

***Escherichia coli* K-12, Strain DC10B**

**Catalog No. NR-49804**

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**Contributor:**

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**Manufacturer:**

BEI Resources

**Product Description:**

Bacteria Classification: *Enterobacteriaceae, Escherichia*

Species: *Escherichia coli*

Strain: K-12, DC10B

Original Source: *Escherichia coli* (*E. coli*) K-12, strain DC10B is a DNA cytosine methyltransferase (*dcm*) deletion mutant that was produced from *E. coli* K-12 derivative strain DH10B via recombination-mediated genetic engineering (recombineering).<sup>1,4</sup>

Comments: *E. coli* K-12, strain DC10B is a universal host for constructing plasmids for introduction into staphylococci and was deposited as  $\Delta dcm$  and resistant to streptomycin.<sup>1,2</sup>

*E. coli* K-12, strain DC10B is a  $\Delta dcm$  mutant of the high-efficiency cloning strain, DH10B, and provides a background for plasmid production in the absence of cytosine methylation. The lack of methylation allows plasmid DNA to bypass a conserved type IV restriction-modification (RM) barrier in Staphylococci which has been identified as a major barrier to transformation with foreign DNA.<sup>1,3,4</sup> *E. coli* K-12, strain DC10B is an ideal host for the construction of recombinant plasmids for subsequent direct transformation into *Staphylococcus aureus* (*S. aureus*) or *Staphylococcus epidermidis*.<sup>1</sup>

Additional transformation efficiency can be gained by the introduction of the *S. aureus* clonal complex (CC) specific methylation profiles observed in type 1 RM. The type 1 RM protein complex is comprised of three protein components: a methylase (HsdM), a specificity protein (HsdS) and a restriction protein (HsdR). The complex recognizes a target recognition motif (TRM) determined by HsdS and detects its methylation status via HsdM. DNA that is correctly hemimethylated will be fully methylated which will prevent the restriction of the DNA by the RM protein complex.<sup>3</sup> *E. coli* K-12 strains recombineered to contain genes from *S. aureus* CC1, CC8, CC30 and CC93, in a  $\Delta dcm$  background, are available from BEI Resources as NR-49805 through NR-49808, respectively.

**Material Provided:**

Each vial contains approximately 0.5 mL of bacterial culture in Tryptic Soy broth containing 25 µg/mL streptomycin supplemented with 10% glycerol.

Note: If homogeneity is required for your intended use, please purify prior to initiating work.

**Packaging/Storage:**

NR-49804 was packaged aseptically, in screw-capped plastic 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:

Tryptic Soy broth or Nutrient broth or equivalent containing 25 µg/mL streptomycin

Tryptic Soy agar or Nutrient agar or Tryptic Soy agar with 5% defibrinated sheep blood or equivalent containing 25 µg/mL streptomycin

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 24 hours.

**Citation:**

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: *Escherichia coli* K-12, Strain DC10B, NR-49804.”

**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/bmbl5/index.htm](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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#### References:

1. Monk, I., et al. "Transforming the Untransformable: Application of Direct Transformation to Manipulate Genetically *Staphylococcus aureus* and *Staphylococcus epidermidis*." MBio. 20 (2012): e00277-11. PubMed: 22434850.
2. Monk, I., Personal Communication.
3. Monk, I., et al. "Complete Bypass of Restriction Systems for Major *Staphylococcus aureus* Lineages." MBio. 26 (2015): e00308-15. PubMed: 26015493.
4. Monk, I. and T. J. Foster. "Genetic Manipulation of Staphylococci-Breaking Through the Barrier." Front Cell Infect. Microbiol. 12 (2012): e00049. PubMed: 22919640.

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