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: 70009085 **Date of Assembly: 29NOV2017**

Table 1: Genetic Toolbox Components

COMPONENT NUMBER	DESCRIPTION	LOT NUMBER	DATE OF MANUFACTURE
NR-49932	pJB38	70009086	20SEP2017
NRC-49933	pTnT	70009084	30SEP2017
NRC-49934	pSPC	70009083	05OCT2017
NRC-49935	pKAN	70009082	06OCT2017
NRC-49936	pTET	70009081	29SEP2017
NRC-49937	pGFP-F	70009080	29SEP2017
NRC-49938	pGFP-R	70009079	04OCT2017
NRC-49939	pYFP-F	70009078	27SEP2017
NRC-49940	pYFP-R	70009077	29SEP2017
NRC-49941	pBFP-F	70009076	04OCT2017
NRC-49942	pBFP-R	70009075	04OCT2017
NRC-49943	pRFP-F	70009074	29SEP2017
NRC-49944	pRFP-R	70009113	29SEP2017
NRC-49945	pFP650-F	70009073	05OCT2017
NRC-49946	pFP650-R	70009072	28SEP2017

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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)	*E. coli*	*E. coli* (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 600 base pairs)	> 99% sequence identity to *E. coli,* strain DH5α (GenBank: JRYM01000066.1)	100% sequence identity to *E. coli,* strain DH5α (GenBank: JRYM01000066.1)
Confirmation of pJB38 plasmid[3,4]	*secY* expression cassette sequence present	*secY* expression cassette sequence confirmed[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 lot 70009086 was produced by inoculation of BEI Resources NRS-49932 lot 63863783 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]Primer sequences used to confirm the presence of the *secY* expression cassette can be found 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 complete sequence and the plasmid map are available as additional information on the BEI website.
[4]Prior to initiating work, it is recommended that the presence and orientation of the insertion be confirmed.
[5]NR-49932 only had one primer, JBSECY2, which produced a sequence read, indicating that the insertion is present. The presence of JBSECY1 priming site cannot be confirmed.
[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.

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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, convex, 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)	*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)	100% sequence identity to *S. aureus,* strain RN4220 (GenBank: AFGU01000017.1)
Confirmation of Plasmid[6,7]	Selectable marker or reporter gene sequence present	Selectable marker or reporter gene sequence present[8,9]
Functional Activity of Antibiotic Resistance Gene in *S. aureus*[3]	Resistant to chloramphenicol	Resistant to chloramphenicol
Purity (post-freeze)[2,10]	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 seed material from BEI Resources NRS-49947 lot 64365843 in Tryptic Soy broth with 10 µg/mL chloramphenicol and grown for 1 to 2 days at 30°C in an aerobic atmosphere. Broth inoculum was used to inoculate 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]Primer sequences used to confirm the presence of the selectable markers and reporter genes can be found 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 complete sequence and the plasmid map are available as additional information on the BEI website.
[7]Prior to initiating work, it is recommended that the presence and orientation of the insertion be confirmed.
[8]NRC-49938 only had one primer, JBTN38, which produced a sequence read, indicating that the insertion is present. The presence of JBTN37 priming site cannot be confirmed.
[9]NRC-49945 and NRC-49946 have the reporter gene present, however the orientation of the insertions could not be determined. BEI Resources is in the process of sequencing both plasmids to confirm the orientation.
[10]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: 19 JAN 2018 **Signature:**

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