Certificate of Analysis for NR-52011

Plasmid pBR322 Containing cDNA from Enterovirus D68, US/IL/14-18952, Infectious Clone pUC-49131

Catalog No. NR-52011

Product Description:

Enterovirus species D type 68 (EV-D68), US/IL/14-18952 (GenBank: KM851230) genome was cloned into the *Escherichia coli (E. coli)* cloning vector pUC19 to generate plasmid pUC-49131. pUC-49131 contains a T7 bacteriophage promoter immediately upstream of the 5' end of the viral genome and beta-lactamase gene TEM-116 to provide transformant selection through ampicillin resistance in *E. coli*. The resulting size of the plasmid is approximately 10,082 base pairs. The deposited plasmid was transformed into NEB® Stable Competent *Escherichia coli* cells (New England Biolabs® C3040H) grown in Luria-Bertani broth containing 50 µg per mL ampicillin 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: 70033863 **Manufacturing Date: 20APR2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	10,082 base pairs (Figure 1)[1]
Genotypic Analysis Sequencing of Enterovirus D68 insert (10,082 base pairs)	Report results ≥ 99% sequence identity to EV-D68, US/IL/14-18952 (GenBank: KM851230.1)	100% sequence identity to depositor's sequence 99.9% sequence identity to EV-D68, US/IL/14-18952 (GenBank: KM851230.1)
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene TEM-116)[2]	TEM-116 sequence present	TEM-116 sequence present
Concentration by PicoGreen® Measurement	Report results	0.71 µg in 100 µL per vial (7.1 µg/mL)
Amount per Vial	Report results	0.71 µg per vial
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation NEB® Stable Competent *Escherichia coli*	≥ 50 colonies per ng	237 colonies per ng

[1]The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence.
[2]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.

Figure 1: Complete Plasmid Sequence of NR-52011

>NR-52011_70033863_complete plasmid sequence

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGC
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BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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