b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52520

Vector pHAGE2 Containing the ZsGreen Gene

Catalog No. NR-52520

Product Description:

The vector includes the green fluorescent protein (GFP) gene ZsGreen1, which was subcloned into the pHAGE2 lentiviral backbone vector under the CMV promoter. In addition, pHAGE2 includes the Woodchuck hepatitis virus post-transcriptional regulatory element to enhance levels of transcription and gene expression. NR-52520 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

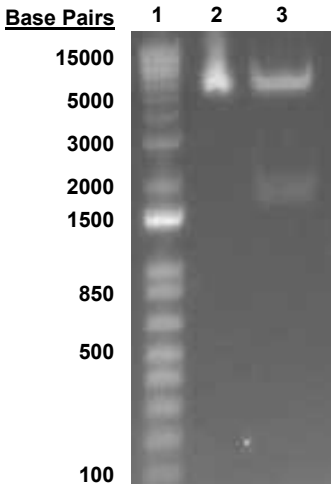
Lot: 70035484 **Manufacturing Date: 29APR2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	~ 7070 base pairs	7073 base pairs[1]
Genotypic Analysis Sequencing of ZsGreen1 gene (~ 700 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[2]	*bla* sequence present	*bla* sequence present
Agarose Gel Electrophoresis (pre-vial) Digestion with *Sap*I	~ 5 kb and ~ 2 kb	~ 5 kb and ~ 2 kb (Figure 1)
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	1.3 µg in 100 µL per vial (11 µg/mL)
Amount per Vial	Report results	1.3 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	> 500 colonies per ng

[1]The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence. The complete plasmid sequence is provided on the BEI Resources webpage.

[2]The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid replication to avoid plasmid loss and increased antibiotic concentrations may be necessary.

Figure 2: Agarose Gel of Undigested and Restriction Enzyme Digested NR-52520



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-52520 undigested
Lane 3: NR-52520 digested

BEI Resources
www.beiresources.org

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

Certificate of Analysis for NR-52520

/Heather Couch/
Heather Couch 24 AUG 2020
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by ATCC® and the contributor to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

BEI Resources
www.beiresources.org

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