Certificate of Analysis for NR-49932

Nebraska Transposon Mutant Library (NTML) Genetic Toolbox Allelic Exchange Plasmid pJB38, Recombinant in *Escherichia coli*

Catalog No. NR-49932

Product Description: NR-49932 is a culture of *Escherichia coli* (*E. coli*), strain DH5α containing the plasmid pJB38. pJB38 is a temperature-sensitive allelic exchange plasmid that is the parent plasmid for the Nebraska Transposon Mutant Library (NTML) Genetic Toolbox plasmid constructs (NR-49947). It can be used for chromosomal mutagenesis of any non-essential gene in *Staphylococcus aureus* (*S. aureus*). pJB38 contains the *E. coli* oriV high-copy-number replication origin and the *S. aureus* pE194ts thermosensitive replication origin. pJB38 was deposited as resistant to ampicillin in *E. coli* and resistant to chloramphenicol in *S. aureus*.

Lot[1]: 63863783 **Manufacturing Date: 12FEB2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology[2]	Report results	Circular, convex, entire, smooth and cream (Figure 1)
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 (Table 1)	Report results	Consistent with pJB38 vector description[3,4]
Presence of Antibiotic Resistance Genes		
Chloramphenicol (encoded by the chloramphenicol acetyl transferase gene)	Gene present	Gene present
Ampicillin (encoded by the beta-lactamase gene)	Gene present	Gene present
Functional Activity of Antibiotic Resistance Genes in *E. coli*		
Ampicillin[2]	Growth	Growth
Chloramphenicol[5]	No growth	No growth
Purity (post-freeze)[6]	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 vector 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 vector sequence has been submitted to GenBank as pJB38.
[5]1 day at 30°C in an aerobic atmosphere on Tryptic Soy agar with 10 µg/mL chloramphenicol
[6]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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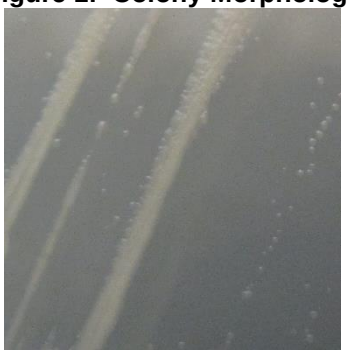
Figure 1: Colony Morphology



Date: 14 JUL 2016 **Signature:**

BEI Resources Authentication

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Table 1: Sequence of plasmid pJB38

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   1 GAATTCGAGC TCGGTACCCG GGGATCCTCT AGAGTCGACC TGCAGGCATG CAAGCTCTGA
  61 TTTCACTTTT TGCATTCTAC AAACTGCATA ACTCATATGT AAATCGCTCC TTTTTAGGTG
 121 GCACAAATGT GAGGCATTTT CGCTCTTTCC GGCGAGGCTA GTTACCCTTA AGTTATTGGT
 181 ATGACTGGCT TTTATAATAT GAGATAATGC CGACTGTAAA AAGTACAGTC GGCATTATCT
 241 CATATTATAA AAGCCAGTCA TTAGGCCTAT CTGACAATTC CTGAATAGAG TTCATAAACA
 301 ATCCTGCATG ATAACCATCA CAAACAGAAT GATGTACCTG TAAAGATAGC GGTAAATATA
 361 TTGAATTACC TTTATTAATG AATTTTCCTG CTGTAATAAT GGGTAGAAGG TAATTACTAT
 421 TATTATTGAT ATTTAAGTTA AACCCAGTAA ATGAAGTCCA TGGAATAATA GAAAGAGAAA
 481 AAGCATTTTC AGGTATAGGT GTTTTGGGAA ACAATTTCCC CGAACCATTA TATTTCTCTA
 541 CATCAGAAAG GTATAAATCA TAAAACTCTT TGAAGTCATT CTTTACAGGA GTCCAAATAC
 601 CAGAGAATGT TTTAGATACA CCATCAAAAA TTGTATAAAG TGGCTCTAAC TTATCCCAAT
 661 AACCTAACTC TCCGTCGCTA TTGTAACCAG TTCTAAAAGC TGTATTTGAG TTTATCACCC
 721 TTGTCACTAA GAAAATAAAT GCAGGGTAAA ATTTATATCC TTCTTGTTTT ATGTTTCGGT
 781 ATAAAACACT AATATCAATT TCTGTGGTTA TACTAAAAGT CGTTTGTTGG TTCAAATAAT
 841 GATTAAATAT CTCTTTTCTC TTCCAATTGT CTAAATCAAT TTTATTAAAG TTCATTTGAT
 901 ATGCCTCCTA AATTTTTATC TAAAGTGAAT TTAGGAGGCT TACTTGTCTG CTTTCTTCAT
 961 TAGAATCAAT CCTTTTTTAA AAGTCAATAT TACTGTAACA TAAATATATA TTTTAAAAAT
1021 ATCCCACTTT ATCCAATTTT CGTTTGTTGA ACTAATGGGT GCTTTAGTTG AAGAATAAAA
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2161 TGTCAACAAA AAGGAGGAAT TAATGATGTC TAGATTAGAT AAAAGTAAAG TGATTAACAG
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2401 GGAAAGCTGG CAAGATTTTT TACGTAATAA CGCTAAAAGT TTTAGATGTG CTTTACTAAG
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3121 TTCGCTGGTG CGAAAAAAGA GTGTCTTGTG ACACTCTTAA ATTCAAAATC TATCGGTCAG
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