Product Information Sheet for NR-50349

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

Catalog No. NR-50349

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-50349 is a preserved culture of *Escherichia coli (E. coli)*, strain 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 has been sequenced (GenBank: KX085043) and the vector map of pKK30 is available in Appendix I.

pKK30 was deposited in conjunction with pKK22 and *E. coli* strains DH5αλpir 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 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*, strains DH5αλpir or GM2163λpir or *S. aureus* USA300 strains containing LAC-p01[2]
NR-50349	pKK30	pKK30 is a variant of pKK22, for use in *E. coli*, strains DH5αλpir or GM2163λpir or *Staphylococcus* species not containing LAC-p01[2]
NR-50350	*E. coli*, Strain DH5αλpir	Host strain containing the *pir* genes for performing genetic manipulations prior to transfer into *Staphylococcus*[3]
NR-50351	*E. coli*, Strain GM2163λpir	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.[3]

Material Provided:

Each vial of NR-50349 contains approximately 0.5 mL of *Escherichia coli – Staphylococcus aureus* Shuttle Vector pKK30, Recombinant in *Escherichia coli* 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 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 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 (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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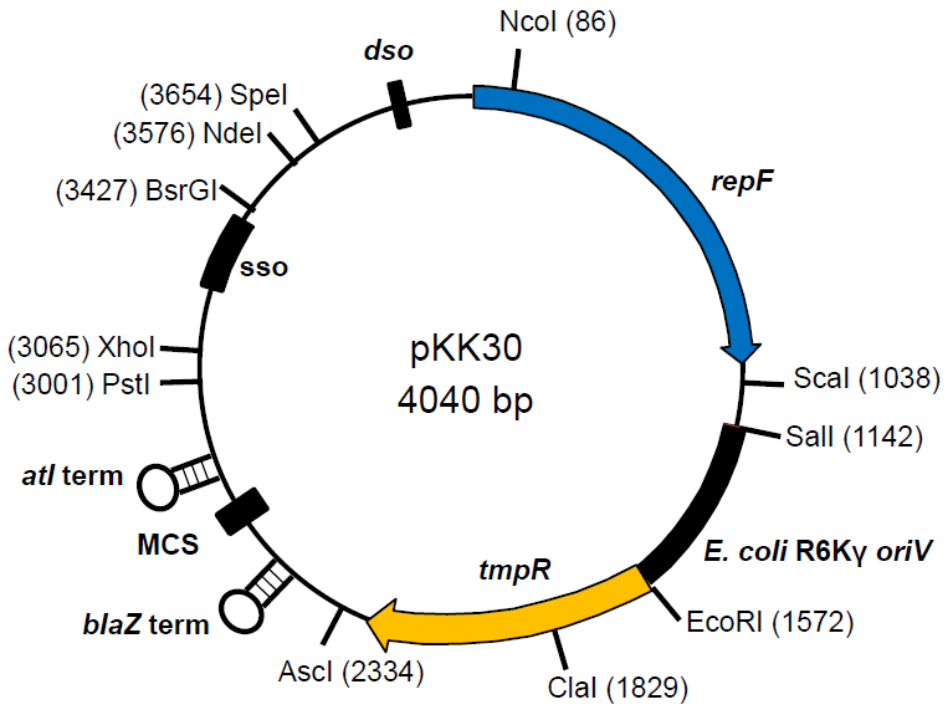
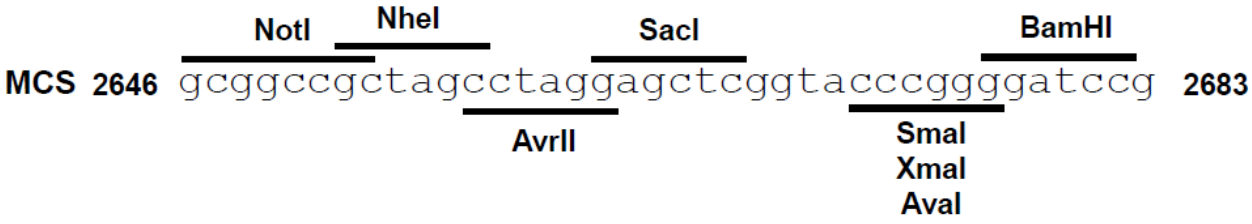
APPENDIX I: VECTOR pKK30 MAP





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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