

***Escherichia coli* – *Staphylococcus aureus*  
Shuttle Vector pCN54, Recombinant in  
*Escherichia coli***

**Catalog No. NR-46154**

**For research use only. Not for human use.**

**Contributor:**

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**Manufacturer:**

BEI Resources

**Product Description:**

NR-46154 is a culture of *Escherichia coli* (*E. coli*) DH5 $\alpha$  (RN9619, NRS619) containing the *E. coli*-staphylococcal shuttle vector pCN54. Vector pCN54 contains the *E. coli* ColE1 replication origin and the *Staphylococcus aureus* (*S. aureus*) pT181 *cop-wt-repC* replicon, the cadmium-inducible promoter *P<sub>cad-cadC</sub>*, the GFP reporter gene *gfpmut2* and the *blaZ* transcriptional terminator. Vector pCN54 was deposited as resistant to ampicillin and erythromycin in *E. coli* and resistant to erythromycin in *S. aureus*.<sup>1</sup>

The complete sequence and vector map of pCN54 have been determined and are available on the Certificate of Analysis for lot 63381374. The BEI Resources vector sequence was deposited into GenBank as NR-46154 (GenBank: KR781470).

Vector pCN54 is a member of a series of novel shuttle vectors that were developed using PCR-designed cassettes to allow for easy exchange of vector components. The base shuttle vectors are comprised of (i) a staphylococcal replicon (pT181-based low-copy number, high-copy-number or thermosensitive replicons or pI258-based low-copy-number theta replicon), (ii) a staphylococcal selectable marker (erythromycin, tetracycline, chloramphenicol, kanamycin or spectinomycin resistance), (iii) an *E. coli* ColE1-based replicon, (iv) an *E. coli* selectable marker (ampicillin resistance) and (v) a pUC19-derived expanded multiple cloning site (MCS). Additionally, some of the vectors may contain a staphylococcal  $\phi$ 11 phage fragment, staphylococcal pathogenicity island SaPI1 fragment, an inducible or constitutive promoter, and reporter genes.<sup>1</sup>

**Material Provided:**

Each vial of NR-46154 contains approximately 0.5 mL of bacterial culture in Luria-Bertani (LB) broth containing 100  $\mu$ g/mL ampicillin supplemented with 10% glycerol.

**Packaging/Storage:**

NR-46154 was packaged aseptically in cryovials. The

product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

**Growth Conditions:**

Media:

LB broth containing 100  $\mu$ g/mL ampicillin

LB agar containing 100  $\mu$ g/mL ampicillin

Incubation:

Temperature: 37°C

Atmosphere: Aerobic

Propagation:

1. Keep vial frozen until ready for use, then thaw.
2. Transfer the entire thawed aliquot into a single tube of broth.
3. Use several drops of the suspension to inoculate an agar slant and/or plate.
4. Incubate the tube, slant and/or plate at 37°C for 18 to 24 hours.

**Citation:**

Acknowledgment for publications should read “The following reagent was provided by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) for distribution by BEI Resources, NIAID, NIH: *Escherichia coli* – *Staphylococcus aureus* Shuttle Vector pCN54, Recombinant in *Escherichia coli*, NR-46154.”

**Biosafety Level: 1**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see [www.cdc.gov/biosafety/publications/bmbli5/index.htm](http://www.cdc.gov/biosafety/publications/bmbli5/index.htm).

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**References:**

1. Charpentier E., et al. "Novel Cassette-Based Shuttle Vector System for Gram-Positive Bacteria." Appl. Environ. Microbiol. 70 (2004): 6076-6085. PubMed: 15466553.

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