

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

Catalog No. NR-50348

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## Contributor:

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## Manufacturer:

BEI Resources

## Product Description:

NR-50348 is a preserved culture of *Escherichia coli* (*E. coli*) DH5 $\alpha$ pir containing the *E. coli*-staphylococcal shuttle vector pKK22. Vector pKK22 contains the *E. coli* R6K $\gamma$  origin of replication and is for use in *E. coli* and *Staphylococcus aureus* (*S. aureus*) USA300 strains that contain LAC-p01, rendering them isogenic.<sup>1,2</sup> Vector pKK22 contains a single trimethoprim resistance cassette that is functional in both *E. coli* and *S. aureus*.<sup>1</sup> The complete pKK22 nucleotide sequence is available (GenBank: [KX085042](#)) and the vector map of pKK22 is available below in Appendix I.

pKK22 was deposited in conjunction with pKK30 and *E. coli* strains DH5 $\alpha$ pir and GM2163 $\lambda$ pir (see Table 1 below for details). 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* R6K $\gamma$  origin of replication of both vectors requires *pir+* for replication which is provided in either DH5 $\alpha$ pir or GM2163 $\lambda$ pir *E. coli* strains.<sup>3</sup>

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

Catalog Number	Vector or Host	Comments
NR-50348	pKK22	For use in <i>E. coli</i> DH5 $\alpha$ pir or GM2163 $\lambda$ pir or <i>S. aureus</i> USA300 strains containing LAC-p01 <sup>2</sup>
NR-50349	pKK30	pKK30 is a variant of pKK22, for use in <i>E. coli</i> DH5 $\alpha$ pir or GM2163 $\lambda$ pir or <i>Staphylococcus</i> species not containing LAC-p01 <sup>2</sup>
NR-50350	<i>E. coli</i> DH5 $\alpha$ pir	Host strain containing the <i>pir</i> genes for performing genetic manipulations prior to transfer into <i>Staphylococcus</i> (F $\phi$ 80dlacZ $\Delta$ M15 $\Delta$ lacZYA-argF U169 deoR supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1) <sup>2</sup>

Catalog Number	Vector or Host	Comments
NR-50351	<i>E. coli</i> GM2163 $\lambda$ pir	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 (F $\phi$ ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 rpsL136 dam13::Tn9 xylA5 mtl-1 thi-1 mcrB1 hsdR2 $\lambda$ pir) <sup>2</sup>

## Material Provided:

Each vial of NR-50348 contains approximately 0.5 mL of *E. coli*, DH5 $\alpha$ pir, in Tryptic Soy broth containing 10  $\mu$ g/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  $\mu$ g/mL trimethoprim

Tryptic Soy agar, nutrient agar, Tryptic Soy agar with 5% defibrinated sheep blood or equivalent; with or without 10  $\mu$ g/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](#). 5th ed.

BEI Resources

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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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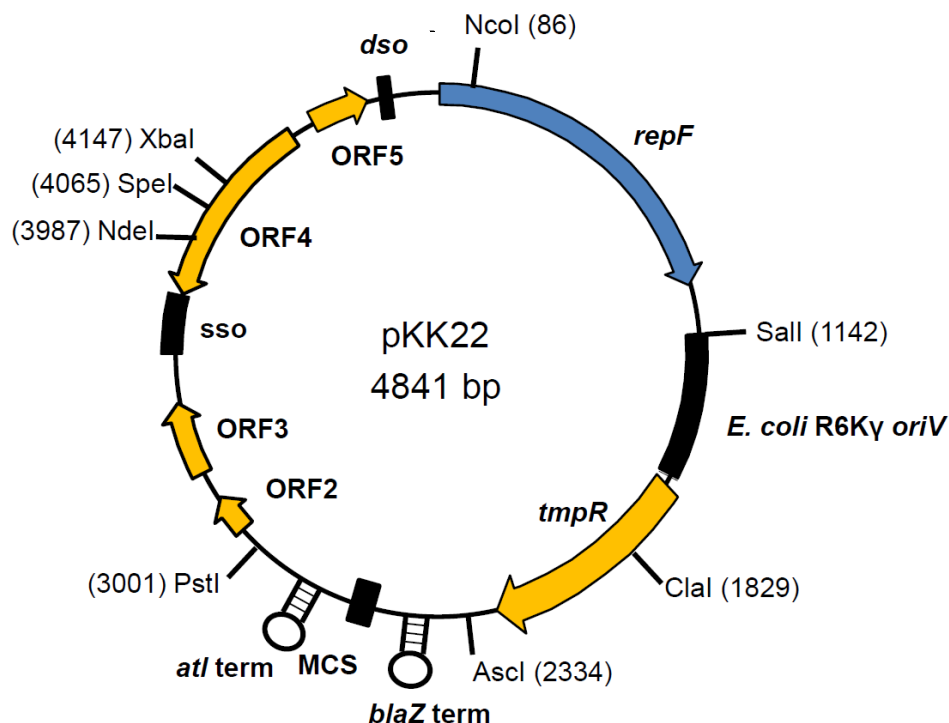
### References:

1. 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



**NotI**    **NheI**    **SacI**    **BamHI**  
MCS 2646    gcggccgctagctaggagctcggtacccgggatccg    2683  
   **AvrII**    **SmaI**  
   **XmaI**  
   **AvaI**

## 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
- ClaI site is methylation blocked and sits between the promoter and *dfrA* gene
- The R6Ky origin of replication requires *pir+* strains of *E. coli* to replicate