

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* (EAggEC) strain NCDC U14-41, serotype O3:K2a,2b(L):H2. The bacterial preparation was produced by propagation of BEI Resources NR-102.

Lot¹: 7642443 Manufacturing Date: 06OCT2006

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis		
Sequencing of 16S rRNA gene (~ 500 bp)	Consistent with Escherichia coli	Consistent with Escherichia coli ²
PCR amplification of plasmid markers		
CVD432 (pAA)	Report results	Positive
aggR (pAA)	Report results	Positive
elt (pJŸ11)	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		
eaeA	Negative	Negative
stx1	Negative	Negative
stx2	Negative	Negative
astA	Report results	Negative
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosoma DNA (Figure 1)
Concentration by PicoGreen [®] Measurement	4 to 6 μg in 25 to 100 μL per vial	5.2 μg in 44 μL per vial (117 μg/mL)
Functional Activity by PCR Amplification 16S ribosomal RNA gene Virulence markers ³ CVD432 (pAA)	~ 1500 bp amplicon ~ 690 bp amplicon	~ 1500 bp amplicon ~ 690 bp amplicon
aggR (pAA)	~ 434 bp amplicon	~ 434 bp amplicon
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 1.9	1.9
Bacterial Inactivation 10% of total yield plated on Trypticase Soy Agar and incubated for 7 days at 37°C	No viable bacteria detected	No viable bacteria detected

¹The bacterial preparation used for extraction of genomic DNA was produced by inoculation of BEI Resources NR-102 (Lot 3670409) into LB Broth (ATCC® 60-2100). After incubation for 19 hours at 37°C, genomic DNA was extracted using proprietary technology.

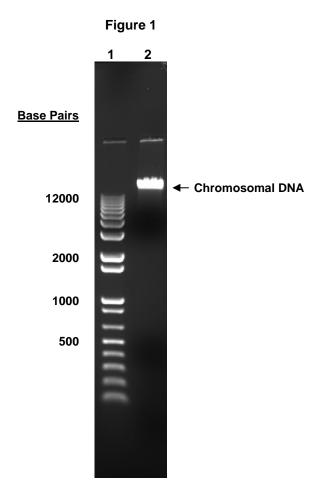
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²Also consistent with *Shigella* species.

³Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." <u>Microbiol. Immunol.</u> 49 (2005): 485–492. PubMed: 15965295.

⁴An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria.

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Lane 1: Invitrogen™ 1Kb Plus DNA Ladder

Lane 2: 200 ng of NR-3052

Date: 10 MAR 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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