

## Certificate of Analysis for NR-10294

## Genomic DNA from Bacillus anthracis, Strain Sterne BA852 (∆asbB)

## Catalog No. NR-10294

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 Sterne BA852 ( $\triangle asbB$ ). This strain is a markerless, nonpolar, 1713 bp deletion mutant of the petrobactin biosynthetic gene ( $\triangle asbB$ ) of the toxigenic acapsulate original Sterne strain (34F2).

Lot<sup>1</sup>: 58253169 Manufacturing Date: 05DEC2008

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1410 bp)	Identical to BEI Resources NR-9990 Consistent with <i>B. cereus</i> group	Identical to BEI Resources NR-9990 Consistent with <i>B. cereus</i> group <sup>2</sup>
Presence or Absence of Plasmids Confirmed by PCR Amplification pXO1 (aat) pXO2 (at, capA, capB, capC)	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	6.0 µg in 46 µL per vial (130 µg/mL)
Functional Activity by PCR Amplification 16S ribosomal RNA gene Virulence markers on plasmid pXO1 (aat)	~ 1500 bp amplicon ~ 125 bp amplicon	~ 1500 bp amplicon ~ 125 bp 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>3,4</sup>	No viable bacteria detected	No viable bacteria detected

B. anthracis, strain BA851 (ΔasbB) was deposited by Philip Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. The bacterial preparation used for extraction of genomic DNA was produced by broth (Tryptic Soy Broth) culture of the deposited material. After incubation for 24 hours at 37°C and aerobic atmosphere, genomic DNA was extracted using proprietary technology.

**Date:** 09 MAY 2011 **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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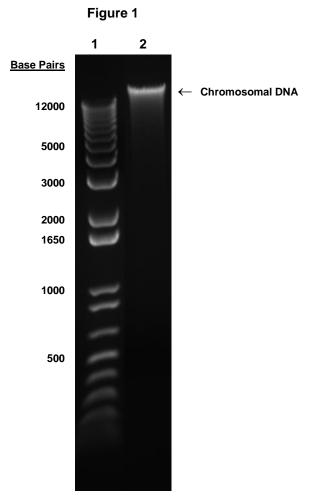
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<sup>&</sup>lt;sup>2</sup>Bacillus cereus group species (*B. cereus, B. thuringiensis, B. mycoides*, and *B. anthracis*) cannot be classified based on 16S sequence [Spencer, R. C. "Bacillus anthracis." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093].

<sup>&</sup>lt;sup>3</sup>7 days at 37°C in an aerobic atmosphere

<sup>&</sup>lt;sup>4</sup>An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative and Gram-positive bacteria.

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Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder Lane 2: 200 ng of NR-10294