b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-10353

Genomic DNA from *Bacillus cereus*, Strain Tor 16585

Catalog No. NR-10353

Product Description: Genomic DNA was isolated from a preparation of *Bacillus cereus (B. cereus)*, strain Tor 16585. *B. cereus*, strain Tor 16585 was isolated from left arm tissue of a patient from Long Island, New York with an open fracture on August 12, 2005.

Lot[1]: 58893069 **Manufacturing Date: 17DEC2009**

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1400 base pairs)	Identical to BEI Resources NR-12151 Consistent with *B. cereus* group	Identical to BEI Resources NR-12151 Consistent with *B. cereus* group[2]
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	7.0 µg in 35 µL per vial (199 µg/mL)[3]
Functional Activity by PCR Amplification[4] 16S ribosomal RNA gene *sspE* chromosomal gene *gyrB* chromosomal gene *groEL* chromosomal gene	 ~ 1500 bp amplicon ~ 70 bp amplicon (*B. cereus* group) ~ 475 bp amplicon (*B. cereus*) ~ 400 bp amplicon (*B. cereus* group)	 ~ 1500 bp amplicon ~ 70 bp amplicon (*B. cereus* group) ~ 475 bp amplicon (*B. cereus*) ~ 400 bp amplicon (*B. cereus* group)
OD_{260}/OD_{280} Ratio	1.7 to 1.9	1.9
Bacterial Inactivation 10% of total yield plated on Tryptic Soy Agar[5,6]	 No viable bacteria detected	 No viable bacteria detected

[1] *B. cereus*, strain Tor 16585 was deposited by George T. Tortora, Ph.D., Professor Emeritus, Department of Clinical Laboratory Sciences, Stony Brook University, Stony Brook, New York. The bacterial preparation used for extraction of genomic DNA was produced by broth (Tryptic Soy Broth; BD 211768) culture of the deposited material. 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] The µg of DNA in the vials is greater than required by current specifications

[4] PCR amplification of the *gyrB* gene yields a 253 bp amplicon for *B. anthracis*, a 604 bp amplicon for *B. mycoides* and a 737 bp amplicon for *B. thuringiensis*. Amplification of the *sspE* gene yields two amplicons for *B. anthracis* that are a 188 bp and 70 bp. Other *B. cereus* group species show only the 70 bp amplicon and non-*B. cereus* group species show no amplicons. For additional PCR information see Park, S.-H. et al. "Simultaneous Detection and Identification of *Bacillus cereus* Group Bacteria Using Multiplex PCR." J. Microbiol. Biotechnol. 17 (2007): 1177-1182. PubMed: 18051330.

[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: 03 JUN 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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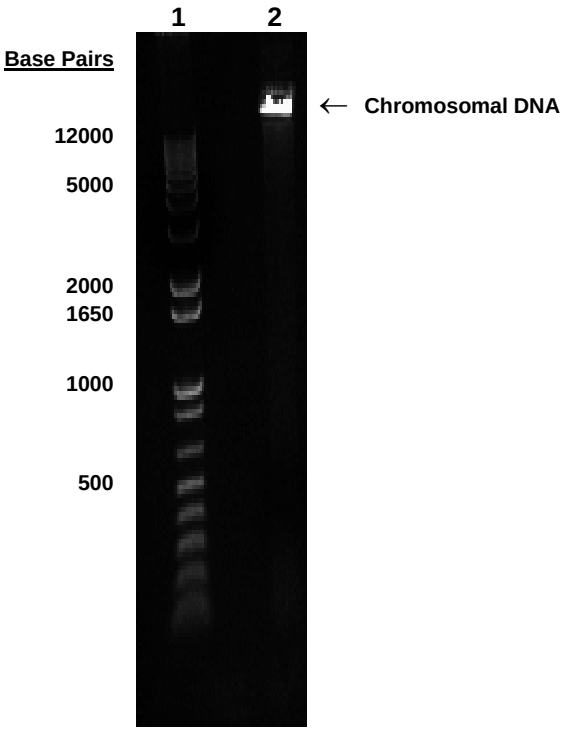
Figure 1



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: 200 ng of NR-10353

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