Certificate of Analysis for NR-3052

Genomic DNA from *Escherichia coli*, Strain NCDC U14-41

Catalog No. NR-3052

Product Description: Genomic DNA was isolated from a preparation of enteroaggregative *Escherichia coli* (EAEC), strain NCDC U14-41, serotype O3:K2a,2b(L):H2.

Lot[1]: 64204563 **Manufacturing Date: 06JUN2016**

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 810 base pairs)	≥ 99% sequence identity to *E. coli* type strain (GenBank: JMDT01000030.1)	99.1% sequence identity to *E. coli* type strain (GenBank: JMDT01000030.1)
PCR amplification of plasmid markers[2,3]		
CVD432 (pAA)	Report results	Positive
aggR (pAA)	Report results	Positive
elt (pJY11)	Report results	Negative
esth (pCS1)	Report results	Negative
estp (pCS1)	Report results	Negative
EAF (pEAF)	Negative	Negative
bfpA (pEAF)	Negative	Negative
invE (pINV)	Negative	Negative
PCR amplification of chromosomal markers[2,3]		
eaeA	Negative	Negative
stx1	Negative	Negative
stx2	Negative	Negative
astA	Report results	Negative
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	0.93 µg in 46.5 µL per vial (20 µg/mL)
Functional Activity by PCR Amplification 16S ribosomal RNA gene	~ 1500 base pairs amplicon	~ 1500 base pairs amplicon
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.7
Bacterial Inactivation 10% of total yield plated on Tryptic Soy agar with 5% defibrinated sheep blood[4,5]	No viable bacteria detected	No viable bacteria detected

[1]The bacterial preparation used for extraction of genomic DNA was produced by inoculation of BEI Resources NR-102, Lot 3670409, into Tryptic Soy broth. After incubation for 24 hours at 37°C, genomic DNA was extracted using proprietary technology.
[2]PCR amplification was not performed on NR-3052, Lot 64204563. PCR amplification data was obtained from NR-3052, Lot 58666833, which was also produced from extraction of BEI Resources NR-102, Lot 3670409.
[3]Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." Microbiol. Immunol. 49 (2005): 485-492. PubMed: 15965295.
[4]14 days at 37°C in an aerobic atmosphere
[5]An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria and Gram-positive bacteria.

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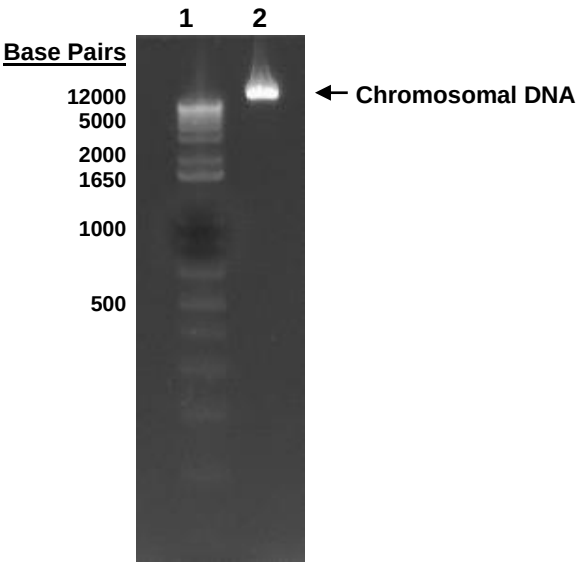
Figure 1: Agarose Gel Electrophoresis



Lane 1: Invitrogen™ 1Kb Plus DNA Ladder
Lane 2: 200 ng of NR-3052

Date: 26 SEP 2016

Signature:

BEI Resources Authentication

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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