

Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Papain-Like Protease Gene

Catalog No. NR-52897

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Product Description:

The papain-like protease [PLpro; amino acids 746 to 1060 of non-structural protein 3 (NSP3); GenPept: [YP_009725299](#)] 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 arginine (AGG/AGA) and isoleucine (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 *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 vialied in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70036139

Manufacturing Date: 20MAY2020

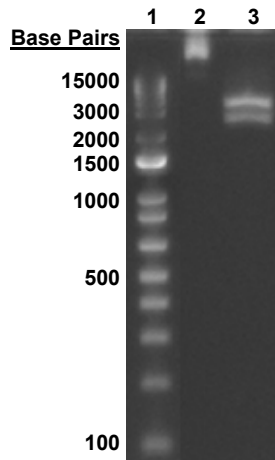
TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5740 base pairs	5733 base pairs ¹
Genotypic Analysis Sequencing of PLpro gene (~ 920 base pairs) Sequencing of pMCSG53 vector (~ 4750 base pairs)	≥ 99% sequence identity to depositor's sequence N-terminal hexa-histidine tag confirmed N-terminal TEV protease cleavage site confirmed	100% sequence identity to depositor's sequence ² N-terminal hexa-histidine tag confirmed N-terminal TEV protease cleavage site confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³	<i>bla</i> sequence present	<i>bla</i> sequence present
Agarose Gel Electrophoresis Digestion with <i>SapI</i> and <i>BamHI</i> (pre-vial)	~ 2.5 kb and ~ 3.5 kb	~ 2.5 kb and ~ 3.5 kb (Figure 1)
Concentration by Qubit™ Measurement	≥ 2 µg/mL	0.2 µg in 20 µL per vial (9 µg/mL)
Amount per Vial	Report results	0.2 µg per vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	412 colonies per ng

¹The 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.

²The NR-52897 insert was codon optimized but is consistent with the PLpro within the NSP3 polyprotein of SARS-CoV-2, Wuhan-Hu-1 (GenPept: YP_009725299).

³The 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.

Figure 1: Agarose Gel Electrophoresis of Digested and Undigested NR-52897



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
 Lane 2: NR-52897 undigested
 Lane 3: NR-52897 digested

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