

## Nebraska Transposon Mutant Library (NTML) Genetic Toolbox

Catalog No. NR-49947

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### Contributor:

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### Manufacturer:

BEI Resources

### 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*).<sup>2,3</sup> 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.<sup>1</sup> 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, transformed into *S. aureus*, strain RN4220 except for pJB38 which was transformed into *Escherichia coli* (*E. coli*), strain DH5 $\alpha$ . pJB38 is the temperature-sensitive allelic exchange plasmid that is the parent plasmid to the toolbox's plasmid constructs and is available as BEI Resources [NR-49932](#). The plasmid [maps](#) and [sequences](#) are available as Additional Information on the BEI website. The complete plasmid sequences have been submitted to GenBank.

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 tube of recombinant *S. aureus* contains approximately 0.5 mL of bacterial culture in Tryptic Soy broth with 10  $\mu$ g/mL chloramphenicol supplemented with 25% glycerol. The recombinant *E. coli* tube contains approximately 0.5 mL of

bacterial culture in Luria-Bertani (LB) broth with 100  $\mu$ g/mL ampicillin supplemented with 25% glycerol.

### Packaging/Storage:

NR-49947 was packaged aseptically, in screw-capped plastic 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:

*E. coli*

Media:

LB broth containing 100  $\mu$ g/mL ampicillin

LB agar containing 100  $\mu$ g/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.

*S. aureus*

Media:

Tryptic Soy broth containing 10  $\mu$ g/mL chloramphenicol

Tryptic Soy agar containing 10  $\mu$ g/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 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, NR-49947."

### 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](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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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.

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**Table 1: Allelic Exchange Plasmids Included in the NTML Genetic Toolbox**

BEI Number	NE Number	Plasmid <sup>1</sup>	Replacement
NR-49932 <sup>2</sup>	NE3001	pJB38	For chromosomal mutagenesis of any non-essential gene in <i>S. aureus</i> ; not for Tn exchange
NRC-49933	NE3002	pTnT	Unmarked
NRC-49934	NE3003	pSPC	Spectinomycin resistance
NRC-49935	NE3004	pKAN	Kanamycin resistance
NRC-49936	NE3005	pTET	Tetracycline resistance
NRC-49937	NE3006	pGFP-F	Superfolder Green Fluorescent Protein
NRC-49938	NE3007	pGFP-R	Superfolder Green Fluorescent Protein
NRC-49939	NE3008	pYFP-F	Enhanced yellow fluorescent protein
NRC-49940	NE3009	pYFP-R	Enhanced yellow fluorescent protein
NRC-49941	NE3010	pBFP-F	Enhanced cyan fluorescent protein
NRC-49942	NE3011	pBFP-R	Enhanced cyan fluorescent protein
NRC-49943	NE3012	pRFP-F	DsRed.T3(DNT), a variant of the DsRed2 red fluorescent protein (RFP)
NRC-49944	NE3013	pRRP-R	DsRed.T3(DNT), a variant of the DsRed2 red fluorescent protein (RFP)
NRC-49945	NE3014	pFP650-F	eqFP650, a far-red fluorescent protein
NRC-49946	NE3015	pFP650-R	eqFP650, a far-red fluorescent protein

<sup>1</sup>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".

<sup>2</sup>pJB38 is available as an individual product, NR-49932.