b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-47991

Staphylococcus aureus subsp. *aureus*, Strain JE2, Transposon Mutant NE1449 (SAUSA300_2366)

Catalog No. NR-47991

Product Description: *Staphylococcus aureus* (*S. aureus*) subsp. *aureus,* transposon mutant NE1449 was derived from *S. aureus* subsp. *aureus,* strain JE2. Mutagenesis occurred through the use of the *mariner*-based transposon *bursa aurealis* resulting in an erythromycin-resistant deletion strain of JE2. *S. aureus* subsp. *aureus,* transposon mutant NE1449 was created by disruption of *hlgC,* which encodes for the pore-forming toxin (PFT) gamma-hemolysin C that is capable of lysing both leukocytes and erythrocytes when associated with gamma-hemolysin B (HglB). Strain JE2 is a plasmid-cured derivative of strain LAC that was isolated in 2002 from a skin and soft tissue infection of an inmate in the Los Angeles County Jail in California, USA.

Lot[1]: 63958884 **Manufacturing Date: 15JAN2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive cocci	Gram-positive cocci
Colony morphology[2]	Report results	Circular, raised, entire, smooth and cream (Figure 1)
Motility (wet mount)	Report results	Non-motile
Confirmation of Transposon Insertion[3]	Resistant to erythromycin	Resistant to erythromycin
Purity (post-freeze)[4]	Consistent with expected colony morphology	Consistent with expected colony morphology
Viability (post-freeze)[2]	Growth	Growth

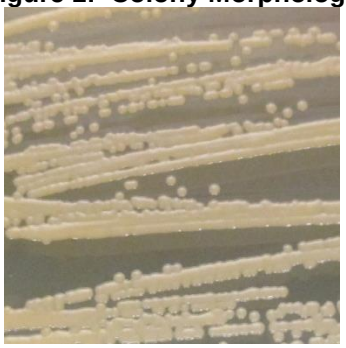
[1]NR-47991 was produced by inoculation of the deposited material into Tryptic Soy broth with 5 µg/mL erythromycin and incubated for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5 µg/mL erythromycin kolles which were grown 1 day at 37°C in an aerobic atmosphere to produce this lot.

[2]1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5 µg/mL erythromycin

[3]Prior to initiating work, it is recommended that the presence and location of the transposon is confirmed. Gene specific primers should be paired with either the "Upstream" primer (5'-CTCGATTCTATTAACAAGGG-3') for transposons in the "plus" orientation or the "Buster" primer (5'-GCTTTTTCTAAATGTTTTTTAAGTAAATCAAGTAC-3') for transposons in the "minus" orientation. For additional information, refer to 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.

[4]Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

Figure 1: Colony Morphology



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Date: 10 FEB 2016 **Signature:**

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