Product Information Sheet for NR-48501

Nebraska Transposon Mutant Library (NTML) Screening Array

Catalog No. NR-48501

For research use only. Not for human use.

Contributor:
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Manufacturer:
University of Nebraska Medical Center

Product Description:
Production in the 384-well format has increased risk of cross-contamination between adjacent wells. Individual clones should be purified (e.g. single colony isolation and purification using good microbiological practices) and sequence-verified prior to use. BEI Resources does not confirm or validate individual clone identities provided by the contributor.

The Nebraska Transposon Mutant Library (NTML) Screening Array consists of approximately 2000 *Staphylococcus aureus* (*S. aureus*) subsp. *aureus* USA300 JE2, transposon (Tn) mutants arrayed in five 384-well microtiter plates. The screening array was developed to be a resource for performing high-throughput phenotypic screens to identify candidate genes for future research. The mutants were derived from *S. aureus* subsp. *aureus,* strain JE2. Mutagenesis occurred through the use of the *mariner*-based transposon *bursa aurealis* resulting in erythromycin resistant deletion strains of JE2.[1,2] The insertion sites were identified by sequencing 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.[2]

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).[3,4] USA300 is the most common cause of community-associated MRSA infection and an increasing cause of hospital-acquired infections.[4]

This screening array is intended for high-throughput phenotypic screens. The mutant strains have not had their Tn-insertion site re-sequenced to confirm the identity of each mutation; therefore it is recommend that array users confirm each mutant selected from a screen. To ensure the quality of this resource for the research community and to reduce the number of passages from user to user, this library is not to be reproduced and distributed to other investigators in any way.

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 well contains approximately 150 µl of bacterial culture in Tryptic Soy broth containing 5 µg/mL erythromycin supplemented with 25% glycerol.

Packaging/Storage:
NR-48501 was packaged in 384-well microtiter plates. 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. Scrape top of frozen well with a pipette tip and streak onto agar plate.
2. Incubate the plates 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: Nebraska Transposon Mutant Library (NTML) Screening Array, NR-48501."

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. 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.
4. 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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