Importance of the Host Cell Line on the Sequence Fidelity of SARS-CoV-2 Genome

Beth Flores*, Michael Parker, Adam Wood, Ciera Albrecht, Sujatha Rashid
BEI Resources, Manassas, VA 20110

Abstract

BEI Resources has propagated over 62 isolates of SARS-CoV-2 variants for use in animal challenge studies since February 2020. African green monkey kidney cells (Vero cells) were initially used for propagating SARS-CoV-2 due to their previous use for the successful propagation of closely related human coronaviruses. Working virus stocks have shown that the sequential propagation of early isolates in Vero cells leads to critical changes in the region of the furin cleavage site in sub-populations. These changes rapidly increase in frequency within a few passages. This undesirable phenotype significantly reduces the utility of such stocks for critical vaccine and therapeutic studies in animals. We have previously reported that SARS-CoV-2 propagation in a human lung cell line (e.g., Calu-3) mitigates this risk, improving the overall genetic stability of working stocks.

Using appropriate virus propagation conditions in Calu-3 to maintain an intact furin cleavage site in SARS-CoV-2 virus populations, we have successfully produced numerous working stocks of several variants of concern (VOC) and variants of interest (VOI) for researchers. However, recent studies have shown that due to a high number of mutations, the Omicron variant exhibits altered cell tropism and mode of entry compared to previous SARS-CoV-2 variants. Here, we report our experience with the propagation of Omicron isolates in Calu-3 compared to commonly used Vero E6 cells overexpressing human ACE2 and TMPRSS2 protease (Vero-AT cells). In addition to virus productivity, we monitor genetic variants using an advanced analytical pipeline capable of detecting indels and point mutations of sub-populations compared to the reference sequence. Our experience with propagating Omicron isolates indicates a much slower growth rate and a diminished ability to induce a cytopathic effect in Calu-3 cells compared to previous variants.

Methods

Table 1: Cell lines used in virus growth assessment and stock production.

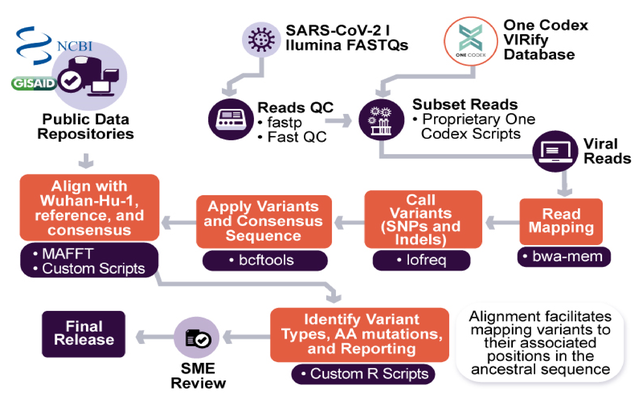
Cell line	Catalog number	Purpose
Vero E6	BEI Resources NR-596	Virus production (previous)
Vero E6/ACE2 /TMPRSS2 (Vero-AT)	BEI Resources NR-54970	Virus production (occasional)
Vero E6/ACE	BEI Resources NR-53726	Optimization studies
A549-hACE2	BEI Resources NR-53821	Optimization studies
HEK-293T-hACE2	BEI Resources NR-52512	Optimization studies
Calu-3	ATCC© HTB-55™	Virus production (routine)
Caco-2	ATCC© HTB-37™	Optimization studies
Vero/TMPRSS2	NIBSC, #100978	Optimization studies
Vero/hSLAM	ECACC (Sigma Aldrich) *	Optimization studies

* For this study, Vero/hSLAM cells were generously shared by the Center for Disease Control and Prevention (CDC).

Initial cell line optimization for virus growth: NR-52281 (USA-WA1/2020) was tested on cells seeded at target confluency of 70%, using MOI of 0.00001. Virus was harvested when 75% of cells were infected, expressed as days post infection (dpi).
Virus seed: Low passage isolates (passage 1 when available) were used to produce stocks.
Virus stock production: Current virus stocks are produced in Calu-3. Later emerging variants were tested in Vero-AT and genome fidelity compared to stocks produced in Calu-3.
Titration: Infectious titer performed on cell line used in virus propagation in triplicate.

NEXT GENERATION SEQUENCING
Library: NEBNext® Ultra II™ RNA Library Prep Kit for Illumina® (NEB #E7775)
Sequencing: Illumina MiSeq platform: MiSeq® Reagent Micro Kit v2 (300 Cycles)
Assembly: Reference-based assembly was performed using as reference sequences: GenBank MN985325.1 Wuhan-Hu-1, and the reference sequence for the interrogated strain (sequence from clinical sample when available or passage 1).
Analysis: See Figure 1 for pipeline.
Sequences generated with reference sequences are tracked for mutations or single nucleotide polymorphisms (SNPs) greater than 10% sample frequency. First, SNPs in which the nucleotide in the sample is identical to the strain reference but differing from the Wuhan-Hu-1 reference was confirmed to authenticate the presence of signature amino acid changes in the variant isolate. Next, genome fidelity was assessed for the whole genome. SNPs that represent mutations introduced due to tissue culture adaptive changes, likely through the passaging of the virus (total) with special attention to the spike (S) region were noted. See tables 2, 3 and 4 for genome fidelity assessment.

Figure 1: Sequencing Pipeline used to analyze NGS data.



Results

Table 2: Cell optimization study with titer and genome fidelity results using hCoV-19/USA-WA1/2020.

Cell line	Titer ($TCID_{50}$/mL)	Adaptive changes: Total / changes in S (*)
Calu-3	$8.89x10^6$	12 / 0
Vero/ACE2	$1.58x10^7$	11 / 3 (2)
Vero E6	$8.89x10^6$	9 / 4 (2, 1 deletion)
Caco-2	$1.58x10^5$	15 / 1
A549/hACE2	$2.81x10^6$	9 / 4 (2, 1 deletion)
Vero/TMPRSS2	$1.58x10^7$	7 / 3 (2)
Vero/hSLAM	$8.89x10^7$	14 / 6 (2, 1 deletion)
HEK-293T-hACE2	$1.58x10^6$	8 / 2 (1)

* Values within a "()" indicate SNPs in the furin cleavage region of spike.

Table 3: Characteristics of representative variants in Calu-3 for virus production

Virus isolate/VOC	Seed virus passage history	Harvest (dpi)	Titer ($TCID_{50}$/mL)	Adaptive changes: Total / changes in S
USA/CA_5574/2020 / alpha	Vero 2	3 days	8.89×10^5	9/0
USA/MD-HP01542/2021 / beta	Vero 1	3 days	1.53×10^5	12/3
USA/MD-HP05285/2021 / delta	Vero 1	3 days	6.86×10^6	2/0
USA/CA-48018/2020 / epsilon	Vero 1	4 days	4.43×10^5	7/1

Table 4: Titers and SNPs in representative Omicron B.1.1.529 lineage viruses using seed virus grown in Vero-AT cells.

Virus isolate	Cell line	Harvest (dpi)	Titer ($TCID_{50}$/mL)	Adaptive changes: Total / changes in S (*)
hCoV-19/USA/MD-HP20874/2021 (BA.1)	Calu-3	7	$4.40x10^6$	6 / 0
	Vero-AT	6	$1.99x10^4$	6 / 0
hCoV-19/USA/CO-CDPHE-2102544747/2021 (BA.2)	Calu-3	5	$2.81x10^5$	5 / 2
	Vero-AT	5	$6.86x10^5$	8 / 2 (1)
hCoV-19/Hong Kong/HKUVOC0589/2022 (BA.2)	Calu-3	6	$2.17x10^5$	5 / 0
	Vero-AT	3	$1.58x10^5$	3 / 0
hCoV-19/USA/COR-22-063113/2022 (BA.5)	Calu-3	6	$2.40x10^5$	8 / 0
	Vero-AT	4	$5.86x10^6$	9 / 0

* Values within a "()" indicate SNPs in the furin cleavage region of spike.

Conclusions

Cell optimization studies show minimal adaptive changes for stocks produced in human cell line Calu-3.

Continuous assessment of other variants in routine virus production in Calu-3 confirmed good genome fidelity across many variants with no changes in furin cleavage site.

A change in growth characteristics with the omicron isolates in Calu-3 was observed where viruses grow at a slower rate with a poor ability to produce cytopathic effect. Vero-AT cells are a good alternative for omicron stock production.

A continual assessment of growth characteristics in cell lines (Calu-3 and Vero-AT) and sequence fidelity check is needed as new SARS-CoV-2 variants emerge.

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BEI RESOURCES 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.359.7370 **Email:** Contact@BEIResources.org **Web:** www.beiresources.org