

***Staphylococcus aureus* subsp. *aureus*,
Strain JE2, Transposon NE1360
(SAUSA300_1255)**

Catalog No. NR-47902

For research use only. Not for use in humans.

Contributor:

Kenneth Bayles, Ph.D., Director, Center for Staphylococcal Research, Department of Pathology and Microbiology and Paul Fey, Ph.D., Medical Director, Nebraska Medical Center Microbiology Section, University of Nebraska Medical Center, Omaha, Nebraska, USA, Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA), and University of Chicago, Chicago, Illinois, USA (contribution of transposon, *bursa aurealis*)

Manufacturer:

BEI Resources

Product Description:

Bacteria Classification: *Staphylococcaceae*, *Staphylococcus*

Species: *Staphylococcus aureus*

Strain: JE2 (also referred to as USA300 JE2)

Transposon Mutant: NE1360

Nebraska Transposon Mutant: SAUSA300_1255

Original Source: *Staphylococcus aureus* (*S. aureus*) subsp. *aureus*, transposon mutant NE1360 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 NE1360 was created by disruption of *fmtC* (also known as *mprF*), which encodes for a phosphatidylglycerol lysyltransferase that affects susceptibility to methicillin and host-produced defensins.^{2,3,4,5}

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), *SCCmec* (subtype IV), *agr* group (I) and *spa* motif (MBQBLO) and typically carry the Pantone-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.⁷ The complete genome of the parental strain, JE2, is available (GenBank: [CP020619](https://www.ncbi.nlm.nih.gov/nuccore/CP020619)).

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. Kenneth Bayles and Dr. Paul Fey at the University of Nebraska Medical Center. Additional information is available at the [NTML](http://ntml.unmc.edu) 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-47902 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 through BEI Resources, NIAID, NIH: *Staphylococcus aureus* subsp. *aureus*, Strain JE2, Transposon Mutant NE1360 (SAUSA300_1255), NR-47902.”

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 \(BMBL\)](#). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

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. Komatsuzawa, H., et al. “Cloning and Sequencing of the Gene, *fmtC*, Which Affects Oxacillin Resistance in Methicillin-Resistant *Staphylococcus aureus*.” *FEMS Microbiol. Lett.* 203 (2001): 49-54. PubMed: 11557139.

4. Peschel, A., et al. “*Staphylococcus aureus* Resistance to Human Defensins and Evasion of Neutrophil Killing via the Novel Virulence Factor MprF is Based on Modification of Membrane Lipids with L-Lysine.” *J. Exp. Med.* 193 (2001): 1067-1076. PubMed: 11342591.

5. Midorikawa, K., et al. “*Staphylococcus aureus* Susceptibility to Innate Antimicrobial Peptides, Beta-Defensins and CAP18, Expressed by Human Keratinocytes.” *Infect. Immun.* 71 (2003): 3730-3739. PubMed: 12819054.

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.

ATCC® is a trademark of the American Type Culture Collection.

