

Certificate of Analysis for NR-2647

Genomic DNA from Escherichia coli, Strain H10407

Catalog No. NR-2647

Product Description: Genomic DNA was isolated from a preparation of enterotoxigenic *Escherichia coli* (ETEC), strain H10407, serotype O78:H11.

Lot¹: 61012595 Manufacturing Date: 12JUN2012

| TEST | SPECIFICATIONS | RESULTS |
|---|--|--|
| Genotypic Analysis | | |
| Sequencing of 16S rRNA gene (~ 1480 base pairs) | Consistent with Escherichia coli | Consistent with Escherichia coli ^{2,3} |
| Sequencing of 16S rRNA gene (~ 430 base pairs) | Identical to NR-4 | Identical to NR-4 |
| PCR amplification of plasmid markers ^{4,5} | | |
| elt (pJY11) | Positive | Positive |
| esth (pCS1) | Positive | Positive |
| estp (pCS1) | Positive | Positive |
| EAF (pEAF) | Negative | Negative |
| bfpA (pEAF) | Negative | Negative |
| invE (pINV) | Negative | Negative |
| CVD432 (pAA) | Negative | Negative |
| aggR (pAA) | Negative | Negative |
| PCR amplification of chromosomal markers ^{4,5} | | |
| eaeA | Negative | Negative |
| stx1 | Negative | Negative |
| stx2 | Negative | Negative |
| astA | Positive | Positive |
| 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 μg in 26 μL per vial (0.04 μg/μL) |
| Functional Activity by PCR Amplification | | |
| 16S ribosomal RNA gene | ~ 1500 bp amplicon | ~ 1500 bp amplicon |
| Virulence markers ^{4,5} | | |
| elt (pJY11) | ~ 263 bp amplicon | ~ 263 bp amplicon |
| esth (pCS1) | ~ 179 bp amplicon | ~ 179 bp amplicon |
| estp (pCS1) | ~ 123 bp amplicon | ~ 123 bp amplicon |
| astA (chromosome) | ~ 106 bp amplicon | ~ 106 bp amplicon |
| OD ₂₆₀ /OD ₂₈₀ Ratio | 1.7 to 2.0 | 2.0 |

| Bacterial Inactivation | | |
|--|-----------------------------|-----------------------------|
| 10% of total yield plated on Tryptic Soy Agar with | No viable bacteria detected | No viable bacteria detected |
| 5% defibrinated sheep blood ^{6,7} | | |

¹The bacterial preparation used for extraction of genomic DNA was produced by culture of BEI Resources NR-4 lot 3561329. Genomic DNA was extracted using proprietary technology.

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²Also consistent with *Shigella* species

³≥ 99% identical to GenBank: FN649414 (*E. coli*, strain H10407)

⁴PCR completed on NR-2647 lot 5107284 and NR-4 lot 3561329.

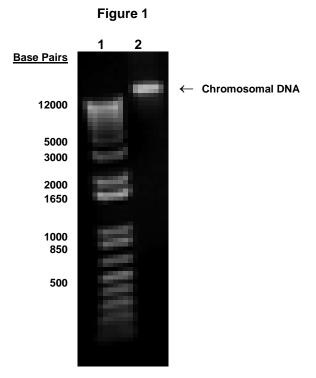
⁵Kimata, K., et al. "Rapid Categorization of Pathogenic *Escherichia coli* by Multiplex PCR." <u>Microbiol. Immunol.</u> 49 (2005): 485–492. PubMed: 15965295.

⁶7 days at 37°C and aerobic atmosphere

⁷An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-positive and Gram-negative bacteria.



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Lane 1: Invitrogen™ Tracklt 1 Kb Plus DNA Ladder™ Lane 2: 200 ng of NR-2647

Date: 12 JUN 2013

Signature:

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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