

**Plasmid pBR322 Containing cDNA from Enterovirus D68, US/MO/14-18947, Infectious Clone pBR-49129**

**Catalog No. NR-52009**

**Product Description:**

Enterovirus species D type 68 (EV-D68), US/MO/14-18947 (GenBank: KM851225) genome was cloned into the *Escherichia coli* (*E. coli*) cloning vector pBR322 to generate plasmid pBR-49129. pBR-49129 contains a T7 bacteriophage promoter immediately upstream of the 5' end of the viral genome and beta-lactamase gene TEM-1 to provide transformant selection through ampicillin resistance in *E. coli*. The resulting size of the plasmid is approximately 11,110 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 vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

**Lot: 70033867**

**Manufacturing Date: 20APR2020**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	Report results	11,110 base pairs (Figure 1) <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of Enterovirus D68 insert (11,110 base pairs)	Report results ≥ 99% sequence identity to EV-D68, US/MO/14-18947 (GenBank: KM851225.1)	100% sequence identity to depositor's sequence 99.9% sequence identity to EV-D68, US/MO/14-18947 (GenBank: KM851225.1)
<b>Antibiotic Resistance</b> Ampicillin (encoded by beta-lactamase gene TEM-1) <sup>2</sup>	TEM-1 sequence present	TEM-1 sequence present
<b>Concentration by PicoGreen® Measurement</b>	Report results	1.46 µg in 100 µL per vial (14.6 µg/mL)
<b>Amount per Vial</b>	Report results	1.46 µg per vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio (pre-vial)</b>	1.7 to 2.1	2.0
<b>Effective Bacterial Transformation</b> NEB® Stable Competent <i>Escherichia coli</i>	> 49 colonies per ng	270 colonies per ng

<sup>1</sup>The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence.

<sup>2</sup>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-52009**

>NR-52009\_70033867\_complete plasmid sequence

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