletion
  - Demonstrates SARS-CoV-2 ORF6 interactivity with specific host IFN factors, even with Δ22-30 mutation

- Appears to disprove our hypothesis



Toward Genomic Fidelity in Cultured SARS-CoV-2

Multiple approaches to reducing novel variants in culture

The Challenge of SARS-CoV-2 Genome Stability

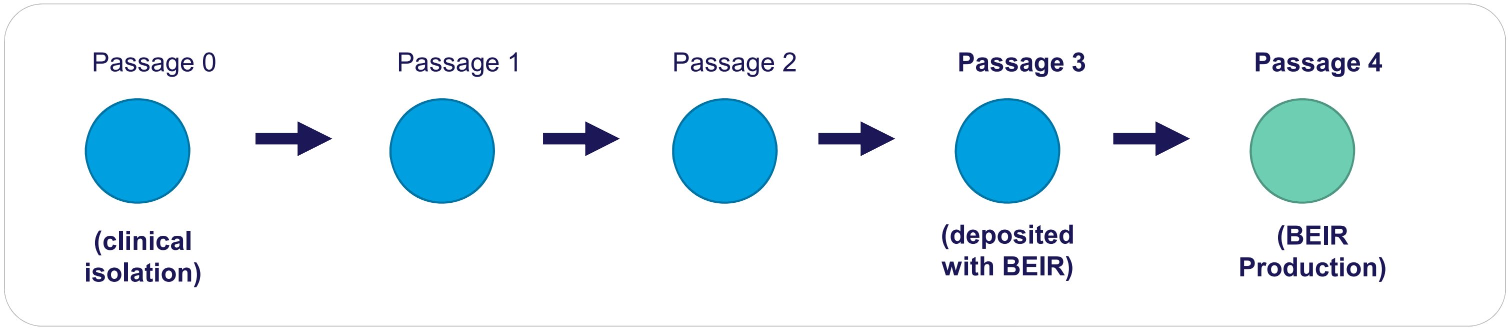
Toward Genomic Fidelity in Cultured SARS-CoV-2

- Genomic stability of RNA viruses is a known challenge, but SARS-CoV-2 is less of a challenge than other RNA viruses
- However, a reasonably stable viral genome is critical for standardized material
- Variables we've investigated to reduce variants in SARS-CoV-2 isolates...
  - Varying MOI and DPI
  - Plaque picking

The WA1 Isolate (BEIR NR-52281 + ATCC® VR-1986D™)

Toward Genomic Fidelity in Cultured SARS-CoV-2

- Originally isolated from an oropharyngeal swab from a patient with a respiratory illness who had recently returned from travel to the affected region of China and developed clinical disease (COVID-19) in January 2020 in Washington, USA.
- Passaged 3 times at CDC in Vero (CCL-81) cells prior to deposit with BEI Resources
- Complete genome of the SARS-CoV-2 USA-WA1/2020 clinical isolate and initial passaging (Passages 0 + 1) were previously sequenced (GenBank: MN985325 + MT020880), and provided as reference genomes



Passage 0 → Passage 1 → Passage 2 → **Passage 3** → **Passage 4**

(clinical isolation)

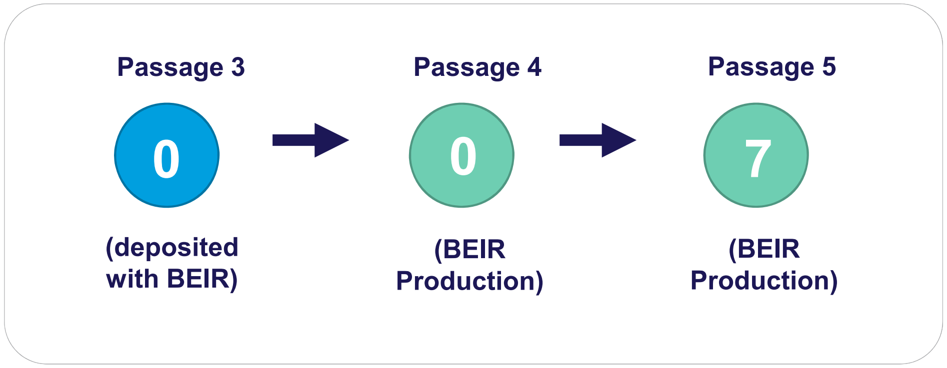
(deposited with BEIR)

(BEIR Production)

The WA1 Isolate (BEIR NR-52281 + ATCC® VR-1986D™)

Toward Genomic Fidelity in Cultured SARS-CoV-2

Encircled numbers indicate # of variants detected at >5% frequency

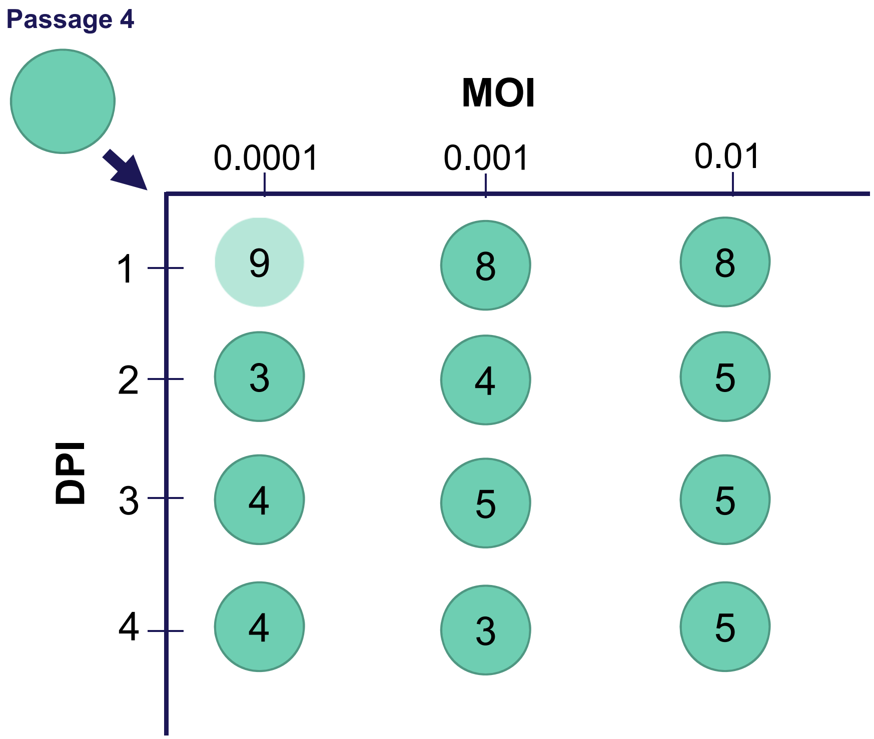


- Passages 4 and 5 were then sequenced for BEI Resources by ATCC SBC
- We saw no variants in passage 4, but 7 variants >5% in passage 5
- What factors can we adjust to increase fidelity to the genome of the deposited material in later passages and reduce the amount of variation?

Varying Multiplicity of Infection and Days Post Infection

Toward Genomic Fidelity in Cultured SARS-CoV-2

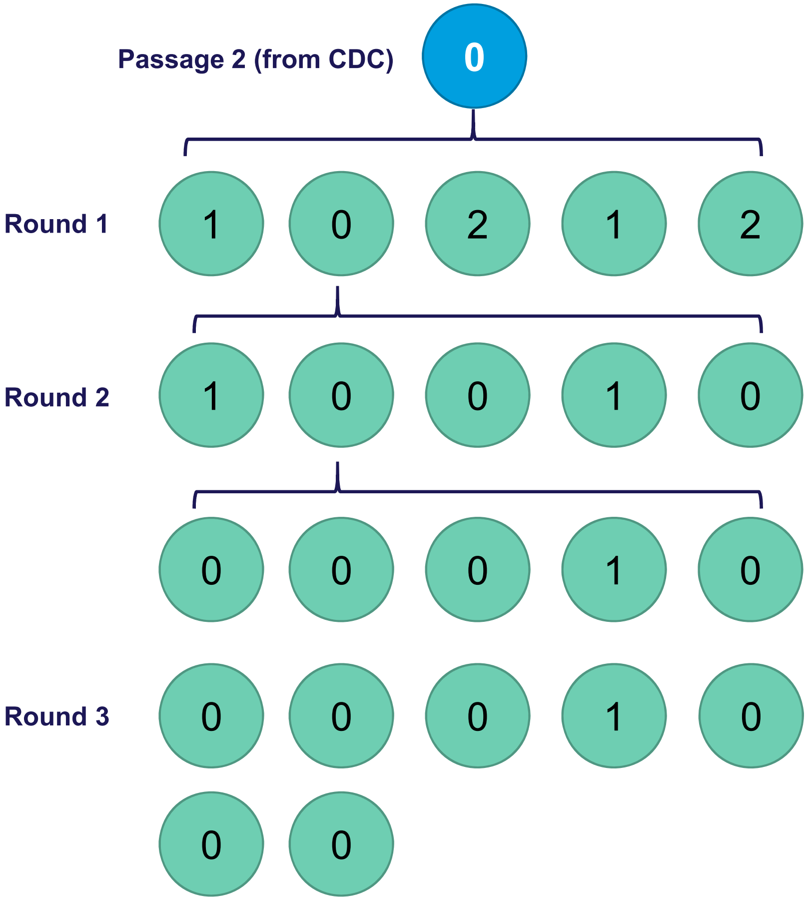
- We wanted to make an educated observation on the effect of MOI and days post infection (DPI) on number of variant nucleotides >5%
- Preliminary results
  - >1 DPI appears to be negatively correlated
  - MOI may be positively correlated
  - 1DPI + very low MOI suffered from poor sequencing coverage
- We continue to work on optimizing MOI and DPI to reduce variation

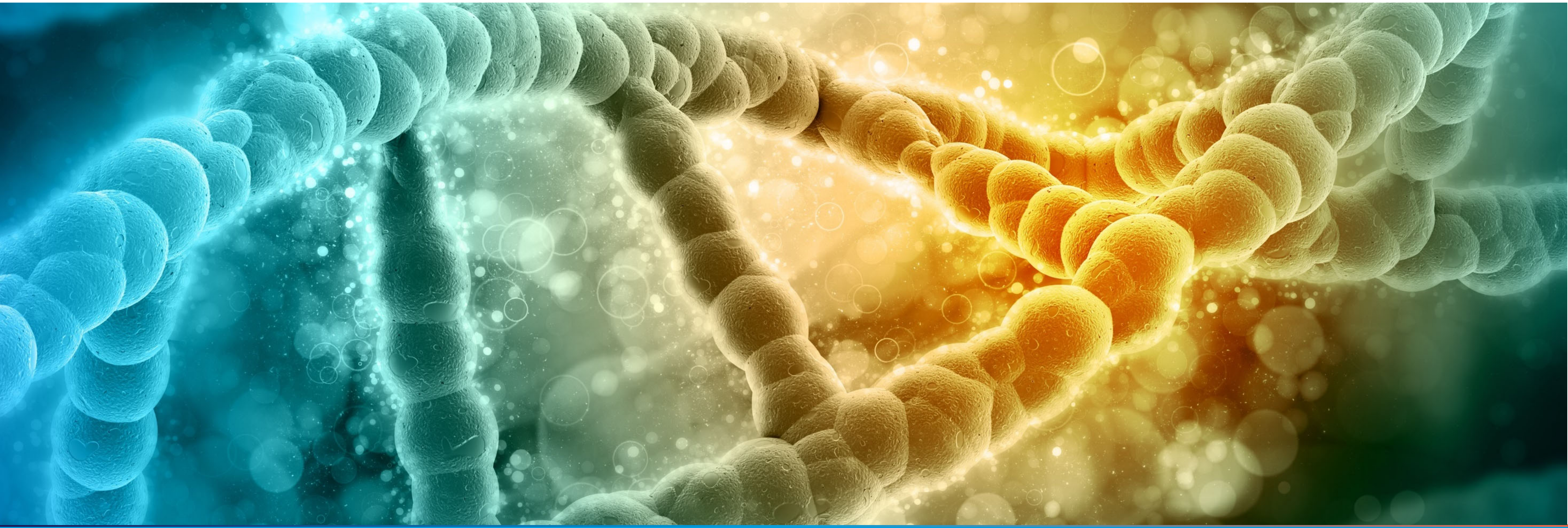


Plaque Picking for Purification

Toward Genomic Fidelity in Cultured SARS-CoV-2

- During production, can selection of viral plaques yield more homogenous SARS-CoV-2 populations?
  - Began with Passage 2 from the CDC, known to contain no variants
  - Performed 3 rounds of plaque picking, selecting the plaque with no variants per round
  - Saw progressively smaller portion of variants per plaque per round, suggests this is a viable approach for application requiring high genomic fidelity
- Selection of plaques without variants for continued propagation is being explored





The ATCC Genome Portal

Reference genomes for the ATCC bacteriology collection and beyond

ATCC's Enhanced Authentication Initiative

In 2019, ATCC made a pledge to raise credibility in science, starting with leveraging NGS to enhance our authentication processes

ATCC embarked on a project to...

1. Enrich the characterization of our biological collections
2. Provide whole-genome sequences paired with specific, authenticated materials researchers need to generate credible data

FA6

Bacteriology Bioinformatics Best Practices

Extract DNA with optimized, proprietary protocols to get the best quality input material
Avoid “garbage in, garbage out” data and results

Sequence on both Illumina® and Oxford Nanopore Technologies®
Impose strict quality control thresholds to save only highest quality reads per instrument

Combine data from both technologies to achieve high-quality, complete genomes
We perform hybrid genome assembly, leveraging the strengths of both platforms while avoiding biases and weaknesses of them individually

Annotate genomes to enable gene-level analyses
Provide users with a reliable annotation, so they can identify genes of interest rapidly to enable gene-specific research

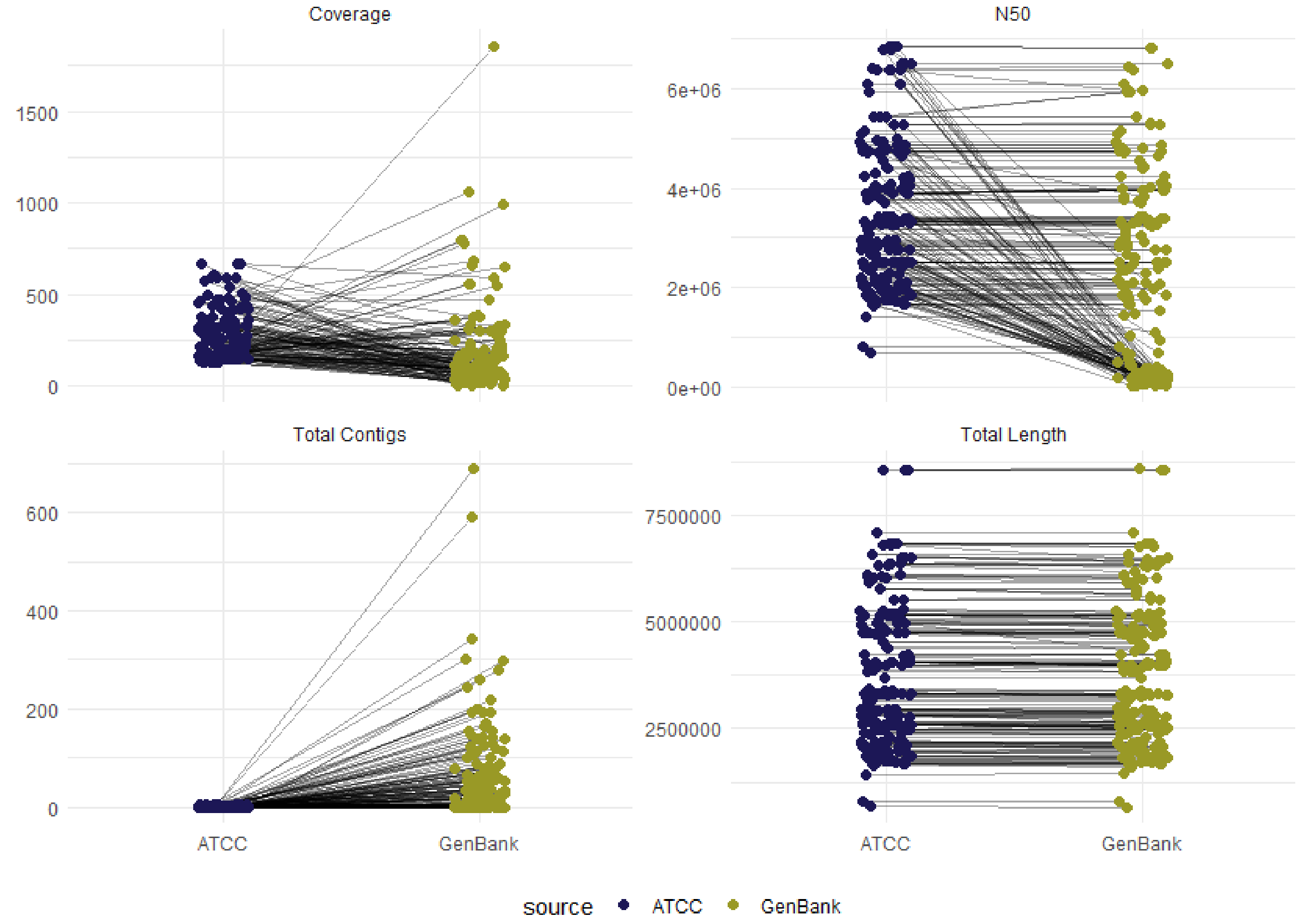
Confirm strain identity against highly curated databases
We’ve partnered with One Codex, an industry leader in bioinformatic bacterial identification, to verify our strain designations by using *k*-mer and genomic distance approaches

Slide 31

FA6 Briana: can this made into a viro focused pipeline?

Frank, Andrew, 10/19/2020

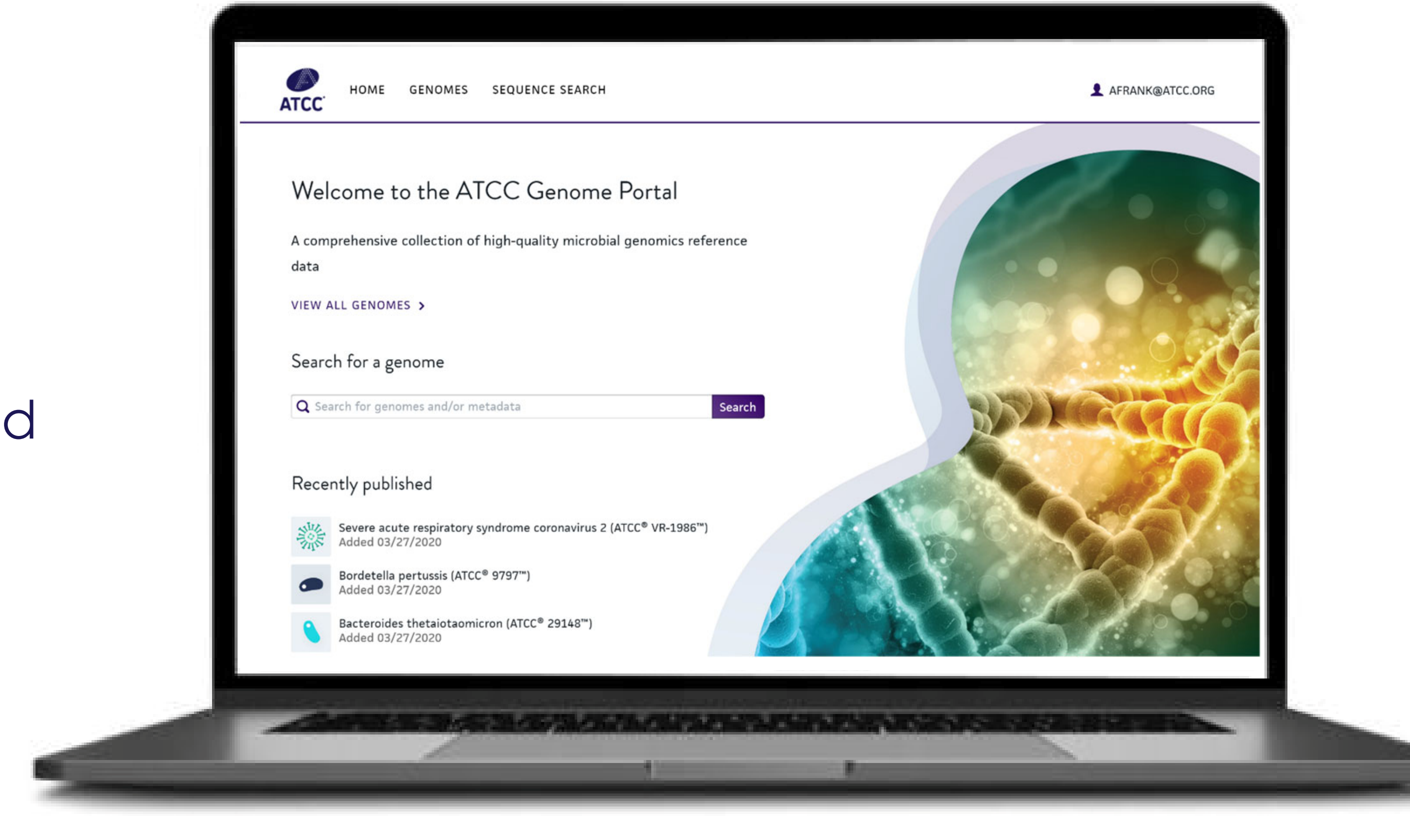
ATCC’s Genomes Substantially Improve On Existing Assemblies



Genomes site

genomes.atcc.org

850+ genomes
tied to authenticated
ATCC material



Welcome to the ATCC Genome Portal

A comprehensive collection of high-quality microbial genomics reference data

Search for a genome

Recently published

Severe acute respiratory syndrome coronavirus 2 (ATCC® VR-1986™)
Added 03/27/2020

Bordetella pertussis (ATCC® 9797™)
Added 03/27/2020

Bacteroides thetaiotaomicron (ATCC® 29148™)
Added 03/27/2020

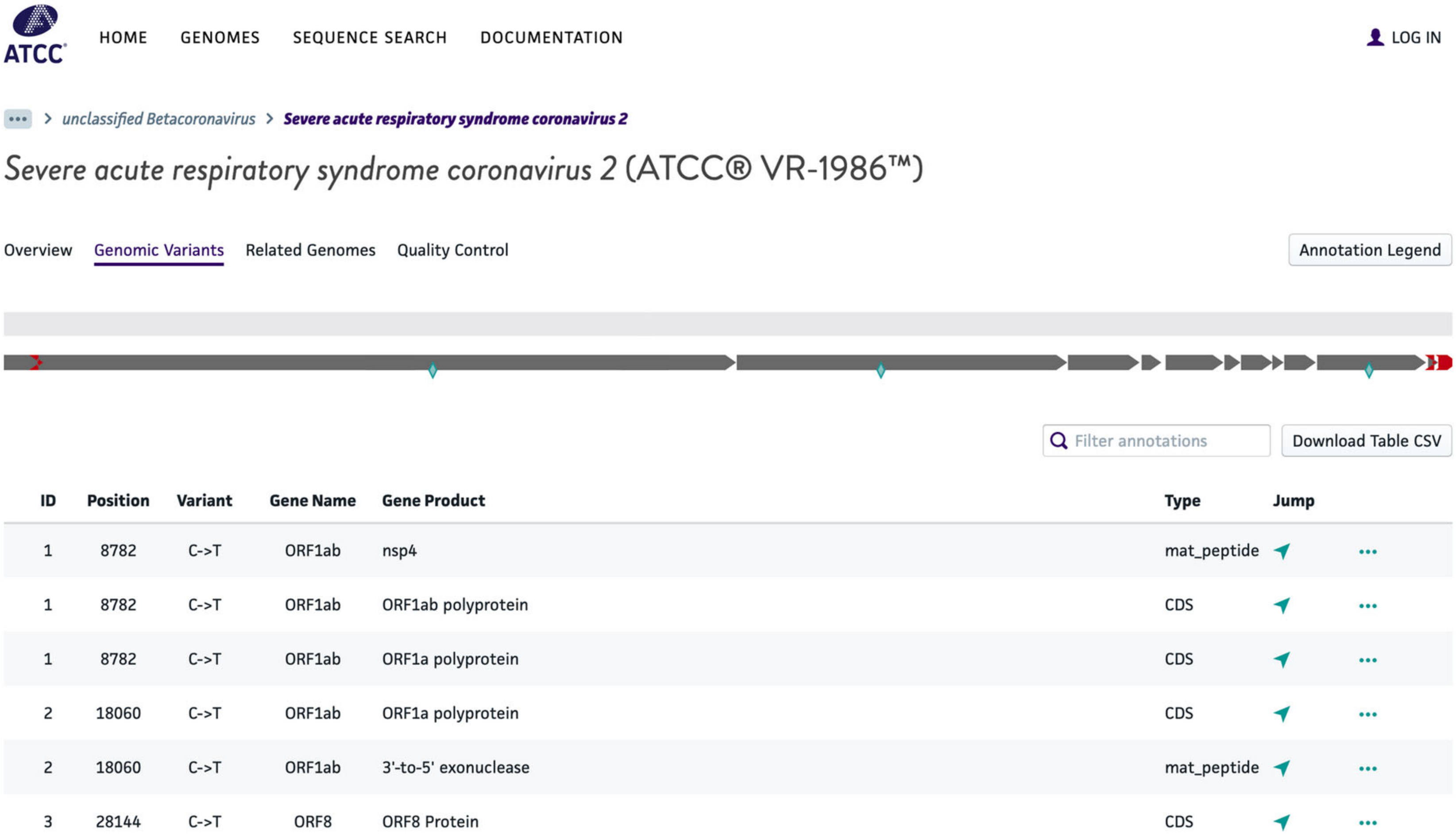
Beyond Bacteriology

The ATCC Genome Portal – genomes.atcc.org

- The ATCC Genome Portal now hosts 6 coronavirus genomes + 36 additional virus genomes from the ATCC virology collection
  - VR-1986™ (2019-nCoV/USA-WA1/2020 RNA)
  - VR-1991™ (2019-nCoV/Hong Kong/VM20001061/2020 RNA)
  - VR-1992™ (2019-nCoV/Italy-INMI1 RNA)
  - VR-1994™ (2019-nCov/Germany/BavPat1/2020 RNA)
  - VR-740™ (Human coronavirus 229E)
  - VR-1558™ (Human coronavirus OC43)

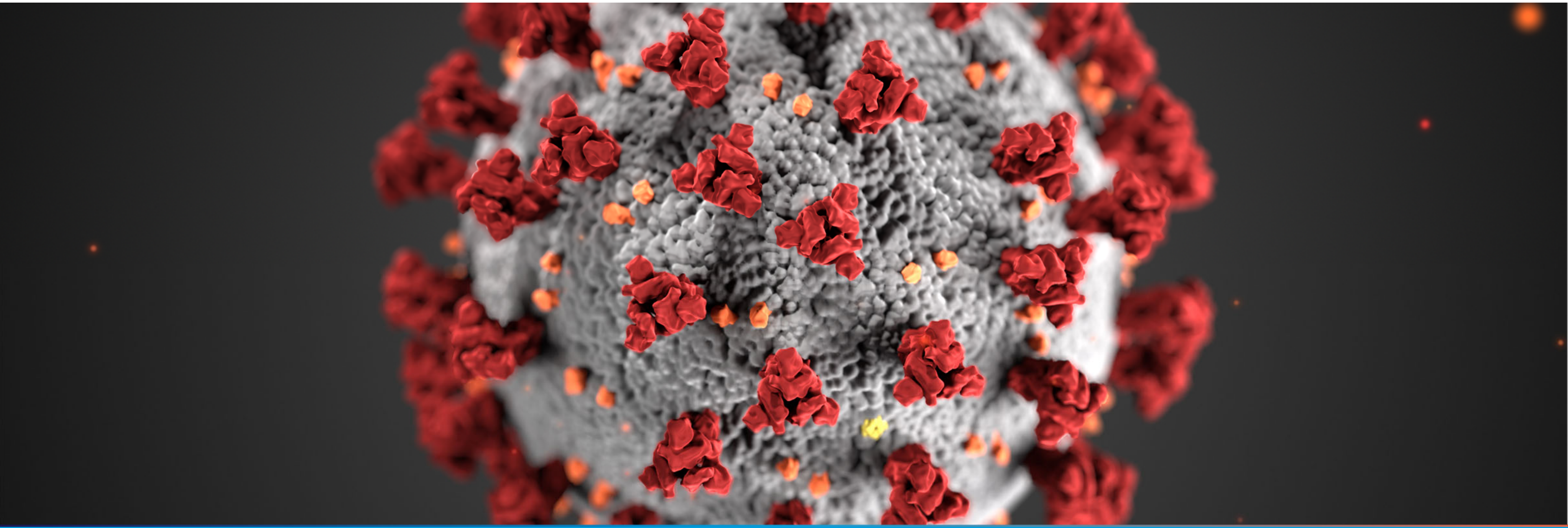
- Coming soon for ATCC viruses...
  - 100 virus genomes from the ATCC virology collection
  - A dedicated tab for virus genomes listing variants + their frequencies to a reference virus genome

ATCC Genome Portal Viral Variant and Annotation View



Severe acute respiratory syndrome coronavirus 2 (ATCC® VR-1986™)

ID	Position	Variant	Gene Name	Gene Product	Type	Jump	
1	8782	C->T	ORF1ab	nsp4	mat_peptide	➤	•••
1	8782	C->T	ORF1ab	ORF1ab polyprotein	CDS	➤	•••
1	8782	C->T	ORF1ab	ORF1a polyprotein	CDS	➤	•••
2	18060	C->T	ORF1ab	ORF1a polyprotein	CDS	➤	•••
2	18060	C->T	ORF1ab	3'-to-5' exonuclease	mat_peptide	➤	•••
3	28144	C->T	ORF8	ORF8 Protein	CDS	➤	•••



Committed to Ending the COVID-19 Pandemic

ATCC and BEI Resources SARS-CoV-2 Work Continues

Committed to Ending the COVID-19 pandemic

- ATCC and BEI Resources remain committed to our public and private partners to continue the fight and ending the COVID-19 pandemic
- Both organizations continue to provide new SARS-CoV-2 standards materials on a regular basis, so check back often

- If you need help with ATCC SARS-CoV-2 materials, please contact tech@atcc.org. If you are unable to find material that fits your need, please contact sales@atcc.org.
- If you need help with BEI Resources SARS-CoV-2 materials, please contact contact@beiresources.org

Acknowledgments

The entire staff of ATCC and BEI Resources, who have worked tirelessly to provide the research community with critical SARS-CoV-2 materials

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  - Marco Riojas, Ph.D.
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  - Beth Flores, B.S.
  - Helen Navin, M.S.
  - Nicholas Tolli, B.S.
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