b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52427

Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 16 Gene

Catalog No. NR-52427

This reagent is the tangible property of the U.S. Government.

Product Description:

The non-structural protein 16 (nsp16) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was codon optimized and cloned into the pMCSG53 plasmid. pMCSG53 is an *Escherichia coli (E. coli)* expression vector that contains an N-terminal hexa-histidine tag, followed by a tobacco etch virus (TEV) protease recognition site prior to the insert coding sequence, resulting in the expression of a cleavable histidine-tagged protein. It also contains tRNA genes covering rare codons for Arg (AGG/AGA) and Ile (AUA) to improve expression in *E. coli*. The beta-lactamase gene, *bla*, provides transformant selection through ampicillin resistance in *E. coli*. The deposited plasmid was transformed into One Shot™ TOP10 *Escherichia 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: 70035123 **Manufacturing Date: 06MAY2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5710 base pairs	5705 base pairs[1]
Genotypic Analysis Sequencing of nsp16 insert (900 base pairs) N-terminal His_6 tag N-terminal TEV protease site	 100% sequence identity to depositor's sequence His_6 tag sequence confirmed TEV protease site sequence confirmed	 100% sequence identity to depositor's sequence[2] His_6 tag sequence confirmed TEV protease site sequence confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[3]	 *bla* sequence present	 *bla* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.2 µg in 20 µL per vial (7 µg/mL)
Amount per Vial	Report results	0.2 µg per vial
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *Escherichia coli*	 ≥ 50 colonies per ng	 366 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-52427 insert was codon optimized, but otherwise is consistent with the SARS-CoV-2, Wuhan-Hu-1 nsp16 protein (GenPept: YP_009725311.1).
[3]The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid expansion to avoid plasmid loss and increased antibiotic concentrations may be necessary.

/Heather Couch/
Heather Couch 28 MAY 2020
Program Manager or designee, ATCC Federal Solutions

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