

## Certificate of Analysis for NR-9541

## Genomic DNA from Bacillus anthracis, Strain Sterne BA695 (△cya244)

## Catalog No. NR-9541

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

**Product Description:** Genomic DNA was isolated from *Bacillus anthracis* (*B. anthracis*), strain Sterne BA695 ( $\Delta cya244$ ). The designation BA695 refers to the numbering system used in the Stibitz laboratory.

Lot<sup>1</sup>: 58109379 Manufacturing Date: 12JUN2008

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1420 base pairs)	Identical to BEI Resources NR-9398 Consistent with <i>B. cereus</i> group <sup>2</sup>	Identical to BEI Resources NR-9398 Consistent with <i>B. cereus</i> group <sup>2</sup>
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	4 to 6 μg in 25 to 100 μL per vial	5.8 μg in 40 μL per vial (144 μg/mL)
PCR Assay of Extracted DNA  16S ribosomal RNA gene Specific chromosomal marker³ Presence of virulence plasmids⁴ pXO1 (three targets) pXO2 (three targets) Verification of Δcya	~ 1500 bp amplicon; ~ 555 bp amplicon Amplicon present Amplicons present No amplicons No amplicon	~ 1500 bp amplicon; ~ 555 bp amplicon Amplicon present Amplicons present No amplicons No amplicon
OD <sub>260</sub> /OD <sub>280</sub> Ratio	1.7 to 1.9	1.8
Bacterial Inactivation 10% of total yield plated on Tryptic Soy Agar with 5% sheep blood <sup>5.6</sup>	No viable bacteria detected	No viable bacteria detected

<sup>&</sup>lt;sup>1</sup>NR-9541 was produced by Tryptic Soy Broth culture of NR-9398 (Lot: 57969327). After incubation for 24 hours at 37°C and 5% CO<sub>2</sub>, genomic DNA was extracted using proprietary technology.

**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.

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<sup>&</sup>lt;sup>2</sup>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].

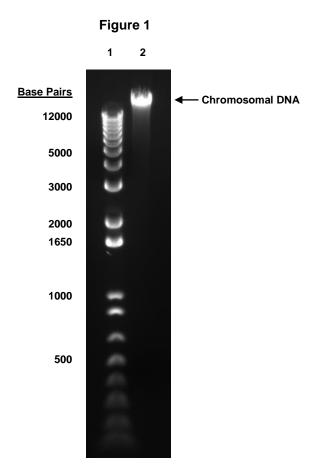
<sup>&</sup>lt;sup>3</sup>This product was verified to a species level using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.

<sup>&</sup>lt;sup>4</sup>Plasmids were verified using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-plasmids pXO1 and pXO2.

<sup>&</sup>lt;sup>5</sup>7 days at 37°C in an aerobic atmosphere

<sup>&</sup>lt;sup>6</sup>An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-positive and Gram-negative bacteria.

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Lane 1: Invitrogen™ Tracklt 1 Kb Plus DNA Ladder™ Lane 2: 200 ng of NR-9541

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