

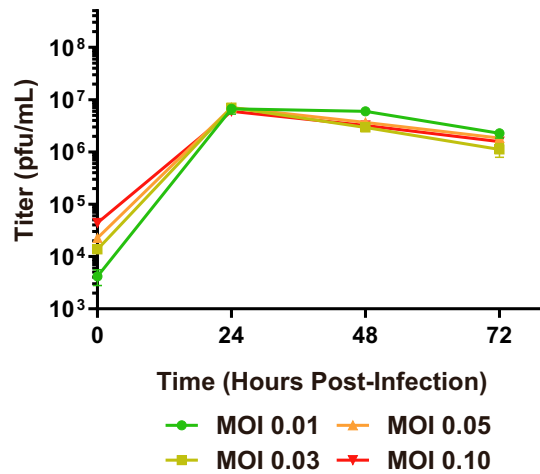
**OMTM, Volume 32**

**Supplemental information**

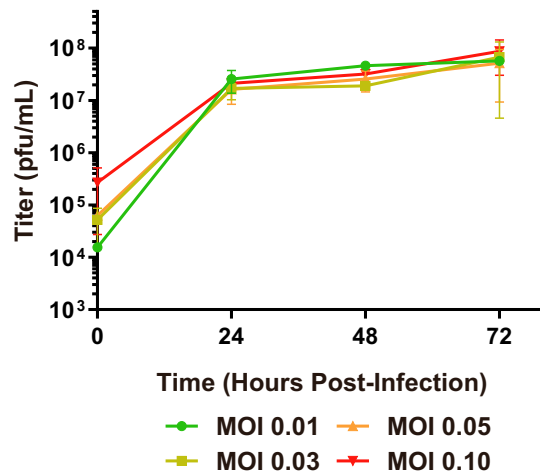
**High-titer manufacturing of SARS-CoV-2**

**Spike-pseudotyped VSV in stirred-tank bioreactors**

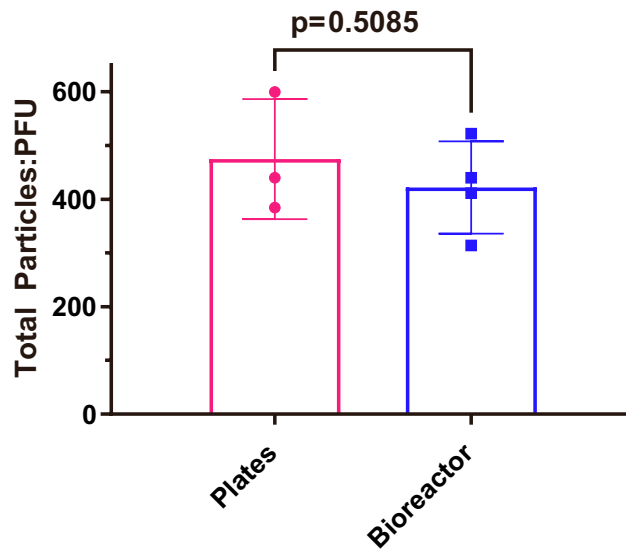
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**Figure S1: Growth curves of VSV-ΔG-S<sub>CTΔ21</sub> in tissue-culture plates.** 145 cm<sup>2</sup> tissue culture plates were infected at indicated MOIs (pfu/cell) with VSV-ΔG-S<sub>CTΔ21</sub>. Cultures were harvested at the indicated times and titered on Vero cells. Mean ± SD is shown (n=2).

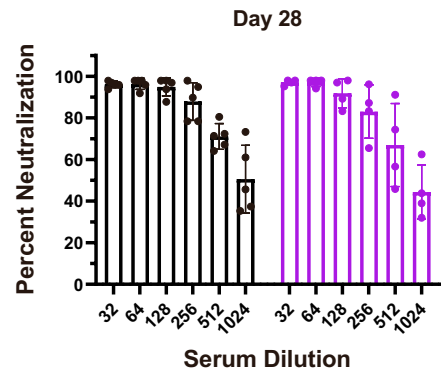
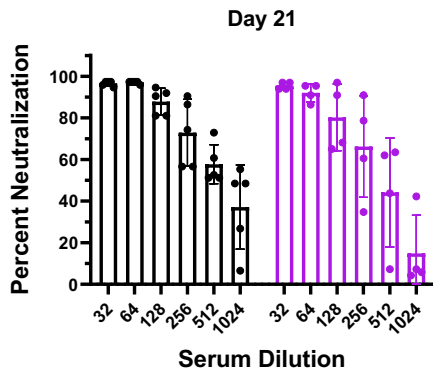
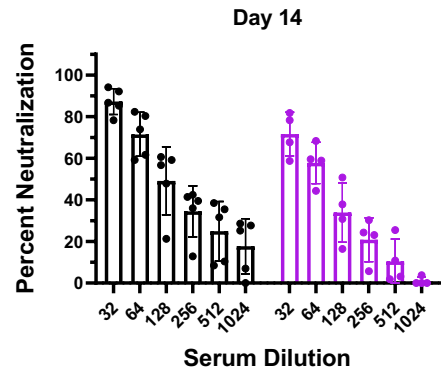
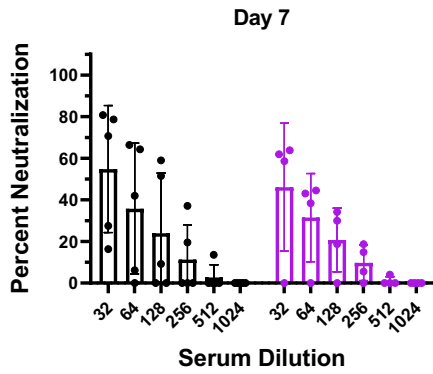


**Figure S2: Growth curves with VSV-ΔG-S<sub>CTΔ21</sub> in 125 mL bioreactors.** Cultures were infected at indicated MOIs (pfu/cell) with VSV-ΔG-S<sub>CTΔ21</sub>. Cultures were harvested at the indicated times and titered on Vero cells. Mean ± SD is shown, n=2.

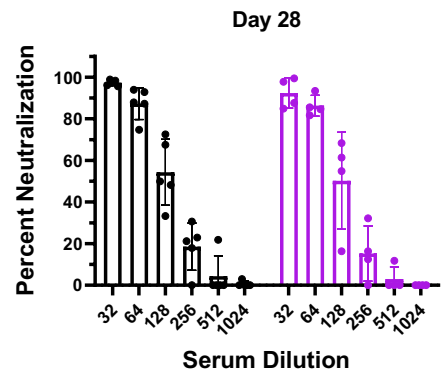
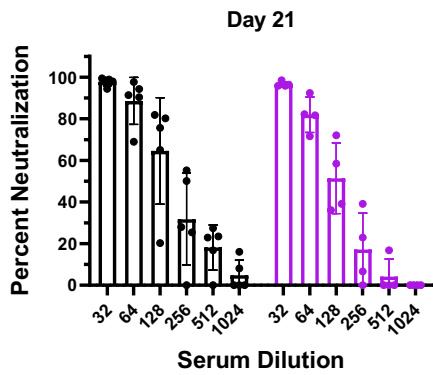
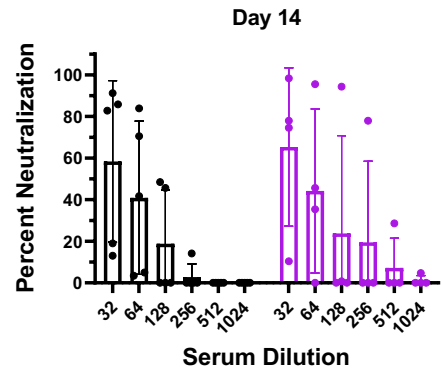
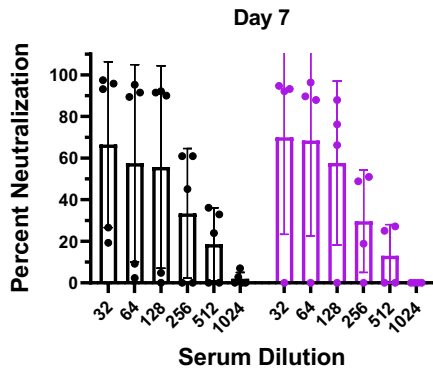


**Figure S3: Particle to pfu ratio of VSV- $\Delta$ G-S<sub>CT</sub> $\Delta$ <sub>21</sub> as measured by Tunable Resistive Pulse Sensing (TRPS).** Virus was produced in 145 cm<sup>2</sup> tissue culture plates or 125 mL Celstir bioreactors as described in Materials and Methods. Mean  $\pm$  SD is shown and two-tailed t-test was used, n=3-4.

VSV-ΔM51-G-S<sub>CTA21</sub>-GFP



VSV-ΔG-S<sub>Omicron</sub>-CTA21-GFP



● Plates ● Bioreactor

**Figure S4: Neutralization against ancestral SARS-CoV-2 pseudovirus measured in serum harvested from hamsters.** Hamsters were vaccinated with a single intranasal (IN) injection of  $1 \times 10^3$  pfu VSV- $\Delta$ G-S<sub>CT $\Delta$ 21</sub> of plate- or bioreactor-based virus. Serum neutralizing antibody titers were monitored over 28 days by pseudovirus PRNT assay using either VSV- $\Delta$ M51- $\Delta$ G-S<sub>CT $\Delta$ 21</sub>-GFP or VSV- $\Delta$ G-S<sub>Omicron-CT $\Delta$ 21</sub>-GFP *in vitro*. Mean  $\pm$  SD is shown, n=4.

**Table S1: Comparison of plate-based and bioreactor-based Vero growth and VSV-ΔG-S<sub>CTΔ21</sub> and VSV-ΔG-S<sub>Omicron-CTΔ21</sub>-GFP production.** \*Determined using the peak virus titers in the culture medium at 24 or 48 hpi for plates and 72 hpi for both bioreactor sizes. \*\*n=1 for 1 L.

	1 145 cm <sup>2</sup> Plate	125 mL Bioreactor	1 L Bioreactor
Available growth area (cm <sup>2</sup> )	145	2 200	26 400
Mean cells per cm <sup>2</sup>	1.00 ± 0. 64 ×10 <sup>5</sup>	2.98 ± 0.47 ×10 <sup>5</sup>	1.49 ± 0.16 ×10 <sup>5</sup>
Mean total cells grown	1.45 ± 0.93 ×10 <sup>7</sup>	6.55 ± 1.02 ×10 <sup>8</sup>	3.95 ± 0.42 ×10 <sup>9</sup>
Mean peak* virus yield for VSV-ΔG-S <sub>CTΔ21</sub> (pfu/mL)	7.02 ± 0.05 ×10 <sup>6</sup>	5.88 ± 2.59 ×10 <sup>7</sup>	2.05 ± 0.58 ×10 <sup>8</sup>
Mean peak* cell-specific virus yield for VSV-ΔG-S <sub>CTΔ21</sub> (pfu/cell)	9.68 ± 4.46	8.57 ± 2.60	45.77 ± 12.2
Mean peak* virus yield for VSV-ΔG- S <sub>Omicron-CTΔ21</sub> (pfu/mL)	1.09 ± 0.02 ×10 <sup>6</sup>	5.58 ± 0.35 ×10 <sup>6</sup>	5.50 ×10 <sup>6**</sup>
Mean peak* cell-specific virus yield for VSV-ΔG-S <sub>Omicron-CTΔ21</sub> (pfu/cell)	3.70 ± 0.07	1.12 ± 0.06	1.82**
Number of plates/runs that would produce an equivalent number of mean total cells	272 plates	6 runs	1 run
Number of plates/runs that would produce an equivalent number of mean total pfu of VSV-ΔG-S <sub>CTΔ21</sub>	1284 plates	32 runs	1 run

**Table S2: Cost considerations for plate-based and bioreactor-based Vero growth and VSV- $\Delta$ G-S<sub>CT $\Delta$ 21</sub> production.**

	1 145 cm <sup>2</sup> Plate	125 mL Bioreactor	1 L Bioreactor
Fixed costs (USD)	\$20.12	\$31.70	\$54.63
Variable costs (USD)	\$31.62	\$143.83	\$965.36
Cost per million pfu (USD)	\$0.37 $\pm$ \$0.00	\$0.03 $\pm$ \$0.02	<\$0.01 $\pm$ \$0.00