Certificate of Analysis for NR-46129

Escherichia coli – *Staphylococcus aureus* Shuttle Vector pCN39, Recombinant in *Escherichia coli*

Catalog No. NR-46129

Product Description: NR-46129 is a culture of *Escherichia coli* (*E. coli*) DH5α (RN9592, NRS592) containing the *E. coli*-staphylococcal shuttle vector pCN39. Vector pCN39 contains the *E. coli* ColE1 replication origin and the thermosensitive *Staphylococcus aureus* (*S. aureus*) pT181 *cop-634ts-repC* replicon. Vector pCN39 was deposited as resistant to ampicillin and erythromycin in *E. coli* and resistant to erythromycin in *S. aureus*.

Lot[1]: 63381349 **Manufacturing Date: 13MAR2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology[2]	Report results	Circular, convex, entire, smooth and cream (Figure 1)
Motility (wet mount)	Report results	Motile
Confirmation of pCN39 Sequence		
Illuminia® MiSeq® sequence (Figure 2, Table 1)	Report results	Consistent with pCN39 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-46129 was produced by inoculation of the deposited material in Luria-Bertani (LB) broth with 100 µg/mL ampicillin and incubated for 25 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 16 hours at 37°C in an aerobic atmosphere with shaking at ~ 250 rpm to produce this lot.

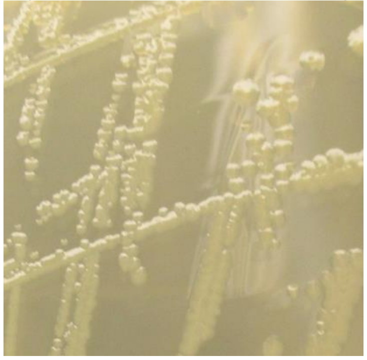
[2] 28 hours at 37°C in an aerobic atmosphere on LB agar with 100 µg/mL ampicillin

[3] Illuminia® MiSeq® sequence was analyzed with CLC Genomics Workbench Version 7.0.2.

[4] pCN39 was sequenced and annotated by ATCC® 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 ATCC® vector sequence was deposited into GenBank as NR-46129 (GenBank: KR781478).

[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

Figure 1: Colony Morphology



Date: 15 JUN 2015 **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

Figure 2: Shuttle Vector pCN39

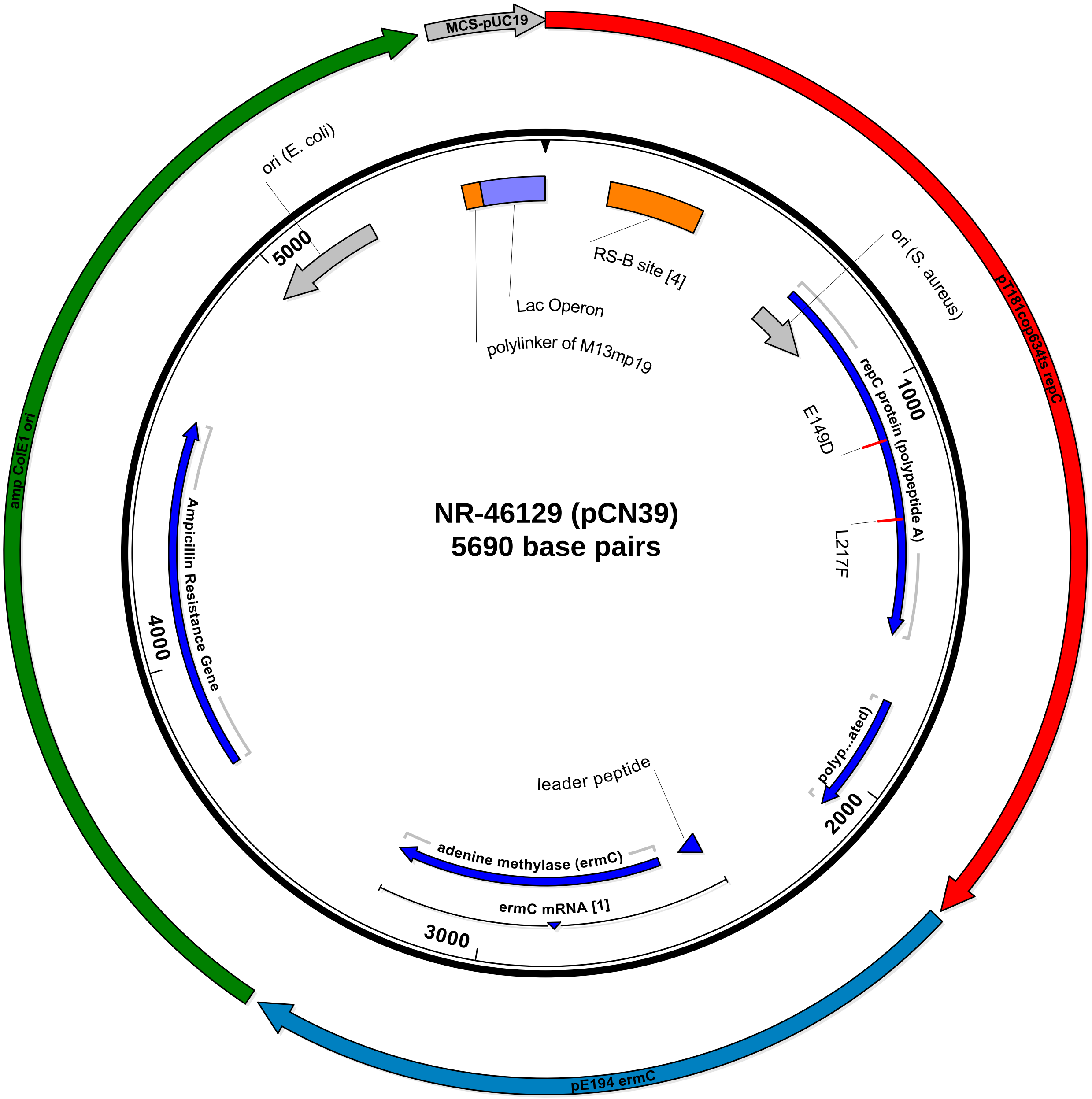


Table 1: Sequence of shuttle vector pCN39

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  51  GGTTTAGCAG GAAACTTAGA TAAAAAAATG AATCCAGAAT TATATTCAGA   100
 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 CACGACCTGA CCAAAGTTTG TGAACGACAT   550
 551  CATTCAAAGA AAAAAACACT GAGTTGTTTT TATAATCTTG TATATTTAGA   600
 601  TATTAAACGA TATTTAAATA TACATCAAGA TATATATTTG GGTGAGCGAT   650
 651  TCCTTAAACG AAATTGAGAT TAAGGAGTCG ATTTTTTATG TATAAAAACA   700
 701  ATCATGCAAA TCATTCAAAT CATTTGGAAA ATCACGATTT AGACAATTTT   750
 751  TCTAAAACCG GCTACTCTAA TAGCCGGTTG GACGCACATA CTGTGTGCAT   800
 801  ATCTGATCCA AAATTAAGTT TTGATGCAAT GACGATCGTT GGAAATCTCA   850
 851  ACCGAGACAA CGCTCAAGCC CTTTCTAAAT TTATGAGTGT AGAGCCCCAA   900
 901  ATAAGACTTT GGGATATTCT TCAAACAAAG TTTAAAGCTA AAGCACTTCA   950
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1051  ATGATTTGGT TAAAACAAAA TATAATAAGC TACATGGAAG ATGACGGTTT  1100
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1951  GGGTAAACAC TGCATATATG TTAACTTTTT CGATAGGAAC AGCAGTATAT  2000
2001  GGAAAATTAT CTGATTATAT AAATATAAAA AAATTGTTAA TTATTGGTAT  2050
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2101  AATTGAATGA GACATGCTAC ACCTCCGGAT AATAAATATA TATAAACGTA  2150
2151  TATAGATTTC ATAAAGTCTA ACACACTAGA CTTATTTACT TCGTAATTAA  2200
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2251  TTCTTTTTTT ACAAGAAAAA AGAAATTAGA TAAATCTCTC ATATCTTTTA  2300
2301  TTCAATAATC GCATCCGATT GCAGTATAAA TTTAACGATC ACTCATCATG  2350
2351  TTCATATTTA TCAGAGCTCG TGCTATAATT ATACTAATTT TATAAGGAGG  2400
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2551  AGTCAAAACT TTATTACTTC AAAACATAAT ATAGATAAAA TAATGACAAA  2600
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