Certificate of Analysis for NR-52376

Plasmid pUC57-Simple Containing cDNA from Enterovirus D68, USA/MN/1989-23220, Infectious Clone EV-D68-R23220

Catalog No. NR-52376

Product Description:

The enterovirus species D type 68 (EV-D68), USA/MN/1989-23220 (GenBank: MN240496) genome was cloned into the *Escherichia coli (E. coli)* cloning vector pUC57-simple to generate plasmid EV-D68-R23220. EV-D68-R23220 contains a T7 bacteriophage promoter immediately upstream of the 5' end of the viral genome. Transfection of cells with RNA transcribed *in vitro* from the linearized plasmid results in production of infectious virus particles. EV-D68-R23220 also contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *E. coli*. The deposited plasmid was transformed into NEB® Stable Competent *E. 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: 70035763 **Manufacturing Date: 28MAY2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 10,130 base pairs	10,130 base pairs[1]
Genotypic Analysis Sequencing of Enterovirus D68 insert (~7400 base pairs)	≥ 99% sequence identity to EV-D68, USA/MN/1989-23220 (GenBank: MN240496.1)	100% sequence identity to EV-D68, USA/MN/1989-23220 (GenBank: MN240496.1)
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[2]	*bla* sequence present	*bla* sequence present
Agarose Gel Electrophoresis Digestion with *Xho*I and *Not*I	~ 7 kb and ~3 kb	~ 7 kb and ~ 3 kb (Figure 1)
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	1.8 µg in 100 µL per vial (18 µg/mL)
Amount per Vial	Report results	1.8 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation NEB® Stable Competent *E. coli*	≥ 50 colonies per ng	144 colonies per ng

[1]The sequence was assembled pre-vial using the predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.
[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.

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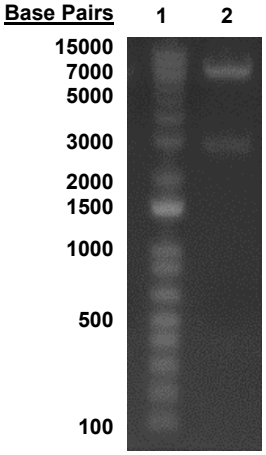
Figure 1: Agarose Gel of Restriction Enzyme Digested NR-52376



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-52376 digested

/Heather Couch/
Heather Couch 03 NOV 2020
Program Manager or designee, ATCC Federal Solutions

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