

Certificate of Analysis for NR-2648

Genomic DNA from Escherichia coli, Strain EDL933

Catalog No. NR-2648

Product Description: Genomic DNA was isolated from a preparation of enterohemorrhagic *Escherichia coli* (EHEC) strain EDL933, serotype O157:H7. The bacterial preparation was produced by propagation of BEI Resources NR-11.

Lot¹: 5107305 Manufacturing Date: 07FEB2006

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis		
Sequencing of 16S rRNA gene (~ 500 bp)	Identical to NR-11	Identical to NR-11
, 3: 3: (3: 7)	Consistent with Escherichia coli	Consistent with Escherichia coli ²
PCR amplification of plasmid markers		
hylA (pO157)	Positive	Positive
elt (pĴY11)	Negative	Negative
esth (pCS1)	Negative	Negative
estp (pCS1)	Negative	Negative
EAF (pEAF)	Negative	Negative
<i>bfpA</i> (pEAF)	Negative	Negative
invE (pINV)	Negative	Negative
CVD432 (pAA)	Negative	Negative
aggR (pAA)	Negative	Negative
PCR amplification of chromosomal markers		
eaeA	Positive	Positive
stx1	Report results	Positive
stx2	Report results	Positive
astA	Report results	Negative
Agarose Gel Electrophoresis	High molecular weight chromosomal	High molecular weight chromosoma
	DNA	DNA (Figure 1)
Concentration by PicoGreen® Measurement	4 to 6 μg in 25 to 100 μL per vial	5.1 μg in 77 μL per vial (66 μg/mL)
Functional Activity by PCR Amplification		
16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Virulence markers ³		
hylA (pO157)	~ 3200 bp amplicon	~ 3200 bp amplicon
eaeA (chromosome)	~ 526 bp amplicon	~ 526 bp amplicon
stx1 (chromosome)	~ 349 bp amplicon	~ 349 bp amplicon
sx2 (chromosome)	~ 404 bp amplicon	~ 404 bp amplicon
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 1.9	1.9
Bacterial Inactivation		
10% of total yield plated on Nutrient 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-11 (Lot 3560110) into LB Broth (ATCC® 60-2100). After incubation for 16 hours at 37°C, genomic DNA was extracted using proprietary technology.

800-359-7370

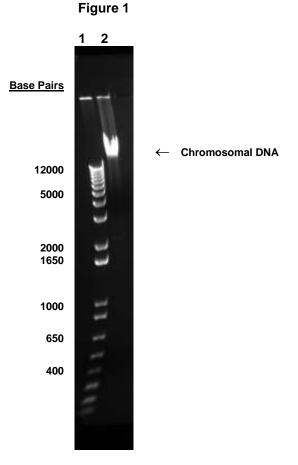
²Also consistent with *Shigella* species.

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 DNA Ladder™ Lane 2: 200 ng of NR-2648

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

Title: Technical Manager, BEI Authentication or designee

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.

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