Nebraska Transposon Mutant Library (NTML) Genetic Toolbox

Catalog No. NR-48850

For research use only. Not for human use.

Contributor:
Kenneth Bayles, Ph.D., Director, Center for Staphylococcal Research, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA, and Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA), NIAID, NIH

Manufacturer:
University of Nebraska Medical Center

Product Description:
The Center for Staphylococcal Research (CSR) at the University of Nebraska Medical Center has generated the Nebraska Transposon Mutant Library (NTML), a collection of sequence-defined transposon (Tn) insertion mutants of *Staphylococcus aureus* (*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 transposons 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 genetic toolbox contains all of the plasmids listed in Table 1. All of the plasmids are in *S. aureus*, with the exception of pJB38 (the temperature-sensitive allelic exchange plasmid that is the parent plasmid of the toolbox's plasmid constructs) which is in *Escherichia coli* (*E. coli*) DH5α.

Additional information about the genetic toolbox is available at the Nebraska Transposon Mutant Library website.

Material Provided:
Each tube of recombinant *S. aureus* contains approximately 25 µL of bacterial culture in Tryptic Soy broth with 10 µg/mL chloramphenicol supplemented with 25% glycerol. The recombinant *E. coli,* DH5α tube contains approximately 25 µL of bacterial culture in Luria-Bertani (LB) broth with 100 µg/mL ampicillin supplemented with 25% glycerol.

Packaging/Storage:
NR-48850 was packaged in PCR tubes. 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:
Recombinant *E. coli*
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 18 to 24 hours.

Recombinant *S. aureus*
Media:
Tryptic Soy broth containing 10 µg/mL chloramphenicol
Tryptic Soy agar containing 10 µg/mL chloramphenicol
Incubation:
Temperature: 30°C
Atmosphere: Aerobic
Propagation:
1. Keep tube(s) 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 18 to 24 hours.

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, NR-48850."

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.

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

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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. 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

Table 1: Exchange Plasmids Included in the NTML Genetic Toolbox

NE number	Plasmid*	Replacement
NE3001	pJB38	Not for Tn exchange, can be used for chromosomal mutagenesis of any non-essential gene in *S. aureus*
NE3002	pTnT	Unmarked
NE3003	pSPC	Spectinomycin resistance
NE3004	pKAN	Kanamycin resistance
NE3005	pTET	Tetracycline resistance
NE3006	pGFP-F	Superfolder Green Fluorescent Protein
NE3007	pGFP-R	Superfolder Green Fluorescent Protein
NE3008	pYFP-F	Enhanced yellow fluorescent protein
NE3009	pYFP-R	Enhanced yellow fluorescent protein
NE3010	pBFP-F	Enhanced cyan fluorescent protein
NE3011	pBFP-R	Enhanced cyan fluorescent protein
NE3012	pRFP-F	DsRed.T3(DNT), a variant of the DsRed2 red fluorescent protein (RFP)
NE3013	pFRP-R	DsRed.T3(DNT), a variant of the DsRed2 red fluorescent protein (RFP)
NE3014	pFP650-F	eqFP650, a far-red fluorescent protein
NE3015	pFP650-R	eqFP650, a far-red fluorescent protein

*Plasmids with the reporter gene in the same orientation as *bursa aurealis ermB* were given the designation "F"; those in the opposite orientation were labeled "R".

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898