

Certificate of Analysis for HM-299D

Genomic DNA from Citrobacter portucalensis, Strain 4_7_47CFAA

Catalog No. HM-299D

Product Description:

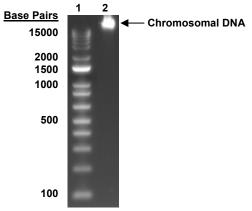
Genomic DNA was extracted from a preparation of *Citrobacter portucalensis* (*C. portucalensis*), strain 4 7 47CFAA. Note: Strain 4 7 47CFAA was previously classified as *Citrobacter freundii*.

Lot: 70016712^{1,2} Manufacturing Date: 28DEC2018

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1490 base pairs) Digital DNA-DNA hybridization (dDDH)	≥ 99% sequence identity to C. portucalensis, strain 4_7_47CFAA (GenBank: ADLG01000001.1) ≥ 70% for species identification	99.6% sequence identity to C. portucalensis, strain 4_7_47CFAA (GenBank: ADLG01000001.1) C. portucalensis (79.5%) 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	1.1 μg in 26 μL per vial (41 μg/mL)
Amount per vial	0.7 to 1.5 μg	1.1 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 100% of total yield plated on agar ^{4,5}	No viable bacteria detected	No viable bacteria detected

Quality control of HMP organisms used for DNA extraction is only performed to demonstrate that the material produced by BEI Resources is identical to the deposited material. It should not be considered a complete characterization of the deposited organism.

Figure 1: Agarose Gel Electrophoresis



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder

Lane 2: 200 ng of HM-299D

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²The bacterial preparation used for extraction of genomic DNA was produced by culture of BEI Resources HM-299 lot 70002700. Genomic DNA was extracted using proprietary technology.

³Relatedness 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." <u>Stand. Genomic Sci.</u> 2 (2010): 117-134. PubMed: 21304684. *C. portucalensis*, strain 4_7_47CFAA (GenBank: ADLG01000001.1), *C. portucalensis*, strain A60T (GenBank: MVFY00000001.1) and *Citrobacter freundii*, strain ATCC® 8090™ (GenBank: JMTA01000001.1) were used for dDDH analysis.

⁴14 days at 37°C in an anaerobic atmosphere on Nutrient agar

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



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/Heather Couch/ Heather Couch

05 SEP 2019

Program Manager or designee, ATCC Federal Solutions

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