Certificate of Analysis for NR-49947

Nebraska Transposon Mutant Library (NTML) Genetic Toolbox

Catalog No. NR-49947

Product Description: The NTML Genetic Toolbox is an allelic exchange system developed for the easy exchange of *bursa aurealis* transposons with either selectable markers or promoterless reporter genes. 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* transposon, the *Escherichia coli* (*E. coli*) oriV high-copy-number replication origin, and the *Staphylococcus aureus* (*S. aureus*) pE194ts thermosensitive replication origin. All of the plasmids were transformed into *S. aureus*, strain RN4220, with the exception of pJB38 which was transformed into *E. coli*, strain DH5α. The plasmids were deposited as resistant to ampicillin in *E. coli* and resistant to chloramphenicol in *S. aureus.*

Lot: 63863786 **Date of Assembly: 29JUL2016**

Table 1: Genetic Toolbox Components

COMPONENT NUMBER	DESCRIPTION	LOT NUMBER	DATE OF MANUFACTURE
NR-49932	pJB38	63863783	12FEB2016
NRC-49933	pTnT	63863787	10FEB2016
NRC-49934	pSPC	63863790	10FEB2016
NRC-49935	pKAN	63863791	05FEB2016
NRC-49936	pTET	63863794	05FEB2016
NRC-49937	pGFP-F	63863795	12FEB2016
NRC-49938	pGFP-R	63863815	12FEB2016
NRC-49939	pYFP-F	63863799	12FEB2016
NRC-49940	pYFP-R	63863802	12FEB2016
NRC-49941	pBFP-F	63863803	10FEB2016
NRC-49942	pBFP-R	63863806	10FEB2016
NRC-49943	pRFP-F	63863807	11FEB2016
NRC-49944	pRFP-R	63863808	05FEB2016
NRC-49945	pFP650-F	63863812	12FEB2016
NRC-49946	pFP650-R	63863813	12FEB2016

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Table 2: pJB38 Plasmid, Recombinant in *E. coli*[1]

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology[2]	Report results	Circular, convex, entire, smooth and cream
Motility (wet mount)	Report results	Motile
VITEK® MS (MALDI-TOF)	Consistent with *E. coli*	*E. coli* (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (790 base pairs)	> 99% sequence identity to *E. coli,* strain DH5α (GenBank: JRYM01000066.1)	99.7% sequence identity to *E. coli,* strain DH5α (GenBank: JRYM01000066.1)
Confirmation of pJB38 Sequence		
Illumina® MiSeq® sequence	Report results	Consistent with pJB38 plasmid description[3-5]
Functional Activity of Antibiotic Resistance Genes in *E. coli*		
Ampicillin[2]	Growth	Growth
Chloramphenicol[6]	No growth	No growth
Purity (post-freeze)[7]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[2]	Growth	Growth

[1]NR-49932 was produced by inoculation of the deposited material in Luria Bertani (LB) broth containing 100 µg/mL ampicillin and grown for 1 day at 30°C in an aerobic atmosphere. Broth inoculum was added to LB agar with 100 µg/mL ampicillin kolles, which were grown for 1 day at 30°C in an aerobic atmosphere to produce this lot.
[2]1 day at 30°C in an aerobic atmosphere on LB agar with 100 µg/mL ampicillin
[3]Illumina® MiSeq® sequence was analyzed with CLC Genomics Workbench Version 7.0.2.
[4]pJB38 was sequenced and annotated by BEI Resources and is consistent with the plasmid described in 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. The plasmid sequence has been submitted to GenBank as pJB38.
[5]The complete sequence and the plasmid map are available as additional information on the BEI website.
[6]1 day at 30°C in an aerobic atmosphere on Tryptic Soy agar with 10 µg/mL chloramphenicol
[7]Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood.

Table 3: NRC-49933 to NRC-49946, Plasmids Recombinant in *S. aureus*[1]

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2]		
Cellular morphology	Gram-positive cocci	Gram-positive cocci
Colony morphology[3]	Report results	Circular, raised, entire, smooth and cream (Figure 2)
Motility (wet mount)	Report results	Non-motile
Hemolysis[4]	Report results	β-hemolytic[5]
Catalase	Positive	Positive
VITEK® MS (MALDI-TOF)	Consistent with *S. aureus*	*S. aureus* (99.9%)
Genotypic Analysis[2]		
Sequencing of 16S ribosomal RNA gene (~ 1490 base pairs)	≥ 99% sequence identity to *S. aureus,* strain RN4220 (GenBank: AFGU01000017.1)	> 99% sequence identity to *S. aureus,* strain RN4220 (GenBank: AFGU01000017.1)
Confirmation of Plasmid Sequences		
Illumina® MiSeq® sequence	Report results	Consistent with plasmid descriptions[6-8]
Functional Activity of Antibiotic Resistance Genes in *S. aureus*		
pTnT (NRC-49933)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pSPC (NRC-49934)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	Growth	Growth
Ampicillin[10]	No Growth	No Growth
pKAN (NRC-49935)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	Growth	Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pTET (NRC-49936)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pGFP-F (NRC-49937)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pGFP-R (NRC-49938)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth

Table 3 (continued): NRC-49933 to NRC-49946, Plasmids Recombinant in *S. aureus*

TEST	SPECIFICATIONS	RESULTS
Functional Activity of Antibiotic Resistance Genes in *S. aureus* (continued)		
pYFP-F (NRC-49939)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pYFP-R (NRC-49940)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pBFP-F (NRC-49941)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pBFP-R (NRC-49942)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pRFP-F (NRC-49943)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pFRP-R (NRC-49944)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pFP650-F (NRC-49945)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
pFP650-R (NRC-49946)		
Chloramphenicol[3]	Growth	Growth
Tetracycline[9]	Growth	Growth
Kanamycin[9]	No Growth	No Growth
Spectinomycin[9]	No Growth	No Growth
Ampicillin[10]	No Growth	No Growth
Purity (post-freeze)[2,11]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[2,3]	Growth	Growth

[1]Plasmid components were produced by inoculation of the deposited material in Tryptic Soy broth with 10 µg/mL chloramphenicol and grown for 1 day at 30°C in an aerobic atmosphere. Broth inoculum was used to inoculated Tryptic Soy agar with 10 µg/mL chloramphenicol kolles, which were grown for 1 day at 30°C in an aerobic atmosphere to produce this lot.
[2]Quality control testing was performed for each component and produced identical results.
[3]1 day at 30°C in an aerobic atmosphere on Tryptic Soy agar with 10 µg/mL chloramphenicol
[4]1 day at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood
[5]Hemolysis may not be detectable on plates incubated at 30°C.
[6]Illumina® MiSeq® sequence was analyzed with CLC Genomics Workbench Version 7.0.2.
[7]The plasmids were sequenced and annotated by BEI Resources and are consistent with the plasmids described in 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. The plasmid sequences have been submitted to GenBank.
[8]The complete sequence and the plasmid map are available as additional information on the BEI website.
[9]1 day at 30°C in an aerobic atmosphere on Tryptic Soy agar with 0.625 µg/mL tetracycline, 75 µg/mL kanamycin or 750 µg/mL spectinomycin, respectively
[10]1 day at 30°C in an aerobic atmosphere on LB agar with 100 µg/mL ampicillin
[11]Purity was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood.

Date: 22 JUL 2016 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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