b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52375

Plasmid pUC19 Containing cDNA from Enterovirus D68, USA/Fermon, Infectious Clone EV-D68-R-Fermon

Catalog No. NR-52375

Product Description:

The enterovirus species D type 68 (EV-D68), USA/Fermon (GenBank: NC_038308) genome was cloned into the *Escherichia coli (E. coli)* cloning vector pUC19 to generate plasmid EV-D68-R-Fermon. EV-D68-Fermon 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-R-Fermon 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: 70035762 **Manufacturing Date: 13MAY2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 10,050 base pairs	10,053 base pairs[1]
Genotypic Analysis Sequencing of Enterovirus D68 insert (~7370 base pairs)	≥ 99% sequence identity to EV-D68, USA/Fermon (GenBank: NC_038308.1)	99.9% sequence identity to EV-D68, USA/Fermon (GenBank: NC_038308.1)[2]
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[3]	*bla* sequence present	*bla* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.3 µg in 30 µL per vial (9 µg/mL)
Amount per Vial	Report results	0.3 µg per vial
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	2.0
Effective Bacterial Transformation NEB® Stable Competent *E. coli*	≥ 50 colonies per ng	79 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]There is an insertion in the 5' untranslated region (UTR) of the EV-D68-R-Fermon insert (C28). It is unknown what effect this has on plasmid function.
[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 05 SEP 2020
Program Manager or designee, ATCC Federal Solutions

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Support Provided by NIAID

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