

***Staphylococcus aureus* subsp. *aureus*,
Strain JE2, Transposon Mutant NE922
(SAUSA300_0124)**

Catalog No. NR-47465

For research use only. Not for human use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Bacteria Classification: *Staphylococcaceae*, *Staphylococcus*

Species: *Staphylococcus aureus*

Strain: JE2 (also referred to as USA300 JE2)

Transposon Mutant: NE922

Nebraska Transposon Mutant: SAUSA300_0124

Original Source: *Staphylococcus aureus* (*S. aureus*) subsp. *aureus*, transposon mutant NE922 was derived from *S. aureus* subsp. *aureus*, strain JE2. Mutagenesis occurred through the use of the *mariner*-based transposon *bursa aurealis* resulting in an erythromycin-resistant deletion strain of JE2.^{1,2} *S. aureus* subsp. *aureus*, transposon mutant NE922 was created by disruption of SAUSA300_0124, which encodes for a pyridoxal-phosphate dependent enzyme superfamily protein. This protein has been identified as SbnG, a citrate synthase. SbnG is expressed under iron-limiting conditions, and provides the precursor citrate for staphyloferrin B biosynthesis.³⁻⁵ Staphyloferrin B is a virulence-associated siderophore necessary to scavenge iron within the host.³

Comments: *S. aureus* subsp. *aureus*, strain JE2 is a plasmid-cured derivative of strain LAC that was isolated in 2002 from a skin and soft tissue infection of an inmate in the Los Angeles County Jail in California, USA.² Strain JE2 is a methicillin-resistant *S. aureus* (MRSA) strain and is a USA300 isolate. USA300 isolates have the same MLST profile (ST 8), SCC*mec* (subtype IV), *agr* group (I) and *spa* motif (MBQBLO) and typically carry the Panton-Valentine leukocidin (PVL) toxin genes and the arginine catabolic mobile element (ACME).^{6,7} USA300 is the most common cause of community-associated MRSA infection and an increasing cause of hospital-acquired infections.⁶

In an effort to enhance the research capabilities of the staphylococcal research community, the Center for Staphylococcal Research (CSR) at the University of Nebraska

Medical Center has generated the Nebraska Transposon Mutant Library, a collection of sequence-defined transposon (Tn) insertion mutants of *S. aureus*. This collection contains mutant derivatives of strain USA300 LAC, in which approximately 2,000 non-essential genes have been disrupted by the insertion of the *mariner*-based transposon *bursa aurealis*.² The insertion sites were identified by determining the nucleotide sequences of the junction fragments containing the end of the transposon and the flanking DNA. The gene names and descriptions associated with each of the Tn mutants were obtained from the National Center for Biotechnology Information.

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 contains approximately 0.5 mL of bacterial culture in Tryptic Soy broth containing 5 µg/mL erythromycin supplemented with 10% glycerol.

Note: If homogeneity is required for your intended use, please purify prior to initiating work. It is recommended that the presence and location of the transposon is confirmed. The Center for Staphylococcal Research has designed primers that anneal to the *bursa aurealis* transposon and are to be used in conjunction with a primer within the gene of interest to generate a PCR product. For transposons in the “plus” orientation, the primer “Upstream” (5'-CTCGATTCTATTAACAAGGG-3') should be paired with a gene-specific primer. For transposons in the “minus” orientation, the primer “Buster” (5'-GCTTTTCTAAATGTTTTTAAGTAAATCAAGTAC-3') should be paired with a gene-specific primer.

Packaging/Storage:

NR-47465 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 containing 5 µg/mL erythromycin or equivalent

Tryptic Soy agar containing 5 µg/mL erythromycin or equivalent

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 provided by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) for distribution by BEI Resources, NIAID, NIH: *Staphylococcus aureus* subsp. *aureus*, Strain JE2, Transposon Mutant NE922 (SAUSA300_0124), NR-47465.”

Biosafety Level: 2

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. 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.

2. 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.

3. Cheung, J., M. E. Murphy and D. E. Heinrichs. “Discovery of an Iron-Regulated Citrate Synthase in *Staphylococcus aureus*.” Chem. Biol. 19 (2012): 1568-1578. PubMed: 23261600.

4. Sheldon, J. R., C. L. Marolda and D. E. Heinrichs. “TCA Cycle Activity in *Staphylococcus aureus* is Essential for Iron-Regulated Synthesis of Staphyloferrin A, but not Staphyloferrin B: The Benefit of a Second Citrate Synthase.” Mol. Microbiol. 92 (2014): 824-839. PubMed: 24666349.

5. Kobylarz, M. J., et al. “SbnG, a Citrate Synthase in *Staphylococcus aureus*: A New Fold on an Old Enzyme.” J. Biol. Chem. 289 (2014): 33797-33807. PubMed: 25336653.

6. Diep, B. A., et al. “Roles of 34 Virulence Genes in the Evolution of Hospital- and Community-Associated Strains of Methicillin-Resistant *Staphylococcus aureus*.” J. Infect. Dis. 193 (2006): 1495-1503. PubMed: 16652276.

7. Diekema, D. J., et al. “Continued Emergence of USA300 Methicillin-Resistant *Staphylococcus aureus* in the United States: Results from a Nationwide Surveillance Study.” Infect. Control Hosp. Epidemiol. 35 (2014): 285-292. PubMed: 24521595.

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