Escherichia coli – Staphylococcus aureus Shuttle Vector pKK30, Recombinant in *Escherichia coli*

Catalog No. NR-50349

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-50349 is a preserved culture of *Escherichia coli* (*E. coli*) DH5αλpir containing the *E. coli*-staphylococcal shuttle vector pKK30. Vector pKK30 contains the *E. coli* R6Kγ origin of replication and is for use in *Staphylococcus* spp. strains without LAC-p01.[1,2] Vector pKK30 contains a single trimethoprim resistance cassette that is functional in both *E. coli* and *Staphylococcus* spp.[1] pKK30 is identical to pKK22 except it lacks open reading frames required by USA300 strains needed for plasmid maintenance.[1] The complete pKK30 nucleotide sequence is available (GenBank: KX085043) and the vector map of pKK30 is available below in Appendix I.

pKK30 was deposited in conjunction with pKK22 and *E. coli* strains DH5αλpir and GM2163λpir (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* R6Kγ origin of replication of both vectors requires *pir*+ for replication which is provided in either DH5αλpir 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 *E. coli,* DH5αλpir or GM2163λpir, or *Staphylococcus aureus* USA300 strains containing LAC-p01
NR-50349	pKK30	pKK30 is a variant of pKK22 for use in *E. coli,* DH5αλpir or GM2163λpir, or *Staphylococcus* spp. without LAC-p01
NR-50350	*E. coli* DH5αλpir	Host strain containing the *pir* genes for performing genetic manipulations prior to transfer into *Staphylococcus* (F⁻Φ80*dlacZ* ΔM15 Δ*lacZYA-argF U169 deoR supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*)
NR-50351	*E. coli* GM2163 λpir	Host strain containing the *pir* genes for performing genetic manipulations. This strain is also a Dam and Dcm methylase mutant for transfer of plasmids into *Staphylococcus* isolates that do not accept *E. coli* DNA easily (F⁻*ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 rpsL136 dam13*::Tn*9 xylA5 mtl-1 thi-1 mcrB1 hsdR2 λpir*)

Material Provided:

Each vial of NR-50349 contains approximately 0.5 mL of *E. coli*, DH5αλpir, in Tryptic Soy broth containing 10 µg/mL trimethoprim supplemented with 10% glycerol.

Packaging/Storage:

NR-50349 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, nutrient agar, 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 pKK30, Recombinant in *Escherichia coli,* NR-50349."

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.

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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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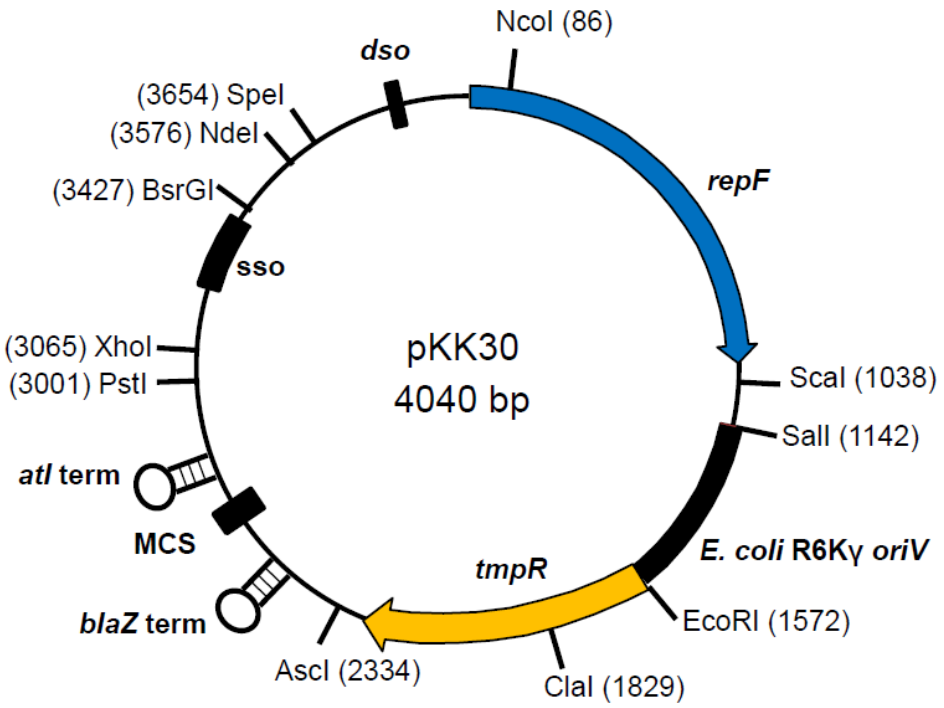
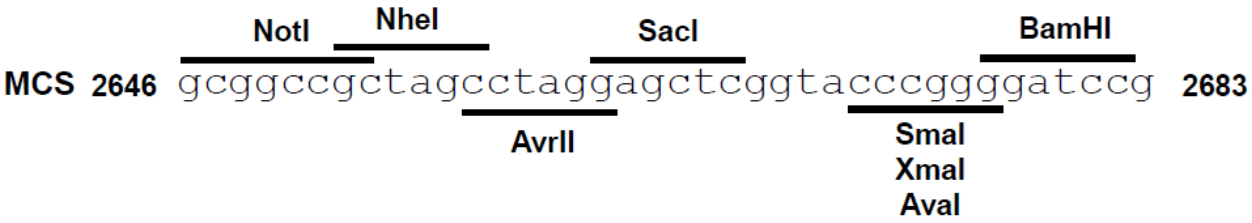
APPENDIX I – Vector pKK30 Map





Notes:

- pKK30 is designed to be used in bacteria not containing LAC-p01 (pUSA01)
- Entire plasmid sequence can be found in GenBank Accession KX085043
- *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 R6Kγ origin of replication requires *pir*+ strains of *E. coli* to replicate

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