Escherichia coli – Staphylococcal Shuttle Vectors and Hosts

Catalog No. NR-50352

For research use only. Not for human use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-50352 is a kit containing two vectors that remain stable during both *in vitro* and *in vivo* experiments without the requirement of antibiotics, and two *Escherichia coli (E. coli)* hosts for genetic manipulations prior to transfer into *Staphylococcus aureus (S. aureus)*. The shuttle vectors are based on the LAC-p01 plasmid. pKK22 is designed to be used with USA300 strains that contain LAC-p01 and will render the strains isogenic. pKK30 is a variant of pKK22 in which open reading frames (ORFs) not needed for vector maintenance have been deleted and is intended for use in Staphylococcal cells not containing LAC-p01. Both vectors contain a single trimethoprim resistance cassette that is functional in both *E. coli* and *Staphylococcus* species. Additionally, they contain the *E. coli* R6Kγ origin of replication, which requires *pir*+ cells for replication thus, DH5α*λpir* and the *pir*+ dam- dcm- strain, GM2163*λpir,* are provided as host strains.[1-3] The complete nucleotide sequences of pKK22 and pKK30 are available (GenBank: KX085042 and KX085043, respectively) and the vector maps are available below in Appendix I.

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

BEI Resources Number	Product	Comments
NR-50348	pKK22 in *E. coli* DH5α*λ*pir	For use in *E. coli* DH5α*λ*pir or GM2163*λ*pir or *S. sureus* USA300 strains containing LAC-p01[2]
NR-50349	pKK30 in *E. coli* DH5α*λ*pir	pKK30 is a variant of pKK22, for use in *E. coli* DH5α*λ*pir or GM2163*λ*pir or *Staphylococcus* species not containing LAC-p01[2]
NR-50350	*E. coli* DH5α*λ*pir	Host strain containing the *pir* genes for performing genetic manipulations prior to transfer into *Staphylococcus* (F⁻Φ80 *dlacZ* Δ*M*15 Δ*lacZYA-argFU169 deoR supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*)[2]
NR-50351	*E. coli* GM2163 *λ*pir	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*)[2]

Material Provided:

NR-50352 is comprised of 4 vials containing:

NR-50348: 0.5 mL of vector pKK22, recombinant in *E. coli* DH5α*λ*pir, in Tryptic Soy broth containing 10 µg/mL trimethoprim supplemented with 10% glycerol.

NR-50349: 0.5 mL of vector pKK30, recombinant in *E. coli* DH5α*λ*pir, in Tryptic Soy broth containing 10 µg/mL trimethoprim supplemented with 10% glycerol.

NR-50350: 0.5 mL of *E. coli* DH5α*λ*pir in Tryptic Soy broth supplemented with 10% glycerol.

NR-50351: 0.5 mL of *E. coli* GM2163*λ*pir in Tryptic Soy broth supplemented with 10% glycerol.

Packaging/Storage:

NR-50352 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, nutrient agar or equivalent

Tryptic Soy agar, nutrient agar, Tryptic Soy agar with 5% defibrinated sheep blood or equivalent

Note: *E. coli* containing pKK22 or pKK30 vectors can be grown 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* – Staphylococcal Shuttle Vectors and Hosts, NR-50352."

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.

Disclaimers:

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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.

Support Provided by NIAID

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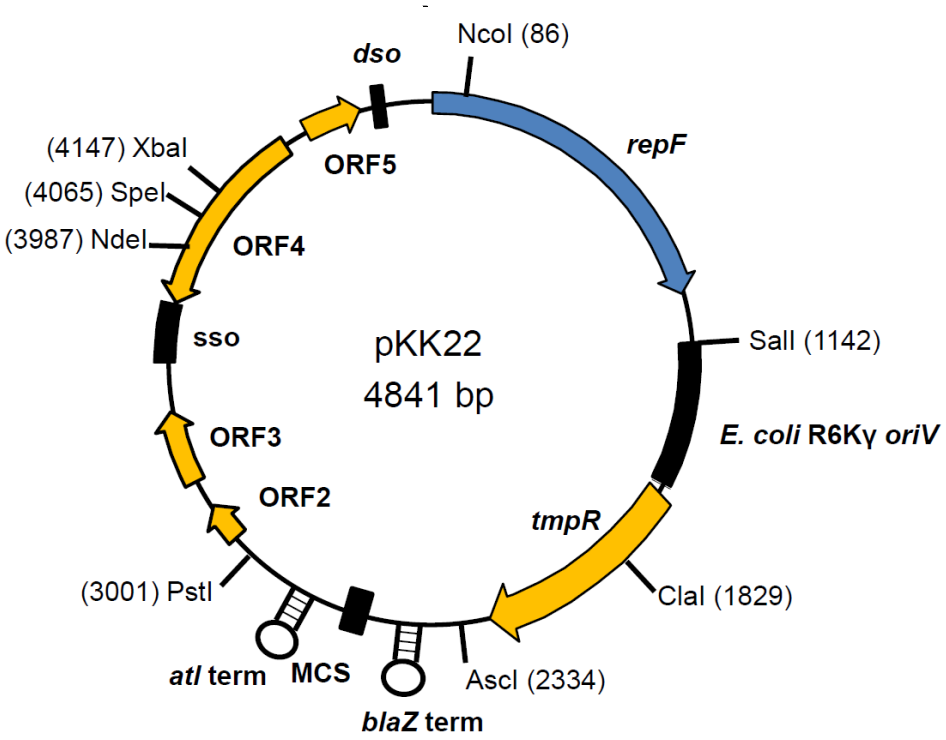
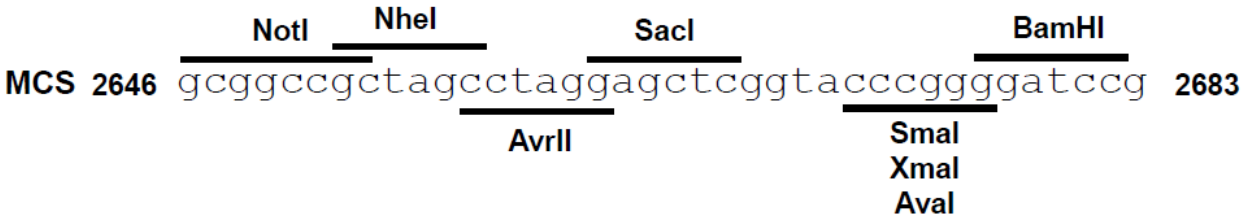
APPENDIX I:

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
- 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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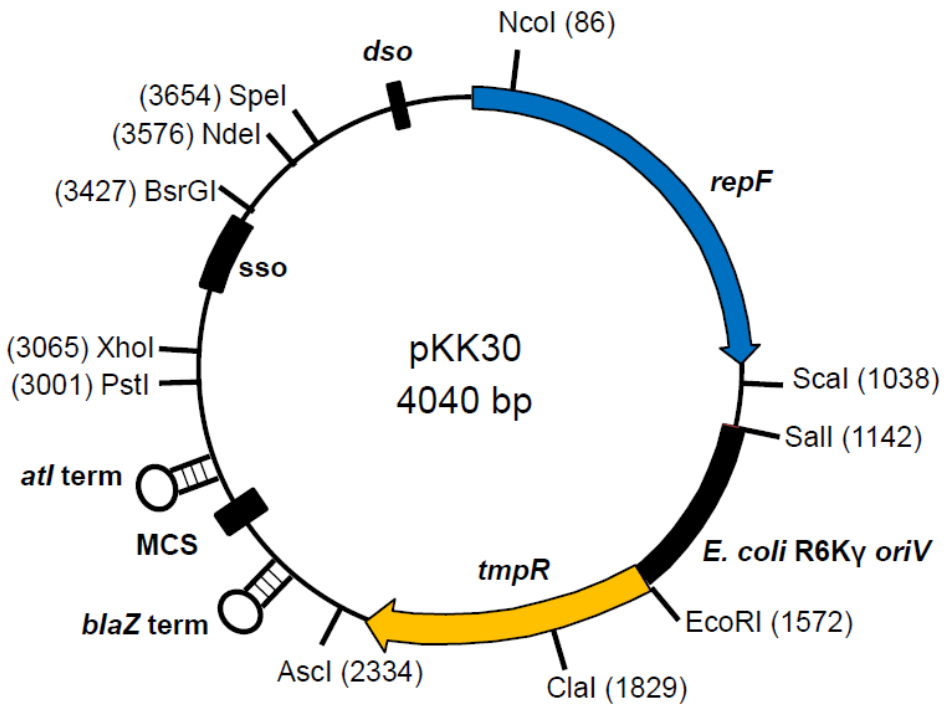
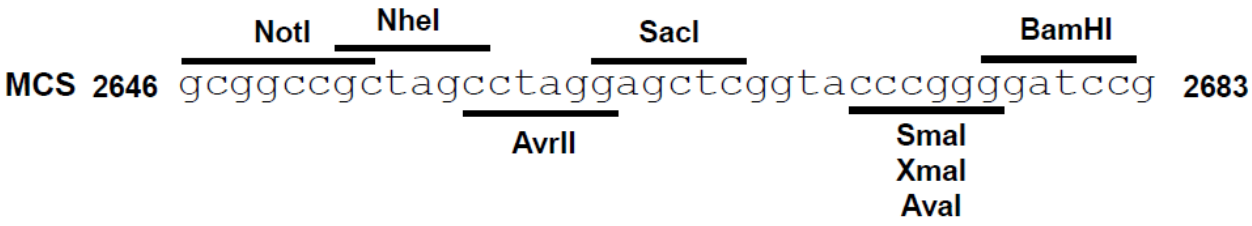
Vector pKK30 Map





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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