

Escherichia coli* – *Staphylococcus aureus* Shuttle Vector pKK22, Recombinant in *Escherichia coli

Catalog No. NR-50348

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-50348 is a preserved culture of *Escherichia coli* (*E. coli*), strain DH5αpir containing the *E. coli* – staphylococcal shuttle vector pKK22. Vector pKK22 contains the *E. coli* R6Kγ origin of replication and is for use in *E. coli* and *Staphylococcus aureus* (*S. aureus*) USA300 strains that contain LAC-p01, rendering them isogenic.^{1,2} Vector pKK22 contains a single trimethoprim resistance cassette that is functional in both *E. coli* and *S. aureus*.¹ The complete pKK22 nucleotide sequence has been sequenced (GenBank: [KX085042](#)) and the vector map of pKK22 is available in Appendix I.

pKK22 was deposited in conjunction with pKK30 and *E. coli* strains DH5αpir and GM2163λpir (Table 1). pKK22 and pKK30 were created to maintain stability in *E. coli* and *Staphylococcus* species without antibiotic selection during *in vitro* and *in vivo* experiments. The *E. coli* R6Kγ origin of replication of both vectors requires *pir+* for replication which is provided in either DH5αpir or GM2163λpir *E. coli* strains.³

Table 1: *E. coli* – *Staphylococcus* Vectors and Hosts

Catalog Number	Vector or Host	Comments
NR-50348	pKK22	For use in <i>E. coli</i> , strains DH5αpir or GM2163λpir or <i>S. aureus</i> USA300 strains containing LAC-p01 ²
NR-50349	pKK30	pKK30 is a variant of pKK22, for use in <i>E. coli</i> , strains DH5αpir or GM2163λpir or <i>Staphylococcus</i> species not containing LAC-p01 ²
NR-50350	<i>E. coli</i> , Strain DH5αpir	Host strain containing the <i>pir</i> genes for performing genetic manipulations prior to transfer into <i>Staphylococcus</i> ³
NR-50351	<i>E. coli</i> , Strain GM2163λpir	Host strain containing the <i>pir</i> genes for performing genetic manipulations. This strain is also a Dam and Dcm methylase mutant for transfer of plasmids into <i>Staphylococcus</i> isolates that do not accept <i>E. coli</i> DNA easily. ³

Material Provided:

Each vial of NR-50348 contains approximately 0.5 mL of *Escherichia coli* – *Staphylococcus aureus* Shuttle Vector pKK22, Recombinant in *Escherichia coli* in Tryptic Soy broth containing 10 µg/mL trimethoprim supplemented with 10% glycerol.

Packaging/Storage:

NR-50348 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:

Tryptic Soy broth or equivalent with or without 10 µg/mL trimethoprim

Tryptic Soy agar or Nutrient agar or Tryptic Soy agar with 5% defibrinated sheep blood or equivalent; with or without 10 µg/mL trimethoprim

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 1 day.

Citation:

Acknowledgment for publications should read “The following reagent was contributed by Dr. J. L. Bose for distribution by BEI Resources, NIAID, NIH: *Escherichia coli* – *Staphylococcus aureus* Shuttle Vector pKK22, Recombinant in *Escherichia coli*, NR-50348.”

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 (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

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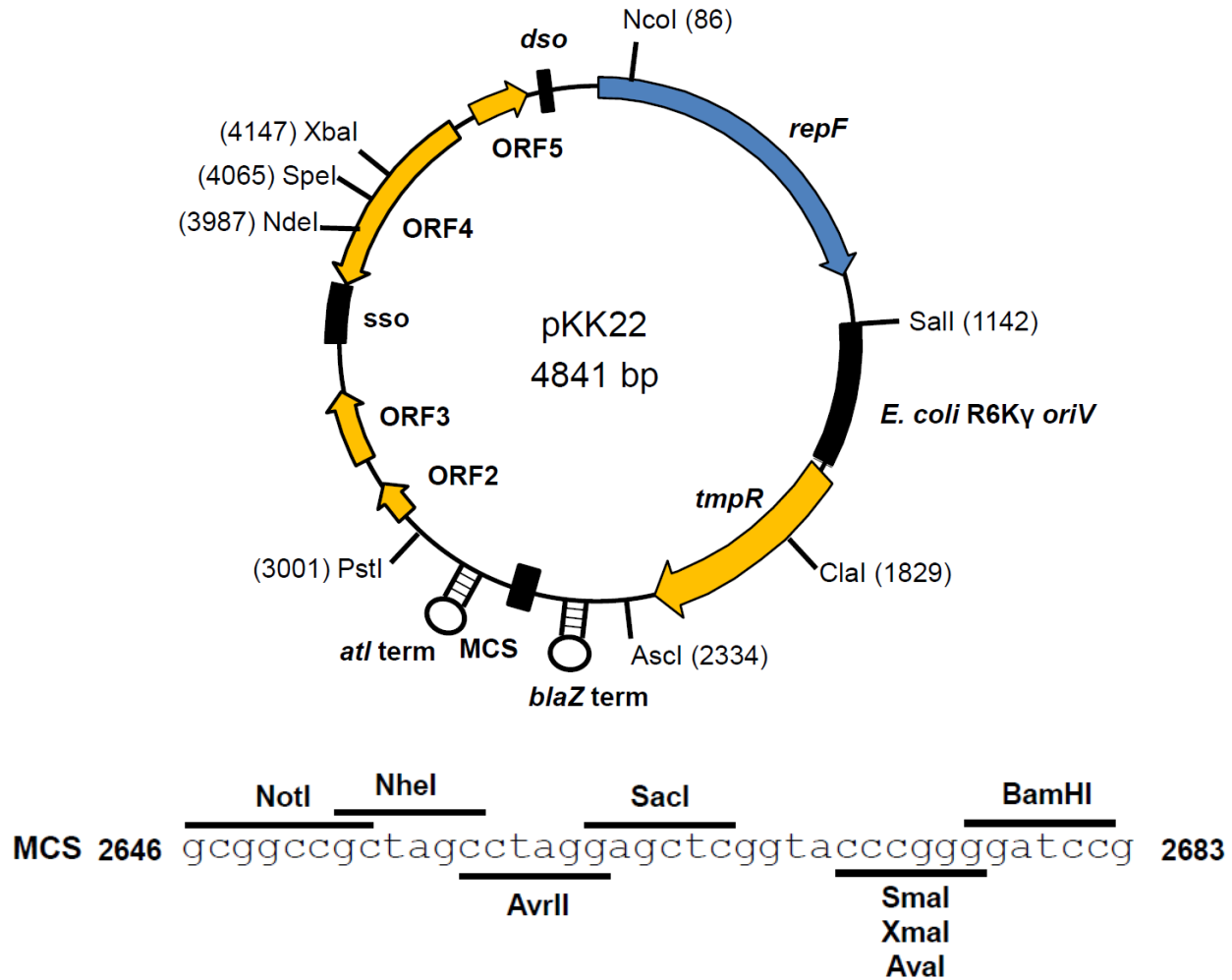
References:

1. Bose, J. L., Personal Communication.
2. Krute, C. N., et al. "Generation of a Stable Plasmid for *In Vitro* and *In Vivo* Studies of *Staphylococcus* Species." Appl. Environ. Microbiol. 82 (2016): 6859-6869. PubMed: 27637878.
3. Dunn, A. K., M. O. Martin and E. V. Stabb. "Characterization of pES213, A Small Mobilizable Plasmid from *Vibrio fischeri*." Plasmid 54 (2005): 114-134. PubMed: 16122560.

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APPENDIX I: VECTOR pKK22 MAP



Notes:

- pKK22 is designed to be used in USA300 strains of *S. aureus* containing LAC-p01 (pUSA01)
- Entire plasmid sequence can be found in GenBank Accession KX085042
- *tmpR* denotes trimethoprim resistance in both *E. coli* and *Staphylococcus* species
- Clal site is methylation blocked and sits between the promoter and *dfrA* gene
- The R6Kγ origin of replication requires *pir+* strains of *E. coli* to replicate