

***Escherichia coli* – *Staphylococcus aureus* Shuttle Vector pCN36, Recombinant in *Escherichia coli***

**Catalog No. NR-46124**

**Product Description:** NR-46124 is a culture of *Escherichia coli* (*E. coli*) DH5α (RN9587, NRS587) containing the *E. coli*-staphylococcal shuttle vector pCN36. Vector pCN36 contains both the *E. coli* ColE1 replication origin and the *Staphylococcus aureus* (*S. aureus*) pT181 *cop-wt-repC* replicon and was deposited as resistant to ampicillin and tetracycline in *E. coli* and *S. aureus*, respectively.

**Lot<sup>1,2</sup>: 62436158**

**Manufacturing Date: 18APR2014**

TEST	SPECIFICATIONS	RESULTS
<b>Confirmation of pCN36 Sequence</b> Illumina <sup>®</sup> MiSeq <sup>®</sup> sequence (Figure 1, Table 1)	Report results	Consistent with pCN36 vector description <sup>3,4</sup>
<b>Antibiotic Resistance</b> Tetracycline (5 µg/mL) Ampicillin (100 µg/mL) <sup>6</sup>	Sensitive (no growth) Resistant (growth)	Growth observed <sup>5</sup> Growth observed
<b>Viability (post-freeze)<sup>7</sup></b>	Growth	Growth

<sup>1</sup>NR-46124 was produced by inoculation of the deposited material in Luria-Bertani (LB) broth with 5 µg/mL tetracycline and incubated for 21 hours at 37°C in an aerobic atmosphere with shaking (250 rpm). Broth inoculum was passaged once in LB broth with 5 µg/mL tetracycline for 21 hours at 37°C in an aerobic atmosphere with shaking at 250 rpm to produce this lot.

<sup>2</sup>NR-46124 was deposited as sensitive to tetracycline and resistant to ampicillin. BEI Resources cultured NR-46124 in the presence of tetracycline and observed growth. Sequencing of the vector identified two tetracycline resistance genes: *tetK* (for expression in *E. coli*) and *tetM* (for expression in *S. aureus*). pCN36 has a transposon insertion which deletes approximately 85% of the *tetK* coding sequence, making it unlikely that the resulting protein is cellularly active and it was reported as sensitive to tetracycline in the original paper describing the construction of this vector (Charpentier E., et al. "Novel Cassette-Based Shuttle Vector System for Gram-Positive Bacteria." *Appl. Environ. Microbiol.* 70 (2004): 6076-6085. PubMed: 15466553.). *tetM* has not been reported to express tetracycline resistance in *E. coli* (Chopra, I. and M. Roberts. "Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance." *Microbiol. Mol. Biol. Rev.* 65 (2001): 232-260. PubMed: 11381101). Sequence analysis identified a point mutation located within *tetM* that results in an amino acid mutation: F109G. This mutation may or may not have functional implication on the activity of the translated protein in *S. aureus*.

<sup>3</sup>Illumina<sup>®</sup> MiSeq<sup>®</sup> sequence was analyzed with CLC Genomics Workbench Version 7.0.2.

<sup>4</sup>pCN36 was sequenced and annotated by BEI Resources and is consistent with the vector described in Charpentier 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 pNR-46124 (GenBank: KM015350). Differences that were not described in the paper include a transposon insertion which deletes approximately 85% of the *tetK* coding sequence and a point mutation located within *tetM* that results in an amino acid mutation: F109G.

<sup>5</sup>NR-46124 was deposited as sensitive to tetracycline in *E. coli*.

<sup>6</sup>24 hours at 37°C in an aerobic atmosphere on LB agar with 100 µg/mL ampicillin

<sup>7</sup>24 hours at 37°C in an aerobic atmosphere on LB agar with 5 µg/mL tetracycline

Date: 27 JUL 2016

Signature:



BEI Resources Authentication

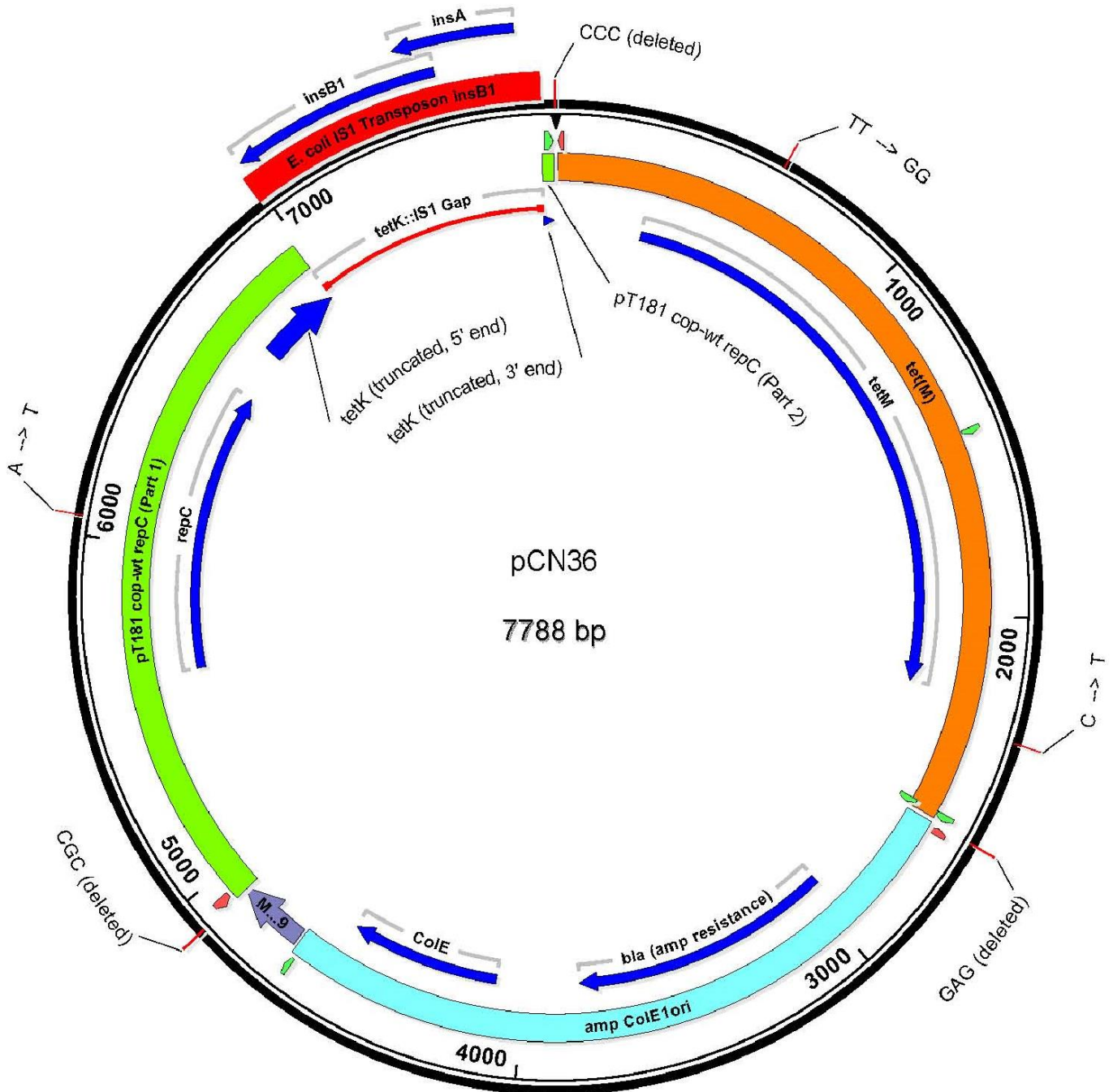
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Figure 1: Shuttle Vector pCN36



**Table 1: Sequence of shuttle vector pCN36**

1	cctaggcaaa	tatgctctta	cgtgctatta	tttaagtgac	tattttaaag	50
51	gagttaataa	atatgctggca	aggtattctt	aaataaactg	tcaatttgat	100
101	agcgggaaca	aataattaga	tgctcttttt	taggagggct	tagttttttg	150
151	taccagttt	aagaatacct	ttatcatgtg	attctaaagt	atccagagaa	200
201	tatctgtatg	ctttgtatac	ctatggttat	gcataaaaaat	cccagtgata	250
251	aaagtattta	tacttgggat	ttttatgccc	ttttggggtt	ttgaatggag	300
301	gaaaatcaca	tgaaaattat	taaatattga	gtttttagctc	atggtgatgc	350
351	aggaaaaact	accttaacag	aaagcttatt	atataacagt	ggagcgatta	400
401	cagaattagg	aagcgtggac	aaaggtacaa	cgaggacgga	taatacgctt	450
451	ttagaacgtc	agagaggaat	tacaattcag	acaggaataa	cctcttttca	500
501	gtgggaaaaat	acgaaggatg	acatcataga	cacgccagga	catatggatt	550
551	tcttagcaga	agtatatcgt	tattatcag	tttttagatgg	ggcaattcta	600
601	ctgatttctg	caaaagatgg	cgtacaagca	caaactcgta	tattatttca	650
651	tgcacttagg	aaaatgggga	ttcccacaat	cttttttatt	aataagattg	700
701	acccaaatgg	aattgattta	tcaacgggtt	atcaggatat	taaagagaaa	750
751	ctttctgccc	aaattgtaat	caaacagaag	gtagaactgt	atcctaatat	800
801	gtgtgtgacg	aactttaccg	aatctgaaca	atgggatacg	gtaatagagg	850
851	gaaacgatga	ccttttagag	aaatatatgt	ccggtaaatac	attagaagca	900
901	ttggaactcg	aacaagagga	aagcataaga	tttcagaatt	gttctctggt	950
951	ccctctttat	catggaagtg	caaaaagtaa	tatagggatt	gataacctta	1000
1001	tagaagttat	tactaataaa	ttttattcat	caacacatcg	aggctccgtc	1050
1051	gaactttgcg	gaaatgtttt	caaaattgaa	tatacaaaaa	aaagacaacg	1100
1101	tcttgcatat	atacgccttt	atagtggagt	actacattta	cgagattcgg	1150
1151	ttagagtatc	agaaaaagaa	aaaataaaag	ttacagaaat	gtatacttca	1200
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1301	aactattgcc	acagagaaaa	aagattgaaa	atccgcaccc	tctactacaa	1350
1351	acaactgttg	aaccgagtaa	acctgaacag	agagaaatgt	tgcttgatgc	1400
1401	ccttttgtaa	atctcagata	gtgatccgct	tctacgatat	tacgtggatt	1450
1451	ctacgacaca	tgaaattata	ctttctttct	tagggaaagt	acaaatggaa	1500
1501	gtgattagtg	cactgttgca	agaaaagtat	catgtggaga	tagaactaaa	1550
1551	agagcctaca	gtcatttata	tggagagacc	gttaaaaaat	gcagaatata	1600
1601	ccattcacat	cgaagtgccg	ccaaatcctt	tctgggcttc	cattggttta	1650
1651	tctgtatcac	cgcttccggt	gggaagtggg	atgcagtatg	agagctcggg	1700
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1751	tacgctatgg	ttgcaacaa	ggattatatg	gttggaatgt	gacggattgt	1800
1801	aaaatctggt	ttaagtacgg	tttatactat	agccctgtta	gtactccagc	1850
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1901	ctggaacaga	attgttagag	ccatatctta	gttttaaagt	ttatgcacca	1950
1951	caggaatatac	tttcacgggc	atataacgat	gctcccaaat	attgtgcaaa	2000
2001	tatcgtaaat	actcaactga	aaaataatga	ggtcattatt	attggagaaa	2050
2051	ttcctgctcg	atgtattcaa	gattatcgca	atgatttaac	tttttttaca	2100
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2401	acttaaaaaat	ttccctttat	actgccccaa	atgtaagaac	gaaactttaa	2450
2451	ttaatgttca	aaaaatgaat	ataataacaa	tcaaagagcc	agacgcccaag	2500
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2601	gcggccgcat	agttaagcca	gccccgacac	ccgccaacac	ccgctgacgc	2650

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