Certificate of Analysis for NR-51176

Plasmid Containing Vaccinia Virus, Western Reserve Genome, VAC(WR)-LoxP-GFP-BAC

Catalog No. NR-51176

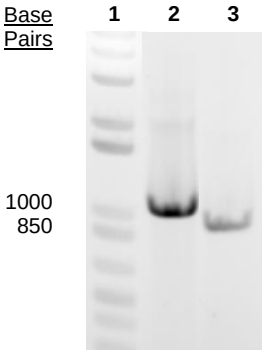
Product Description: The entire vaccinia virus (VACV) Western Reserve (WR) genome with a green fluorescent protein (GFP) sequence and two loxP sites was cloned into a plasmid vector and grown in *Escherichia coli* DH10β cells as a bacterial artificial chromosome (BAC).

Lot[1]: 70011271 **Manufacturing Date: 26JAN2018**

TEST	SPECIFICATIONS	RESULTS
PicoGreen® Measurement DNA content DNA concentration	 Report results Report results	 4 ng 4 ng in 100 µL per vial (0.004 µg per mL)
Restriction Digestion Analysis	Expected restriction pattern with *Hin*dIII-HF	Expected restriction pattern with *Hin*dIII-HF
Functional Activity by PCR Amplification[2]	Vaccinia-specific amplicon (~ 1100 base pairs) GFP amplicon (~ 1000 base pairs)	Vaccinia-specific amplicon (~ 1100 base pairs) (Figure 1) GFP amplicon (~ 1000 base pairs) (Figure 1)
Sequencing of Vaccinia-Specific Region (~ 920 base pairs)	≥ 99% sequence identity to VACV WR	100% identity with VACV WR (Genbank: AY243312)
Sequencing of GFP Amplicon (~ 890 base pairs)	≥ 99% sequence identity to GFP gene	100% identity with GFP sequence from pSV-EGFP vector (Genbank: GU062789)
Functional Activity by Recovery of Vaccinia Virus with Helper Virus by Cytopathic Effect and Immunofluorescence[3]	Recovery of vaccinia virus with fowlpox virus	Recovery of vaccinia virus with fowlpox virus

[1]Produced in *Escherichia coli* DH10β cells and extracted using a Plasmid *Plus* Maxi Kit (QIAGEN® 12963). The plasmid is functional in transfection into mammalian cells and recovery of vaccinia virus in the presence of a helper virus but transformation into bacteria for expansion purposes may not be successful.
[2]Amplified using iTaq DNA Polymerase (Bio-Rad 170-8870) and dNTP mix (Bio-Rad 170-8874)
[3]NR-51176 was transfected into fowlpox virus strain C (ATCC® VR-250™) infected *Cercopithecus aethiops* kidney CV1 (ATCC® CCL-70™) cells. Assembly of functional virus was monitored by cytopathic effect and immunofluorescence using monoclonal anti-vaccinia virus E3L (BEI Resources NR-4547 lot 57846250).

Figure 1: PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 100 base pairs DNA Ladder
Lane 2: NR-51176 Vaccinia-specific amplicon
Lane 3: NR-51176 loxP-GFP amplicon

BEI Resources
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Heather Couch

Digitally signed by Heather Couch
Date: 2018.06.15 08:15:23 -04'00'

15 JUN 2018

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 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

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