Nebraska Transposon Mutant Library (NTML) Genetic Toolbox Allelic Exchange Plasmid pJB38, Recombinant in *Escherichia coli*

Catalog No. NR-49932

For research use only. Not for human use.

Contributor:
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Manufacturer:
BEI Resources

Product Description:
NR-49932 is a culture of *Escherichia coli (E. coli)*, strain DH5α containing the plasmid pJB38. pJB38 is a temperature-sensitive allelic exchange plasmid that is the parent plasmid for the Nebraska Transposon Mutant Library (NTML) Genetic Toolbox plasmid constructs (NR-49947). It can be used for chromosomal mutagenesis of any non-essential gene in *Staphylococcus aureus (S. aureus)*. pJB38 contains the *E. coli* oriV high-copy-number replication origin and the *S. aureus* pE194ts thermosensitive replication origin. pJB38 was deposited as resistant to ampicillin in *E. coli* and resistant to chloramphenicol in *S. aureus*.[1]

The complete sequence of pJB38 has been determined and is available on the Certificate of Analysis for NR-49932, Lot 63863783. The BEI Resources plasmid sequence has been submitted to GenBank as pJB38 and the plasmid map is shown in Appendix I.

The Center for Staphylococcal Research (CSR) at the University of Nebraska Medical Center has generated the NTML, a collection of sequence-defined transposon (Tn) insertion mutants of *S. aureus*.[2,3] To increase the functionality of the NTML, an allelic exchange system, the NTML Genetic Toolbox, was developed for the easy exchange of the transposon with either selectable markers or promoterless reporter genes. The selectable markers can be used to create multiple defined mutations within the *S. aureus* chromosome, whereas the reporter genes allow for the generation of single copy reporter constructs within any gene included in the NTML.[1] The exchange plasmids are comprised of either a selectable marker or a reporter gene, flanked by the 5' and 3' ends of the *bursa aurealis* Tn.

The Nebraska Transposon Mutant Library (NTML) was constructed in the laboratories of Dr. Ken Bayles and Dr. Paul Fey at the University of Nebraska Medical Center. Additional information is available at the NTML website.

Material Provided:
Each vial of NR-49932 contains approximately 0.5 mL of bacterial culture in Luria-Bertani (LB) broth containing 100 µg/mL ampicillin supplemented with 25% glycerol.

Packaging/Storage:
NR-49932 was packaged aseptically, in screw-capped plastic cryovial. 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:
LB broth containing 100 µg/mL ampicillin
LB agar containing 100 µg/mL ampicillin
Incubation:
Temperature: 30°C
Atmosphere: Aerobic
Propagation:
1. Keep tube 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 30°C for 1 day.

Citation:
Acknowledgment for publications should read "The following reagent was provided by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) for distribution by BEI Resources, NIAID, NIH: Nebraska Transposon Mutant Library (NTML) Genetic Toolbox Allelic Exchange Plasmid pJB38, Recombinant in *Escherichia coli*, NR-49932."

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., P. D. Fey and K. W. Bayles. "Genetic Tools to Enhance the Study of Gene Function and Regulation in *Staphylococcus aureus*." Appl. Environ. Microbiol. 79 (2013): 2218-2224. PubMed: 23354696.
2. Bae, T., et al. "*Staphylococcus aureus* Virulence Genes Identified by *bursa aurealis* Mutagenesis and Nematode Killing." Proc. Natl. Acad. Sci. USA 101 (2004): 12312-12317. PubMed: 15304642.
3. Fey, P. D., et al. "A Genetic Resource for Rapid and Comprehensive Phenotype Screening of Nonessential *Staphylococcus aureus* Genes." MBio 4 (2013): e00537-12. PubMed: 23404398.

Support Provided by NIAID

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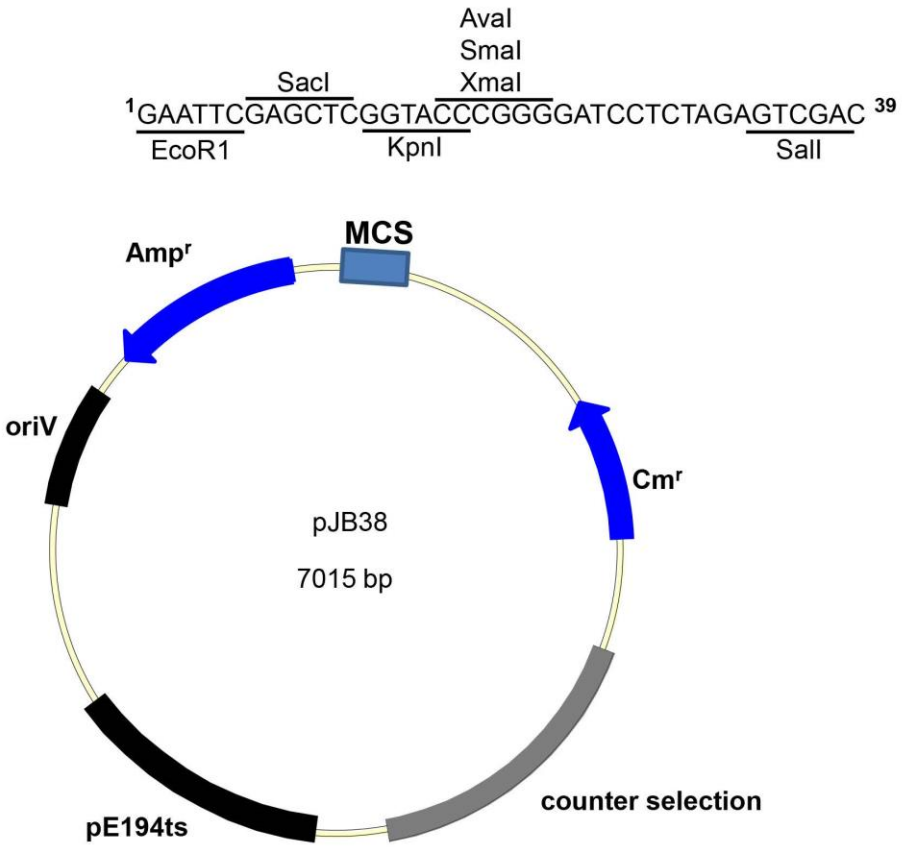
Appendix I: Plasmid Map of pJB38



E. coli Features
-oriV: high copy origin
-Amp^r: Ampicillin resistance (100 µg ml^{-1})
-MCS: shown multi-cloning site

S. aureus Features
-pE194ts: temperature-sensitive origin
-counterselection: anhydrotetracycline-inducible (100 ng ml^{-1})
-Cm^r: chloramphenicol resistance (10 µg ml^{-1})

Note: inserts into the multi-cloning site can be confirmed with:
Forward primer: CCCGAAAAGTGCCACCTGACGTC
Reverse primer: CGAAAATGCCTCACATTTGTGCCACC