b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for HM-84D

Genomic DNA from Clostridiales bacterium, Strain 3_1_39B/D5

Catalog No. HM-84D

Product Description: Genomic DNA was extracted from a preparation of Clostridiales bacterium, strain 3_1_39B/D5. This isolate was obtained from healthy biopsy tissue from the gastrointestinal tract of a 44-year-old woman undergoing a colon cancer screen procedure in Alberta, Canada in 2007. [HM-84 was deposited to BEI Resources as unclassified *Clostridium*; digital DNA-DNA hybridization (dDDH) analysis, performed at BEI Resources, could not confirm the species-level classification.]

Lot[1,2]: 58984210 **Manufacturing Date: 09JUN2010**

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1350 base pairs) Digital DNA-DNA hybridization (dDDH)[4]	≥ 99% identical to depositor's sequence ≥ 70% for species identification	≥ 99% identical to depositor's sequence[3] *Faecalicatena fissicatena* (92.9%)[5]
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	1.0 µg in 50 µL per vial (20 µg/mL)
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 1.9	1.9
Bacterial Inactivation 10% of total yield plated on Tryptic Soy agar with 5% defibrinated sheep blood[6,7]	No viable bacteria detected	No viable bacteria detected

[1]Quality control of HMP material is only performed to demonstrate that the material distributed by BEI Resources is identical to the deposited material. It should not be considered a complete characterization of the deposited organism.
[2]The bacterial preparation used for extraction of genomic DNA was produced by Modified Reinforced Clostridial broth culture of the deposited material. After incubation for 2 days at 37°C in an anaerobic atmosphere (80% N_2:10% CO_2:10% H_2), genomic DNA was extracted using proprietary technology.
[3]HM-84D 16S sequence aligns favorably with GenBank sequences for *Eubacterium* (99%), *Clostridium* (97%), *Ruminococcus* (96%) and *Hespellia* (95%) species
[4]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.
[5]The required whole genome sequence for the type strain of this species is not available. *Faecalicatena fissicatena*, strain KCTC 15010 (GenBank: LDAQ00000000.1) was used for dDDH analysis. Because this strain is not the type strain and therefore, may be identified incorrectly, the dDDH only indicates Clostridiales bacterium, Strain 3_1_39B/D5 belongs to the same species as KCTC 15010.
[6]7 days at 37°C in an anaerobic atmosphere
[7]An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-positive and Gram-negative bacteria.

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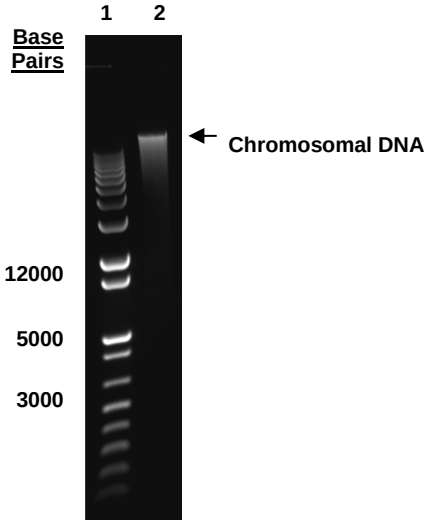
Figure 1: Agarose Gel Electrophoresis



Lane 1: Invitrogen™ TrackIt 1 Kb Plus DNA Ladder™
Lane 2: 200 ng of HM-84D

/Heather Couch/
Heather Couch 20 SEP 2018
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 by ATCC® 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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