

Vector pCC1-BAC-HIS-mNeonGreen containing the SARS-Related Coronavirus 2 Δ Spike and NSP12 Mutations (D760N/D761N) Replicon

Catalog No. NR-58663

Lot No. 70055802

For research use only. Not for use in humans.

Contributor and Manufacturer:

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Product Description:

The vector for the non-infectious replicon from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) was designed by assembling a replicon consisting of all non-structural genes (nsp1 to nsp16) and the structural genes membrane (M), envelope (E) and nucleocapsid (N) using an RNA virus reverse genetics system in *Saccharomyces cerevisiae* (*S. cerevisiae*).¹ The replicon is flanked by a T7 promoter upstream and a self-cleaving hepatitis delta virus poly A/HDV ribozyme downstream. NR-58663 contains an inactivating mutation (D760N/D761N), [Pol(-)] in Nsp12, the viral RNA-dependent RNA polymerase.^{1,2} The spike (S) gene was replaced by a gene cassette consisting of a neomycin-resistance (*neoR*) gene and nuclear-localized monomeric NeonGreen (mNeonGreen), separated by a T2A ribosome sequence shift. Expression of the cassette is under the control of the S transcription-regulating sequence (TRS). NR-58663 also contains the chloramphenicol acetyltransferase (*cat*) gene for selection in *Escherichia coli* (*E. coli*) and the imidazoleglycerol-phosphate dehydratase (HIS3) gene for selection in *S. cerevisiae*. The resulting size of the plasmid is approximately 36,000 base pairs.^{1,2} The complete plasmid sequence and map are provided on the BEI Resources webpage.

The SARS-CoV-2 replicon system enables the production of non-infectious self-replicating RNAs or replicons enabling the study of the virus outside the BSL3 setting. The replicon plasmids allow testing of various spike variants and the use of cell types not ordinarily susceptible to live SARS-CoV-2, in addition to their use in antiviral screening, host factor validation and viral mutant phenotype assessment. Trans-complementation of these replicons with viral glycoprotein can be used to generate Replicon Delivery Particles (RDPs) for single-cycle delivery into a range of cell types.² Protocols for this system can be requested through [Bio-protocol](#).²

Please refer to Appendix I for isolation of plasmid DNA from glycerol stock and Appendix II for reagents and growth media formulations.

Material Provided:

Each vial contains approximately 0.5 mL of NR-58663 transformed *S. cerevisiae* in Yeast Extract-Peptone-Dextrose broth without histidine (YPD-HIS) supplemented with 15% glycerol.

Packaging/Storage:

NR-58663 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -60°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Vector pCC1-BAC-HIS- mNeonGreen containing the SARS-Related Coronavirus 2 Δ Spike and NSP12 Mutations (D760N/D761N) Replicon, NR-58663."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. [Biosafety in Microbiological and Biomedical Laboratories](#), 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

Note: Transfection of cells with the product of *in vitro* transcription requires institutional biosafety committee approval.

Disclaimers:

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NR-58663 is claimed in U.S. Provisional Patent Application numbers 63/083,852 and 63/187,233.

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This item contains mNeonGreen. Registrants from non-profit organizations need to sign licensing agreement before BEI distributes the material. Registrants from for-profit companies need to contact info@allelebiotech.com to obtain a license before ordering items containing mNeonGreen.

References:

1. Rice, C. M., Personal Communication.
2. Ricardo-Lax, I., et al. "Replication and Single-Cycle Delivery of SARS-CoV-2 Replicons." *Science* 374 (2021): 1099-1106. PubMed: 34648371.

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APPENDIX I: PLASMID DNA ISOLATION FROM GLYCEROL STOCK

1. Thaw the glycerol stock by placing it in a 37°C water bath. Immediately after thawing, inoculate an SD-HIS agar plate using a sterile pick/tip.
2. Incubate the plates at 30°C for 2 to 4 days until colonies form.
3. For plasmid DNA maxi-prep, select a colony from the SD-HIS agar plate and transfer it to a tube containing 40mL SD-HIS media.
4. Incubate overnight at 30°C with agitation.
5. Transfer the culture to 500 mL fresh SD-HIS media. Incubate overnight at 30°C with agitation until optical density reaches approximately 2.0.
6. Extract the plasmid DNA using a column-based commercial plasmid DNA extraction kit with the following modifications:
 - a) Harvest the culture at 24,000 × g (or maximum speed for the rotor) for 30 minutes at 4°C.
 - b) Discard the supernatant and resuspend the pellet in 14 mL fresh Lysis solution.
 - c) Incubate at 37°C for 1 hour without agitation.

Note: It is recommended to centrifuge the tube after the neutralization step to remove most of the debris to avoid clogging the column during elution.

7. Elute in the minimal possible volume, as plasmid yield is typically low.

Note: The plasmid DNA in the eluate may also contain residual yeast genomic DNA at this stage. The plasmid DNA can be used for bacterial transformation or for further purification and amplification steps. Ricardo-Lax, I., et al. "Replication and Single-Cycle Delivery of SARS-CoV-2 Replicons." *Science* 374 (2021): 1099-1106. PubMed: 34648371.

APPENDIX II: REAGENTS AND GROWTH MEDIA

Zymolase® Solution:

Zymolase® 100-T	10 mg/mL
Tris-HCl, pH 7.5	50 mM
Glycerol	50% (v/v)

Lysis Solution:

To prepare the Lysis Solution add the following to the resuspension buffer (from a commercial kit - typically called P1 or similar)

Zymolase® Solution	10%
β-mercaptoethanol	1%

SD-HIS Medium:

Succinic acid	10 g
NaOH	6 g
SC-HIS dropout powder	1.37 g

Dissolve the components completely in ddH₂O and bring up the volume to 800 mL (for 1 liter).

For solid growth medium, add 20 g/L agar. Autoclave for 35 minutes.

When the media cools down (42°C for plates), add 100 mL of 20% D-glucose and 100 mL of filter-sterilized 10x YNB+AS Solution.

10X YNB+AS Solution:

Yeast Nitrogen Base (YNB) without amino acids and without ammonium sulfate	17 g
Ammonium sulfate	50 g

Dissolve the components completely in ddH₂O to a final volume of 1 liter and filter sterilize using a 0.22 µm filter. Store at 4°C.

Alternatively, dissolve 67 g of the YNB containing ammonium sulfate to a final volume of 1 liter and filter sterilize using a 0.22 µm filter. Store at 4°C.

D-Glucose solution:

Dissolve 20% (w/v) D-glucose solution in ddH₂O. Autoclave and store at room temperature.