Certificate of Analysis for NR-51165

Staphylococcus aureus Fluorescent Reporter Plasmid pSFRFPS1, Recombinant in *Staphylococcus aureus*

Catalog No. NR-51165

Product Description: NR-51165 is a glycerol stock of *Staphylococcus aureus (S. aureus)*, strain RN4220 containing the eqFP650 far-red fluorescent protein (FRFP) reporter plasmid pSFRFPS1, a derivative of the *Escherichia coli (E. coli)* - staphylococcal shuttle vector pKK30.

Lot[1]: 70010752 **Manufacturing Date: 22NOV2017**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive cocci	Gram-positive cocci
Colony morphology[2]	Report results	Circular, convex, entire, smooth and cream to slight lavender (Figure 1)
Motility (wet mount)	Report results	Non-motile
Hemolysis[3]	Report results	Non-hemolytic[4]
Biochemical characterization		
Catalase	Positive	Positive
VITEK® MS (MALDI-TOF)	*S. aureus*	*S. aureus* (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 770 base pairs)	≥ 99% sequence identity to *S. aureus,* strain RN4220 (GenBank: AFGU01000017.1)	100% sequence identity to *S. aureus,* strain RN4220 (GenBank: AFGU01000017.1)[5]
Confirmation of pSFRFPS1 plasmid	Report results	Consistent with pSFRFPS1 plasmid description (Figure 2, Table 1)[6,7]
Functional Activity of Antibiotic Resistance Gene in *S. aureus*		
Trimethoprim[2]	Growth	Growth
Purity (post-freeze)[8]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[2]	Growth	Growth

[1]NR-51165 was produced by inoculation of the deposited material in Tryptic Soy broth containing 10 µg/mL trimethoprim and incubated for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was used to inoculate Tryptic Soy agar with 10 µg/mL trimethoprim kolles, which were grown for 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 10 µg/mL trimethoprim
[3]1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.
[4]Limited β-hemolysis was observed after 2 days
[5]Also consistent with *S. argenteus* and *S. simiae*
[6]Illumina® MiSeq® sequence was analyzed with CLC Genomics Workbench Version 7.0.2.
[7]pSFRFPS1 was sequenced and annotated by BEI Resources and is consistent with the vector described in Rodriguez, M. D., et al. "Construction of Stable Fluorescent Reporter Plasmids for Use in *Staphylococcus aureus*." Front. Microbiol. 8 (2017): 2491. PubMed: 29312199.
[8]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

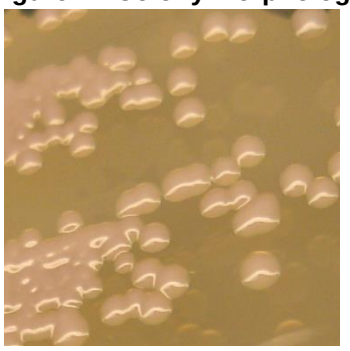

Figure 2: Fluorescent Report Plasmid pSFRFPS1

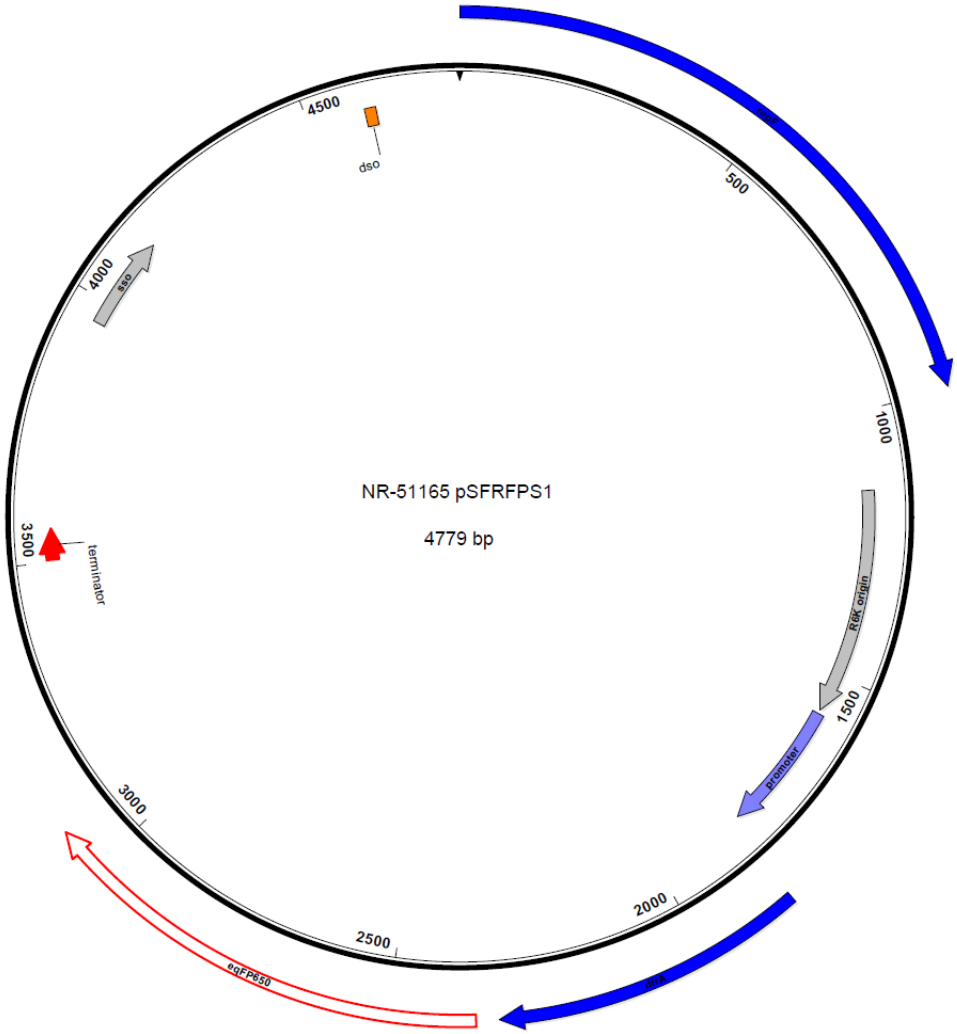

Table 1: Sequence of pSFRFPS1

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 121 GCAGATATAC TAGAAATTTT AAAAATCAAA AAGGCTTTTA ATGTAAAACA ATGTGGTAAT
 181 ATTTTAGAAT TTAAGCCAAC TGATGAAGGC TATTTGAAGT TACATAAGAC ATGGTTTTGT
 241 AAATCAAAAT TATGTCCGGT TTGTAATTGG AGACGTGCTA TGAAAAATAG TTATCAAGCT
 301 CAAAAAGTGA TTGAAAAAGT AATTAAGGAA AAGCCAAAAG CACGTTGGTT GTTTTTAACA
 361 CTTTCAACAA AAAATGCGAT AGATGGAGAT ACTTTAGAAC AAAGTTTGAA GCATCTAACT
 421 AAAGCATTTG ATAGGTTGAG TAGATATAAA AAGGTTAAAC AAAATCTTGT TGGATTTATG
 481 CGTTCAACAG AAGTTACCGT TAATAAAAAT GACGGTAGTT ATAATCAGCA CATGCATGTT
 541 TTGTTATGTG TTGAAAATGC ATATTTTAGA AAAAAAGAGA ATTATATAAC TCAAGAAGAA
 601 TGGGTTAATT TATGGCAAAG AGCATTACAA GTTGACTATC GACCTGTTGC TAATGTTAAA
 661 GCGATCAAAC CGAATAGAAA AGGCGATAAA GACATTGAAT CGGCAATCAA AGAGACCTCA
 721 AAATATTCGG TTAAATCATC TGATTTTTTA ACTGATGATG ATGAAAAAAA TCAAGAAATT
 781 GTAAGTGATT TAGAAAAAGG TTTGTATCGA AAACGTATGT TAAGTTATGG TGGATTGCTT
 841 AAACAAAAAC ATAAAATTTT AAACTTAGAC GATGTCGAAG ATGGTAATTT GATTAATGCA
 901 AGTGATGAAG ATAAAACAAC AGACGAAGAA GAAAAAGCAC ATTCAATTAC CGCAATTTGG
 961 AATTTCGAAA AGCAAAATTA TTATTTAAGA CATTAGTGTT GACTAATGTC TTTTTTGTTG
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2281 ATACTATACC GCATACATTC TTACATTTAG TGCGTAGAAA AGGGAAATAG GCGCGCCTGA
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2461 AGGTGAAGGT AAACCTTATG AAGGTACACA AACTGCAAAA ATTAAAGTTG TAGAAGGTGG
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2581 TATTAATCAT ACTCAAGGTA TTCCAGATTT CTTTAAACAA TCTTTTCCTG AAGGTTTTAC
2641 ATGGGAACGT ATTACAACTT ATGAAGATGG TGGTGTTTTA ACAGCAACTC AAGATACAAG
2701 TTTACAAAAT GGTTGTTTAA TTTATAATGT TAAAATTAAT GGTGTAAATT TTCCATCAAA
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3181 CTATTATTTT CGAGATTTAT TTTCTTAATT CTCTTTAACA AACTAGAAAT ATTGTATATA
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3361 ATATCAAGCA AAGTGACAGG CGATGCGGCC GCTAGCCTAG GAGCTCGGTA CCCGGGGATC
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Heather Couch

Digitally signed by Heather Couch
Date: 2018.04.17 12:01:03 -04'00'

17 APR 2018

Program Manager or designee, ATCC Federal Solutions

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