Certificate of Analysis for NR-52514

Vector pHDM Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein

Catalog No. NR-52514

Product Description:

The vector for the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: NC_045512) was designed by codon optimizing the S sequence for mammalian expression and subcloned into the pHDM vector under the CMV promoter. The Kozak sequence in pHDM has been corrected. NR-52514 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. The deposited plasmid was transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404003), grown in Luria-Bertani broth with ampicillin (50 µg per mL) for 1 day at 37°C in an aerobic atmosphere, extracted using a Plasmid *Plus* Maxi Kit (QIAGEN® 12963) and vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70035472 **Manufacturing Date: 29APR2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 8380 base pairs	8386 base pairs[1]
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3820 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence[2]
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[3]	*bla* sequence present	*bla* sequence present
Agarose Gel Electrophoresis (pre-vial) Digestion with *Sap*I	~ 5 kb and ~ 4 kb	~ 5 kb and ~ 4 kb (Figure 1)
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.7 µg in 100 µL per vial (7 µg/mL)
Amount per Vial	Report results	0.7 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	129 colonies per ng

[1]The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.
[2]The NR-52514 insert was codon optimized for mammalian expression, but otherwise is 100% identical to the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: YP_009724390).
[3]The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid replication to avoid plasmid loss and increased antibiotic concentrations may be necessary.

BEI Resources
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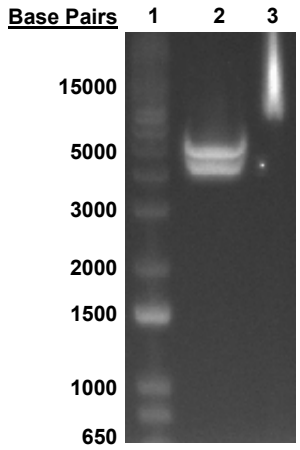
Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-52514



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-52514 digested
Lane 3: NR-52514 undigested

/Heather Couch/
Heather Couch 04 NOV 2020
Program Manager or designee, ATCC Federal Solutions

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