Table S1. Bacterial strains, plasmids and primers used in this study.

|  |  |  |
| --- | --- | --- |
| Strain/ Plasmid/ Primer | Description | Source (Reference) |
| **Strains*****E. coli***DH5αS17-1***P. aeruginosa***PA14*fis*::Tn*fis*::Tn/*att7*::*fis*Δ*fis*/pMMB67EH-*fis*-His*fis*::Tn/pUCP20-P*tac*-*exsA*PA14 T0T1*fis*::Tn T0T1**Plasmid**pEX18TcpUC18T-mini-Tn7T-TcpUC18T-mini-Tn7T-Tc-*fis*pMMB67EHpMMB67EH-*fis*-HispUCP20-P*tac*-*exsA*pEX18Tc-T0T1 insertion*exsA*-Flag-A*exsA*-Flag-SpDN19lacΩP*exsA*-*lacZ*P*exsC*-*lacZ*P*exsC-A*-*lacZ*P*exsC-Am1*-*lacZ*P*exsC-Am2*-*lacZ*pUCP20-P*exsA*-*exsA*-HispUCP20-P*exoU*-*exoU*-His**Primer**PA4853pupPA4853pdownPA4853upPA4853downPA4853sense2PA4853antisense2PA4852SENSEPA4852ANTISENSEexsC senseexsC antisenseexsU senseexsU antisenseexsA’ senseexsA’ antisensePexsA fis up2PexsA-flag-hisB+A UPB+A DOWNPexsC-AupPexsC-AdownT0T1upstreamFT0T1upstreamRT0T1downstreamFT0T1downstreamRT0T1upT0T1downOverlap shang m1Overlap xia m1Overlap shang m2Overlap xia m2 | F-, φ80d*lacZ*ΔM15, Δ(*lacZYA-argF*)U169, *deoR*, *recA1*, *endA1*, *hsdR17*(*r*k-,*m*k+), *phoA*, *supE44*, λ-, *thi-1*, *gyrA96*, *relA1**recA*, *pro*, *hsdR*, RP4-2-Tc::Mu-Km::Tn7Wild type strain of *Pseudomonas aeruginosa*PA14 with MAR2xT7 transposon inserted at *fis*; Gmr*fis*::Tn with *fis* inserted on chromosome with mini-Tn7T insertion; Gmr, TcrPA14 knockout *fis* with *fis*-His is driven by an inducible *tac* promoter; Cbr*fis*::Tn with *exsA* overexpressionPA14 with terminators T0T1 inserted at *exsB*-*exsA* intergenic region before *exsA* promoter*fis*::Tn with terminators T0T1 inserted at *exsB*-*exsA* intergenic region before *exsA* promoter; GmrGene replacement vector; Tcr, *oriT*+, *sacB*+mini-Tn7 base vector from insertion into chromosome attTn7 site; TcrpUC18T-mini-Tn7T-Tc with *fis*; TcrExpression vector with *tac* promoter; AprpMMB67EH with *fis*-His driven by an inducible *tac* promoter; AprpUCP20 with *exsA* driven by *tac* promoterT0T1 insertion at PA14 *exsB*-*exsA* intergenic region on pEX18Tc; Tcr*exsA*-Flag-CTC (containing *exsA* ORF only) fused with pDN19; Apr , Tcr*exsA*-Flag-CTC (containing *exsA* ORF and 225bp upstream fragment) fused with pDN19; Apr , TcrPromoterless lacZ fusion vector, Spr, Smr, Tcr*exsA* promoter-*lacZ* fusion reporter in pDN19lacΩ; Spr, Smr, Tcr*exsC* promoter*-lacZ* fusion reporter in pDN19lacΩ; Spr, Smr, Tcr*exsC* promoter to *exsA* promoter*-lacZ* fusion reporter in pDN19lacΩ; Spr, Smr, Tcrpoints mutation in P*exsC-A*-*lacZ*; Spr, Smr, Tcrpoints mutation in P*exsC-A*-*lacZ*; Spr, Smr, Tcr*exsA* promoter of PA14 fused to *exsA-His* on promoterless pUCP20; Apr*exoU* promoter of PA14 fused to *exoU-His* on promoterless pUCP20; Apr**Sequence (5’→3’)**CGCGGATCCGGTCCGCAGCCATCCCGAATTCAGCAAAACTGCAGGGGGCTTCCCTGTCTTGCGGTGCTGAAAACTGCAGACAACGGAACAGGGGTGGCCGCATGCCGGAATTCGGAGCCCGCCGCCGCTCTTAAAGAATTACAACATGGTGCTCTGATCGTATTGCTTGAGTTTCTCAATGTGGAACTCGGTGCGCTGCCTTGTTGCATACCATGGATTTAACGAGCAAGGTCAAGAGGGACAGGGAAGGCAAACTTCAGAGCGTCATACCTCAACACTGGTGAGCATACAAGGAGCCAAATCTCTTGCTTGTTTACCCTGTATTCGGCTCTAGATGATACATTGCCTGCTCCCAAGCTTTCAGTGGTGGTGGTGGTGGTGCTTATCGTCGTCATCTCGCCCGGAAGAAAGATCTGGCCCCTGTATTCGAAAGTTGGAATGTCCGGAATTCGGTGATCCAGTCCTTCGTCCAGATGCGCGGATCCTTATAAGAACCCCAACACTTCCCGTCCCGGAATTCGCACCGTTTCGATCTGCATTTCCGCGGATCCCGAGACTTGCACTTCTTTAATCTCCATACTAGTCTAGAGTTCGTTGTCAGGGAAGGCCTCGCCCAAGCTTACTGACTGGAAAAGCCCGCCTCCGCGGATCCGACTCCTGTTGATAGATCCAGTAATGACCTCCTAGTCTAGAGGCGGATTTGTCCTACTCAGGAGAGCACGGAGTCCATTTTATAATAATGAGGATTATCGATAATCCTCATTATTATAAAATGGACTCCGTGCACGGAGTCGATATTATAATTATCAGGATTATCGATAATCCTGATAATTATAATATCGACTCCGTG | TransGen([Simon et al., 1983](#_ENREF_7))([Liberati et al., 2006](#_ENREF_6))([Liberati et al., 2006](#_ENREF_6))this studythis studythis studythis studythis study([Hoang et al., 1998](#_ENREF_3))([Choi and Schweizer, 2006](#_ENREF_1))this study([Furste et al., 1986](#_ENREF_2))this studythis studythis study([Li et al., 2013](#_ENREF_4))([Li et al., 2013](#_ENREF_4))([Totten and Lory, 1990](#_ENREF_8))this studythis studythis studythis study this studythis study([Li et al., 2016](#_ENREF_5))**Purpose***fis* promoter cloning*fis* promoter cloning*fis* cloning*fis* cloningreal-time PCRreal-time PCRreal-time PCRreal-time PCRreal-time PCRreal-time PCRreal-time PCRreal-time PCRreal-time PCRreal-time PCR*exsA* cloning*exsA* cloningRT-PCRRT-PCR*exsC-A* promoter cloning*exsC-A* promoter cloningT0T1 insertionT0T1 insertionT0T1 insertionT0T1 insertionT0T1 insertionT0T1 insertionsite-directed mutagenesissite-directed mutagenesissite-directed mutagenesissite-directed mutagenesis |

Choi, K.H., and Schweizer, H.P. (2006). mini-Tn7 insertion in bacteria with single attTn7 sites: example Pseudomonas aeruginosa. *Nat Protoc* 1(1)**,** 153-161. doi: 10.1038/nprot.2006.24.

Furste, J.P., Pansegrau, W., Frank, R., Blocker, H., Scholz, P., Bagdasarian, M., et al. (1986). Molecular cloning of the plasmid RP4 primase region in a multi-host-range tacP expression vector. *Gene* 48(1)**,** 119-131.

Hoang, T.T., Karkhoff-Schweizer, R.R., Kutchma, A.J., and Schweizer, H.P. (1998). A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. *Gene* 212(1)**,** 77-86.

Li, K., Xu, C., Jin, Y., Sun, Z., Liu, C., Shi, J., et al. (2013). SuhB is a regulator of multiple virulence genes and essential for pathogenesis of Pseudomonas aeruginosa. *MBio* 4(6)**,** e00419-00413. doi: 10.1128/mBio.00419-13.

Li, M., Long, Y., Liu, Y., Liu, Y., Chen, R., Shi, J., et al. (2016). HigB of Pseudomonas aeruginosa Enhances Killing of Phagocytes by Up-Regulating the Type III Secretion System in Ciprofloxacin Induced Persister Cells. *Front Cell Infect Microbiol* 6**,** 125. doi: 10.3389/fcimb.2016.00125.

Liberati, N.T., Urbach, J.M., Miyata, S., Lee, D.G., Drenkard, E., Wu, G., et al. (2006). An ordered, nonredundant library of Pseudomonas aeruginosa strain PA14 transposon insertion mutants. *Proc Natl Acad Sci U S A* 103(8)**,** 2833-2838. doi: 10.1073/pnas.0511100103.

Simon, R., Priefer, U., and Pühler, A. (1983). A Broad Host Range Mobilization System for In Vivo Genetic Engineering: Transposon Mutagenesis in Gram Negative Bacteria. *Nature Biotechnology* 1(9)**,** 784-791.

Totten, P.A., and Lory, S. (1990). Characterization of the type a flagellin gene from Pseudomonas aeruginosa PAK. *J Bacteriol* 172(12)**,** 7188-7199.