b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52275

Genomic DNA from *Bacillus licheniformis*, Strain Gibson 46

Catalog No. NR-52275

Product Description:

Genomic DNA was extracted from a preparation of *Bacillus licheniformis*, strain Gibson 46. The bacterial preparation used for extraction of genomic DNA was produced by culture of BEI Resources NR-52262 lot 70033110. Genomic DNA was extracted using proprietary technology and is provided in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8).

Lot: 70033337 **Manufacturing Date: 24MAR2020**

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Digital DNA-DNA hybridization (dDDH)[1] Sequencing of 16S ribosomal RNA gene (~ 1310 base pairs)	 ≥ 70% for species identification ≥ 99% sequence identity to *B. licheniformis,* strain Gibson 46 (GenBank: CP000002.3)	 *B. licheniformis* (96.6%)[2] 99.9% sequence identity to *B. licheniformis,* strain Gibson 46 (GenBank: CP000002.3)[3]
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)
Concentration by PicoGreen® Measurement	0.7 to 1.5 µg in 25 to 100 µL per vial	1.2 µg in 28.5 µL per vial (35.1 µg per mL)
Amount per Vial	0.7 to 1.5 µg	1.2 µg
Functional Activity by PCR Amplification 16S ribosomal RNA gene	 ~ 1500 base pair amplicon	 ~ 1500 base pair amplicon
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Bacterial Inactivation 10% of total yield plated on agar for 14 days[4,5]	 No viable bacteria detected	 No viable bacteria detected

[1]Relatedness between bacterial strains has traditionally been determined using DDH. For additional information, refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684. dDDH analysis was performed using the Type (Strain) Genome Server.

[2]The whole genome of *B. licheniformis*, strain Gibson 46 was sequenced using the Illumina® MiSeq® system. *De novo* contig sequences were generated using Unicycler v0.4.8-beta.

[3]Also consistent with *B. aerius*

[4]14 days under propagation conditions

[5]An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative and Gram-positive bacteria.

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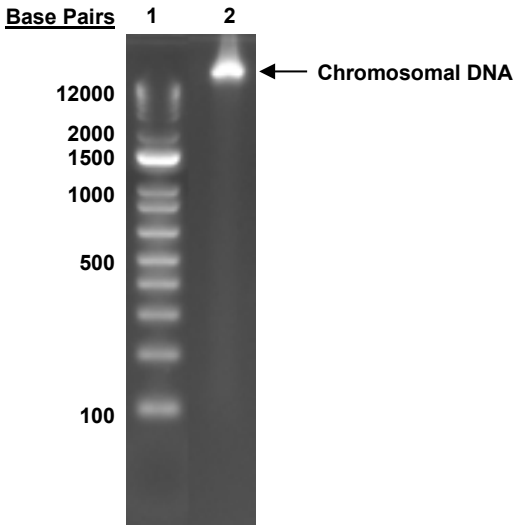
Figure 1: Agarose Gel Electrophoresis



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: ~ 200 ng of NR-52275

/Heather Couch/
Heather Couch 23 JUN 2021
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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