b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-10450

Genomic DNA from *Bacillus anthracis*, Strain Ames35

Catalog No. NR-10450

This reagent is the tangible property of the U.S. Government.

Product Description: Genomic DNA was isolated from a preparation of *Bacillus anthracis* (*B. anthracis*), strain Ames35. *B. anthracis*, strain Ames35 is a derivative of *B. anthracis*, strain Ames that was treated with novobiocin to cure it of the pXO2 plasmid.

Lot[1]: 59557827 **Manufacturing Date: 22APR2011**

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1400 base pairs)	Consistent with *B. cereus* group[2]	Consistent with *B. cereus* group[2]
Presence or Absence of Plasmids Confirmed by PCR Amplification pXO1 pXO2	 Positive Negative	 Positive Negative
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)
Content by PicoGreen® Measurement	0.7 to 1.5 µg in 25 to 100 µL per vial	0.9 µg in 31 µL per vial (30.5 µg/mL)
PCR Assay of Extracted DNA 16S ribosomal RNA gene Specific chromosomal marker[3] Presence of virulence plasmids[4] pXO1 (four targets) pXO2 (three targets)	 ~ 1500 bp amplicon; ~ 555 bp amplicon Amplicon present Amplicons present No amplicons	 ~ 1500 bp amplicon; ~ 555 bp amplicon Amplicon present Amplicons present No amplicons
OD_{260}/OD_{280} Ratio	1.7 to 2.0	1.9
Bacterial Inactivation 10% of total yield plated on Tryptic Soy Agar with 5% sheep blood[5,6]	 No viable bacteria detected	 No viable bacteria detected

[1] *B. anthracis*, strain Ames35 was deposited by Stephen Leppla, Laboratory of Bacterial Diseases, NIAID/NIH. The bacterial preparation used for extraction of genomic DNA was produced by Tryptic Soy Broth culture of the NR-10355 lot 58485555. After incubation for 24 hours at 37°C and aerobic atmosphere, genomic DNA was extracted using proprietary technology.
[2] *Bacillus cereus* group species (*B. cereus, B. thuringiensis, B. mycoides*, and *B. anthracis*) cannot be classified based on 16S sequence [Spencer, R. C. "*Bacillus anthracis*." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093].
[3] This product was verified to a species level using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.
[4] Plasmids were verified using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-plasmids pXO1 and pXO2.
[5] 7 days at 37°C in an aerobic atmosphere
[6] An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-positive and Gram-negative bacteria.

Date: 05 SEP 2012 **Signature:**

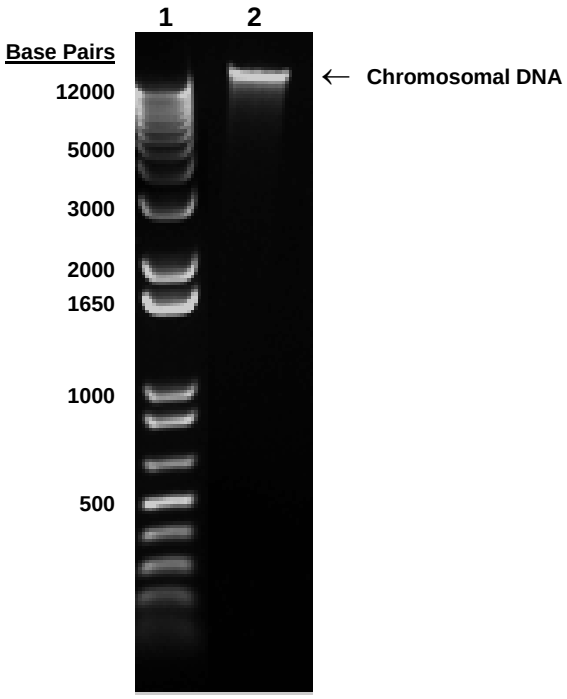
Title: Technical Manager, BEI Authentication or designee

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

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

Figure 1



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: 200 ng of NR-10450

BEI Resources
www.beiresources.org

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