Certificate of Analysis for NR-46136

Escherichia coli – *Staphylococcus aureus* Shuttle Vector pCN44, Recombinant in *Escherichia coli*

Catalog No. NR-46136

Product Description: NR-46136 is a culture of *Escherichia coli* (*E. coli*) DH5α (RN9600, NRS600) containing the *E. coli*-staphylococcal shuttle vector pCN44. Vector pCN44 contains the *E. coli* ColE1 replication origin, the staphylococcal Φ11 phage fragment for high frequency transduction, the *Staphylococcus aureus (S. aureus*) pT181 *cop-wt-repC* replicon, the cadmium-inducible promoter P_{cad}-*cadC* and the *blaZ* transcriptional terminator. Vector pCN44 was deposited as resistant to ampicillin and erythromycin in *E. coli* and resistant to erythromycin in *S. aureus*.

Lot[1]: 63381371 **Manufacturing Date: 19MAR2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology[2]	Report results	Circular, slight peaked, entire and cream (Figure 1)
Motility (wet mount)	Report results	Motile
Confirmation of pCN44 Sequence		
Illumina® MiSeq® sequence (Figure 2, Table 1)	Report results	Consistent with pCN44 vector description[3,4]
Antibiotic Resistance		
Erythromycin (encoded by the adenine methylase gene *ermC*)	*ermC* sequence present	*ermC* sequence confirmed
Ampicillin (encoded by the beta-lactamase gene *amp)*	*amp* sequence present	*amp* sequence confirmed
Antibiotic Resistance		
Ampicillin (100 µg/mL)[2]	Resistant (growth)	Growth observed
Purity (post-freeze)[5]	Growth consistent with *E. coli*	Growth consistent with *E. coli*
Viability (post-freeze)[2]	Growth	Growth

[1]NR-46136 was produced by inoculation of the deposited material in Luria-Bertani (LB) broth with 100 µg/mL ampicillin and incubated for 20 hours at 37°C in an aerobic atmosphere with shaking at ~ 250 rpm. Broth inoculum was passaged once in LB broth with 100 µg/mL ampicillin for 22 hours at 37°C in an aerobic atmosphere with shaking at ~ 250 rpm to produce this lot.
[2]23 hours at 37°C in an aerobic atmosphere on LB agar with 100 µg/mL ampicillin
[3]Illumina® MiSeq® sequence was analyzed with CLC Genomics Workbench Version 7.0.2.
[4]pCN44 was sequenced and annotated by BEI Resources and is consistent with the vector described in Charpentier, E., et al. "Novel Cassette-Based Shuttle Vector System for Gram-Positive Bacteria." Appl. Environ. Microbiol. 70 (2004): 6076-6085. PubMed: 15466553. The BEI Resources vector sequence was deposited into GenBank as NR-46136 (GenBank: KR781465).
[5]Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood at 37°C in an aerobic atmosphere

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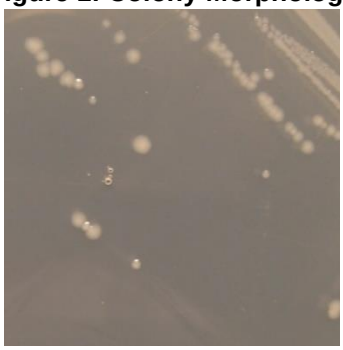
Figure 1: Colony Morphology



Date: 27 JUL 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.

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Figure 2: Shuttle Vector pCN44

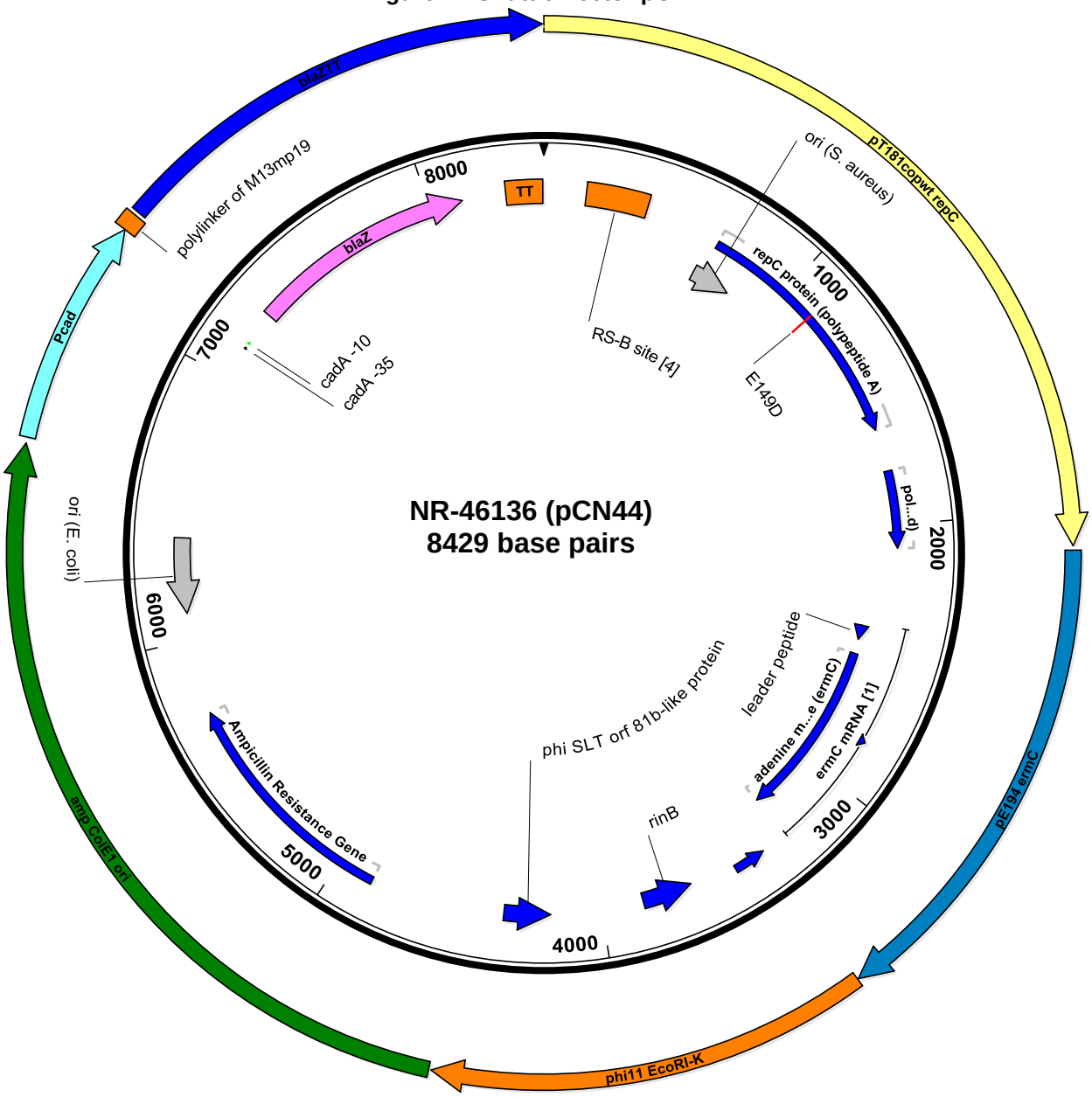

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Table 1: Sequence of shuttle vector pCN44

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 101  ACAGGAACAG CAACAAGAAC AACAAAAGAA TCAAAAACGA GATAGAGGTA   150
 151  TGCACTTATA GAACATGCAT TTATGCCGAG AAAACTTATT GGTTGGAATG   200
 201  GGCTATGTGT TAGCTAACTT GTTAGCGAGT TGGTTGGACT TGAATTGGGA   250
 251  TTAATCCCAA GAAAGTACCA ACTCAACAAC ACATAAAGCC CTGTAGGTTC   300
 301  CGACCAATAA GGAAATTGGA ATAAAGCAAT AAAAGGAGTT GAAGAAATGA   350
 351  AATTCAGAGA AGCCTTTGAG AATTTTATAA CAAGTAAGTA TGTACTTGGT   400
 401  GTTTTAGTAG TTTTAACTGT TTACCAGATA ATACAAATGC TTAAATAAAA   450
 451  AAAGACTTGA TCTGATTAGA CCAAATCTTT TGATAGTGTT ATATTAATAA   500
 501  CAAAATAAAA AGGAGTCGCT CACGCCCTGA CCAAAGTTTG TGAACGACAT   550
 551  CATTCAAAGA AAAAAACACT GAGTTGTTTT TATAATCTTG TATATTTAGA   600
 601  TATTAAACGA TATTTAAATA TACATCAAGA TATATATTTG GGTGAGCGAT   650
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 751  TCTAAAACCG GCTACTCTAA TAGCCGGTTG GACGCACATA CTGTGTGCAT   800
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1851  TGTATTCTTT CATTTTTTAG TGTATTAAAT GAAATGGTTT TAAATGTTTC  1900
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2051  TAGTTTGAGC TGTCTTGGTT CATTGATTGC TTTTATTGGG CCCACCTAGG  2100
2101  AATTGAATGA GACATGCTAC ACCTCCGGAT AATAAATATA TATAAACGTA  2150
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