**Supplementary Data**

**Table S1**. Bacterial strains and plasmids used in this study.

|  |  |  |
| --- | --- | --- |
| Strain or plasmid | Relevant genotype or phenotype | Source or reference |
| ***P.aeruginose* strains** |  |  |
| PAO1 | Wild type strain | Lab collection |
| ∆*fleS* | *fleS* deletion mutant of PAO1 with 1208-nt internal coding region deleted  | This study |
| ∆*fleR* | *fleR* deletion mutant of PAO1 with 1422-nt internal coding region deleted  | This study |
| ∆*fleS*∆*fleR* | ∆*fleS* ∆*fleR* double-deletion mutant  | This study |
| ∆*fleS*(*fleS*) | Mutant ∆*fleS* harboring the expression construct pBBR1-MCS5-*fleS* | This study |
| ∆*fleR*(*fleR*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleS* | This study |
| ∆*fleS*∆*fleR*(*fleS*) | Double-deletion mutant ∆*fleS*∆*fleR* harboring the expression construct pBBR1-MCS5-*fleS* | This study |
| ∆*fleS*∆*fleR*(*fleR*) | Double-deletion mutant ∆*fleS*∆*fleR* harboring the expression construct pBBR1-MCS5-*fleR* | This study |
| ∆*REC* | REC domain of *fleR* deletion mutant | This study |
| ∆*AAA* | AAA domain of *fleR* deletion mutant | This study |
| ∆*HTH* | HTH domain of *fleR* deletion mutant  | This study |
| ∆*REC*(*REC*) | ∆*REC* harboring the expression construct pBBR1-MCS5-REC domain of *fleR* | This study |
| ∆*AAA*(*AAA*) | ∆*AAA* harboring the expression construct pBBR1-MCS5-AAA domain of *fleR* | This study |
| ∆*HTH*(*HTH*) | ∆*HTH* harboring the expression construct pBBR1-MCS5-HTH domain of *fleR* | This study |
| ∆*REC*(*fleR*) | ∆*REC* harboring the expression construct pBBR1-MCS5-*fleR* | This study |
| ∆*AAA*(*fleR*) | ∆*AAA* harboring the expression construct pBBR1-MCS5-*fleR* | This study |
| ∆*HTH*(*fleR*) | ∆*HTH* harboring the expression construct pBBR1-MCS5- *fleR* | This study |
| ∆*fleS*(*fleS*∆*PAS*) | Mutant ∆*fleS* harboring the expression construct pBBR1-MCS5-*fleS* ofabsence PAS domain  | This study |
| ∆*fleS*(*fleS*∆*HisKA*) | Mutant ∆*fleS* harboring the expression construct pBBR1-MCS5-*fleS* ofabsence HisKA domain | This study |
| ∆*fleS*(*fleS*∆*HATPase*) | Mutant ∆*fleS* harboring the expression construct pBBR1-MCS5-*fleS* ofabsence HATPase domain | This study |
| ∆*fleR*(*fleR*∆*REC*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleR* ofabsence REC domain | This study |
| ∆*fleR*(*fleR*∆*AAA*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleR* ofabsence AAA domain | This study |
| ∆*fleR*(*fleR*∆*HTH*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleR* ofabsence HTH domain | This study |
| ∆*fleS*(*fleSH191A*) | Mutant ∆*fleS* harboring the expression construct pBBR1-MCS5-*fleSH191A* | This study |
| ∆*fleS*(*fleSI135A*) | Mutant ∆*fleS* harboring the expression construct pBBR1-MCS5-*fleSI135A* | This study |
| ∆*fleS*(*fleSL143A*)  | Mutant ∆*fleS* harboring the expression construct pBBR1-MCS5-*fleSL143A* | This study |
| ∆*fleS*(*fleSI135AL143A*) | Mutant ∆*fleS* harboring the expression constructpBBR1-MCS5-*fleSI135AL143A* | This study |
| ∆*fleR*(*fleRD10A*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD10A* | This study |
| ∆*fleR*(*fleRD53A*)  | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD53A* | This study |
| ∆*fleR*(*fleRD99A*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD99A* | This study |
| ∆*fleR*(*fleRD11A*)  | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD11A* | This study |
| ∆*fleR*(*fleRD20A*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD20A* | This study |
| ∆*fleR*(*fleRD33A*)  | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD33A* | This study |
| ∆*fleR*(*fleRD60A*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD60A* | This study |
| ∆*fleR*(*fleRD87A*)  | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD87A* | This study |
| ∆*fleR*(*fleRD112A*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD112A* | This study |
| ∆*fleR*(*fleRK180A*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRK180A* | This study |
| ∆*fleR*(*fleRT208A*) | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRT208A* | This study |
| ∆*fleR*(*fleRD229AE230A*)  | Mutant ∆*fleR* harboring the expression construct pBBR1-MCS5-*fleRD229AE230A* | This study |
| ∆*fleQ* | *fleQ* deletion mutant of PAO1 which the gene *fleQ* was in frame deleted  | This study |
| ∆*fleS*∆*fleQ* | *fleS* and *fleQ* double deletion mutant which *fleQ* was deleted in ∆*fleS* background | This study |
| ∆*fleR*∆*fleQ* | *fleR* and *fleQ* double deletion mutant which *fleQ* was deleted in ∆*fleR* background | This study |
| ***E. coli* strains**  |  |  |
| DH5α | *spuE44 ∆lacU169(φ80lacZ∆M15) hsdR17λpir recA1 endA1 gyrA96 thi-1 relA1*  | Lab collection |
| BL21(DE3) | F-*ompT* *hsdS* (rB-mB-) *dcm*+ Tetr *gal* (DE3) *endA* | Lab collection |
| pRK2013 | Tra+, Mob-, ColE1-replicon, Kanr, Sper | Lab collection |
| **Plasmids** |  |  |
| pBBR1-MCS5 | Broad host-range cloning vector; Gmr | Lab collection |
| pBBR1-MCS5-*fleS* | pBBR1-MCS5 containing *fleS* under control of Plac  | This study |
| pBBR1-MCS5-*fleR* | pBBR1-MCS5 containing *fleR* under control of Plac  | This study |
| pK18mobsacB  | Broad-host-range gene replacement vector, sacB,Gmr  | Lab collection  |
| pK18-*fleS* | pK18 containing *fleS* flanking regions for generation of *fleS* in-frame deletion  | This study |
| pK18-*fleR* | pK18 containing *fleR* flanking regions for generation of *fleR* in-frame deletion  | This study |
| pBBR1-MCS5-Gm-*lacZ* | lacZ transcriptional fusion vector, Gmr  | Lab collection |
| pBBR1-MCS5-*fleS* | Single-copy Tn7 insertion plasmid containing *fleS* | This study |
| pBBR1-MCS5-*fleR* | Single-copy Tn7 insertion plasmid containing *fleR* | This study |
| pBBR1-MCS5-*fleSH191A* | pBBR1-MCS5 containing *fleS* derivative with H191A substitution  | This study |
| pBBR1-MCS5-*fleSI135A* | pBBR1-MCS5 containing *fleS* derivative with I135A substitution  | This study |
| pBBR1-MCS5-*fleSL143A* | pBBR1-MCS5 containing *fleS* derivative with L143A substitution  | This study |
| pBBR1-MCS5-*fleSI135AL143A* | pBBR1-MCS5 containing *fleS* derivative with I135A and L143A substitution  | This study |
| pBBR1-MCS5-*fleRD10A* | pBBR1-MCS5 containing *fleR* derivative with D10A substitution  | This study |
| pBBR1-MCS5-*fleRD53A* | pBBR1-MCS5 containing *fleR* derivative with D53A substitution  | This study |
| pBBR1-MCS5-*fleRD99A* | pBBR1-MCS5 containing *fleR* derivative with D99A substitution  | This study |
| pBBR1-MCS5-*fleRD11A* | pBBR1-MCS5 containing *fleR* derivative with D11A substitution  | This study |
| pBBR1-MCS5-*fleRD20A* | pBBR1-MCS5 containing *fleR* derivative with D20A substitution  | This study |
| pBBR1-MCS5-*fleRD33A* | pBBR1-MCS5 containing *fleR* derivative with D33A substitution  | This study |
| pBBR1-MCS5-*fleRD60A* | pBBR1-MCS5 containing *fleR* derivative with D60A substitution  | This study |
| pBBR1-MCS5-*fleRD87A* | pBBR1-MCS5 containing *fleR* derivative with D87A substitution  | This study |
| pBBR1-MCS5-*fleRD112A* | pBBR1-MCS5 containing *fleR* derivative with D112A substitution  | This study |
| pBBR1-MCS5-*fleRK180A* | pBBR1-MCS5 containing *fleR* derivative with K180A substitution  | This study |
| pBBR1-MCS5-*fleRT208A* | pBBR1-MCS5 containing *fleR* derivative with T208A substitution  | This study |
| pBBR1-MCS5-*fleRD229AE230A* | pBBR1-MCS5 containing *fleR* derivative with D229A and E230A substitution  | This study |

Gmr, gentamicin resistance; Kanr, kanamycin resistance; Ampr,ampicillin resistance

**Table S2.** PCR primers used in this study.

|  |  |  |
| --- | --- | --- |
| Primers | Sequence (5’-3’) | Application |
| *fleS*-Up-F | gagctcggtacccggggatccGGAGCGCCTGGCGATCAT | For amplification of the 5’-region of *fleS* |
| *fleS*-Up-R | aatcgcagaaagGGCGTTGAGGGCTGGTTG |
| *fleS*-Dn-F | tcaacgccCTTTCTGCGATTCAGGAGTAACC | For amplification of the 3’-region of *fleS* |
| *fleS*-Dn-R | acgacggccagtgccaagcttCGCCGGAGATCAGCACGG |
| *fleR*-Up-F | gagctcggtacccggggatccGCTGGTGTTCGCCCGCGG | For amplification of the 5’-region of *fleR* |
| *fleR*-Up-R | agcacGGGGTTACTCCTGAATCGCAG |
| *fleR*-Dn-F | ttcaggagtaaccccGTGCTCGCCATGTTCCCC | For amplification of the 3’-region of *fleR* |
| *fleR*-Dn-R | acgacggccagtgccaagcttACGCTGGCCTTCTGGCTG |
| *fleR*-De-REC-1 | gagctcggtacccggggatccGCTGGTGTTCGCCCGCGG | For amplification of the 5’-region REC domain of *fleR* |
| *fleR*-De-REC-2 | TGCCATGGGGTTACTCCTGA  |
| *fleR*-De-REC-3 | tcaggagtaaccccatggcaGCGCGCCATGCGCTGGGC | For amplification of the 3’-region REC domain of *fleR* |
| *fleR*-De-REC-4 | acgacggccagtgccaagcttACGCTGGCCTTCTGGCTG |
| *fleR*-De-AAA-1 | gagctcggtacccggggatccGCTGGTGTTCGCCCGCGG | For amplification of the 5’-region AAA domain of *fleR* |
| *fleR*-De-AAA-2 | ccagcgggaaGCGCGCGACCCGCGCGGC |
| *fleR*-De-AAA-3 | ggtcgcgcgcTTCCCGCTGGCCTGGCGG | For amplification of the 3’-region AAA domain of *fleR* |
| *fleR*-De-AAA-4 | acgacggccagtgccaagcttACGCTGGCCTTCTGGCTG |
| *fleR*-De-HTH-1 | gagctcggtacccggggatccGCTGGTGTTCGCCCGCGG | For amplification of the 5’-region HTH domain of *fleR* |
| *fleR*-De-HTH-2 | tccatccccgcgtcgcgGTCGCCCAGCGCGCCGCT |
| *fleR*-De-HTH-3 | gacCGCGACGCGGGGATGGAC | For amplification of the 3’-region HTH domain of *fleR* |
| *fleR*-De-HTH-4 | acgacggccagtgccaagcttACGCTGGCCTTCTGGCTG |
| *fleS*-FC-F | gtcgacggtatcgataagcttGGGGTGATCGGGGTCGGC | For construction of pBBR1-MCS5-*fleS* |
| *fleS*-FC-R | cgctctagaactagtggatccCGCGTAGTGCGCGGTCGT |
| *fleR*-FC-F | gtcgacggtatcgataagcttTGCGGGCCCGAACTGCGC | For construction of pBBR1-MCS5-*fleR* |
| *fleR*-FC-R | cgctctagaactagtggatccGCGGACGCAAAAGGCCCG |
| *sadC*-PC-F | gtcgacggtatcgataagcttCGGGTCGGCCAGGATCGA | For construction of pBBR1-MCS5-*sadC* |
| *sadC*-PC-R | cgctctagaactagtggatccAGGTTGCTGCCGGCGGCG |
| *bifA*-PC-F | gtcgacggtatcgataagcttCGACGTCTGGGAACACGC | For construction of pBBR1-MCS5-*bifA* |
| *bifA*-PC-R | cgctctagaactagtggatccCTGGGCAGCGCGCTATTG |
| *siaD*-PC-F | gtcgacggtatcgataagcttCAAGGCCATGATGGACATCC | For construction of pBBR1-MCS5-*siaD* |
| *siaD*-PC-R | cgctctagaactagtggatccCTCAGCCGAGGCCAGGTG |
| *fleS*-De-PAS-1 | gtcgacggtatcgataagcttATGCAACCAGCCCTCAACG | For construction the 5’-region of pBBR-*fleS*(∆PAS) |
| *fleS*-De-PAS-2 | gtcCGCCAGGCGCTCCTTTTC |
| *fleS*-De-PAS-3 | aaaaggagcgcctggcgGACGGACGTCGCCTGTCC | For construction the 3’-region of pBBR-*fleS*(∆PAS) |
| *fleS*-De-PAS-4 | cgctctagaactagtggatccTTACTCCTGAATCGCAGAAA |
| *fleS*-De-HisKA-1 | gtcgacggtatcgataagcttATGCAACCAGCCCTCAACG | For construction the 5’-region of pBBR-*fleS*(∆HisKA) |
| *fleS*-De-HisKA-2 | gtcggtcagCGACAGGCGCTCGTGGCG |
| *fleS*-De-HisKA-3 | agcgcctgtcgCTGACCGACCGGGTGGCG | For construction the 3’-region of pBBR-*fleS*(∆HisKA) |
| *fleS*-De-HisKA-4 | cgctctagaactagtggatccTTACTCCTGAATCGCAGAAA |
| *fleS*-De-HATPase-F | gtcgacggtatcgataagcttATGCAACCAGCCCTCAACG | For construction the of pBBR-*fleS*(∆HATPase) |
| *fleS*-De-HATPase-R | cgctctagaactagtggatccTTACTCCTGAATCGCAGAAAGAGGCGCCGCCAGCAGCTCGCCGCCGCG |
| *fleR*-De-REC-F | gtcgacggtatcgataagcttATGGCAGCGCGCCATGCGCTGGGC | For construction the of pBBR-*fleR*(∆REC) |
| *fleR*-De-REC-R | cgctctagaactagtggatccTCAGATGGCGTAGAGATAGGCC |
| *fleR*-De-AAA-UF | gtcgacggtatcgataagcttATGGCAGCCAAAGTCCTGC | For construction the 5’-region of pBBR-*fleR*(∆AAA) |
| *fleR*-De-AAA-UR | ccagcgggaaGCGCGCGACCCGCGCGGC |
| *fleR*-De-AAA-DF | ggtcgcgcgcTTCCCGCTGGCCTGGCGG | For construction the 3’-region of pBBR-*fleR*(∆AAA) |
| *fleR*-De-AAA-DR | cgctctagaactagtggatccTCAGATGGCGTAGAGATAGGCC |
| *fleR*-De-HTH-F | gtcgacggtatcgataagcttATGGCAGCCAAAGTCCTGC | For construction the of pBBR-*fleR*(∆HTH) |
| *fleR*-De-HTH-R | cgctctagaactagtggatccTCAGATGGCGTAGAGATAGGCCTCCACGTCCATCCCCGCGTCGCGGTCGCCCAGCGCGCCGCT |
| *fleRD10A*-F | gtcgacggtatcgataagcttATGGCAGCCAAAGTCCTGCTGGTCGAAGCCGACCGCGCACTACGCGAA | For construction of pBBR-*fleR*(D10A) |
| *fleRD10A*-R | cgctctagaactagtggatccTCAGATGGCGTAGAGATAGGCC |
| *fleRD11A*-F | gtcgacggtatcgataagcttATGGCAGCCAAAGTCCTGCTGGTCGAAGACGCCCGCGCACTACGCGAAGCC | For construction of pBBR-*fleR*(D11A) |
| *fleRD11A*-R | cgctctagaactagtggatccTCAGATGGCGTAGAGATAGGCC |
| *fleRD20A*-2 | tGGCGCTGAGGGCTTCGCGTAGT | For construction the 5’-region of pBBR-*fleR*(D20A) with *fleR*-PC-F primer |
| *fleRD20A*-3 | actacgcgaagccctcagcGCCACCCTGCTGCTGGGCGGT | For construction the 3’-region of pBBR-*fleR*(D20A) with *fleR*-PC-R primer |
| *fleRD33A*-2 | aGGCCACGGCGACGAACTCGTG | For construction the 5’-region of pBBR-*fleR*(D33A) with *fleR*-PC-F primer |
| *fleRD33A*-3 | tcacgagttcgtcgccgtgGCCTCGGCGGAGGCGGCGCTG | For construction the 3’-region of pBBR-*fleR*(D33A) with *fleR*-PC-R primer |
| *fleRD53A*-2 | catgttcacGCCGCTGATCACCAGGCTGAAGG | For construction the 5’-region of pBBR-*fleR*(D53A) with *fleR*-PC-F primer |
| *fleRD53A*-3 | tggtgatcagcGGCGTGAACATGCCGGGCATG | For construction the 3’-region of pBBR-*fleR*(D53A) with *fleR*-PC-R primer |
| *fleRD60A*-2 | agcaactggtgtccGGCCATGCCCGGCATGTTCAC | For construction the 5’-region of pBBR-*fleR*(D60A) with *fleR*-PC-F primer |
| *fleRD60A*-3 | ggcatgGCCGGACACCAGTTGCTCGGCC | For construction the 3’-region of pBBR-*fleR*(D60A) with *fleR*-PC-R primer |
| *fleRD87A*-2 | atcgcctcgacggcgcgGGCGACCGCGCCGTAGGCGGT | For construction the 5’-region of pBBR-*fleR*(D87A) with *fleR*-PC-F primer |
| *fleRD87A*-3 | gtcGCCCGCGCCGTCGAGGCGATG | For construction the 3’-region of pBBR-*fleR*(D87A) with *fleR*-PC-R primer |
| *fleRD99A*-2 | tcgaacggcttgaccaggtaGGCGGCGGCGCCCTGGCGCAT | For construction the 5’-region of pBBR-*fleR*(D99A) with *fleR*-PC-F primer |
| *fleRD99A*-3 | GCCTACCTGGTCAAGCCGTTCGA | For construction the 3’-region of pBBR-*fleR*(D99A) with *fleR*-PC-R primer |
| *fleRD112A*-2 | atggcgcgccaccagGGCGAGCAGCGCCCGCGCCTC | For construction the 5’-region of pBBR-*fleR*(D112A) with *fleR*-PC-F primer |
| *fleRD112A*-3 | tgctcGCCCTGGTGGCGCGCCATGCG | For construction the 3’-region of pBBR-*fleR*(D112A) with *fleR*-PC-R primer |
| *fleRK164A*-2 | agttggccaggacttcTGCGCCGGTCCCGGACTCGCC | For construction the 5’-region of pBBR-*fleR*(K164A) with *fleR*-PC-F primer |
| *fleRK164A*-3 | cGCAGAAGTCCTGGCCAACTATATCCA | For construction the 3’-region of pBBR-*fleR*((K164A) with *fleR*-PC-R primer |
| *fleRT208A*-2 | ggcgccTGCGAAGGAACCTTTCTCGTGGCC | For construction the 5’-region of pBBR-*fleR*(T208A) with *fleR*-PC-F primer |
| *fleRT208A*-3 | aaggttccttcGCAGGCGCCATTGCCGCCCAG | For construction the 3’-region of pBBR-*fleR*(T208A) with *fleR*-PC-R primer |
| *fleRD229AE230A*-2 | tttccgatatTGCTGCGAGAAGAATGGTGCCGCCG | For construction the 5’-region of pBBR-*fleR*(D229AE230A) with *fleR*-PC-F primer |
| *fleR*D229AE230A-3 | tctcGCAGCAATATCGGAAATGCCCCTCG | For construction the 3’-region of pBBR-*fleR*(D229AE230A) with *fleR*-PC-R primer |
| *fleSI135A*-2 | cagggaCGCCTCGTGACCGTCATCCTCGC | For construction the 5’-region of pBBR-*fleR*(I135A) with *fleS*-PC-F |
| *fleSI135A*-3 | atgacggtcacgaggcgTCCCTGCGCGACGGACGT | For construction the 3’-region of pBBR-*fleR*(I135A) with *fleS*-PC-R |
| *fleSL143*A-2 | tggcgatggaCGCGCGACGTCCGTCGCGCAG | For construction the 5’-region of pBBR-*fleR*(L143A) with *fleS*-PC-F |
| *fleSL143A*-3 | cggacgtcgcgcgTCCATCGCCACCCGCTCG | For construction the 5’-region of pBBR-*fleR*(L143A) with *fleS*-PC-R |
| *fleSH191A*-2 | aacggcgtgcggatctgcgcGGCCAGCGAGGCGACCAT | For construction the 5’-region of pBBR-*fleR*(H191A) with *fleS*-PC-F |
| *fleSH191A*-3 | GCGCAGATCCGCACGCCG | For construction the 5’-region of pBBR-*fleR*(H191A) with *fleS*-PC-R |
| *fleR*-Up-F | gagctcggtacccggggatccATCGGTGAGCTGGATCAGGTC | For amplification of the 5’-region of *fleQ* |
| *fleR*-Up-R | atccgaTTCGCGCCACATTTTGATC |
| *fleR*-Dn-F | aaatgtggcgcgaaTCGGATGATTGACAGGTCGTT | For amplification of the 3’-region of *fleQ* |
| *fleR*-Dn-R | acgacggccagtgccaagcttCCTCGCGCGGAGCGAAGC |
| q-*flgB*-F | GAGCACTCGGTATCCACCAG | For qRT-PCR analysisFor qRT-PCR analysis |
| q-*flgB*-R | GATATGCCGCTCGTTGGTG |
| q-*flgC*-F | CCTCGCCAGTGTCTTCAACA |
| q-*flgC*-R | CTGCTGGAACATGGTGGAGA |
| q-*flgD*-F | GTCGCTGAACAAGAGCATGG |
| q-*flgD*-R | TTGACCCATACGTTGCTGCT |
| q-*flgF*-F | GTGCTCATGCCAACAACCTG |
| q-*flgF*-R | GCCATGGCTGAAATCGGT |
| q-*cdrA*-F | CCAGTTCAACCCCAACGAGA |
| q-*cdrA*-R | GTCGAAGCCCTTCCAGTTGA |
| *rplU*-F | GCAGCACAAAGTCACCGAAG | Internal control for qRT-PCR analysis |
| *rplU*-R | CCGATTTTCACGTCTTCGCC |
| *W909-14945*-FC-F | GTCGACGGTATCGATAAGCTTAAATATTAGCCAGGCTTATGT | For construction of pBBR1-MCS5-*W909-14945* |
| *W909-14945*-FC-R | CGCTCTAGAACTAGTGGATCCCTAGTGGTGGTGGTGGTGGTGCTGGTGTGTATTCAGATCA |
| *W909-14950*-FC-F | GTCGACGGTATCGATAAGCTTTACACAGCGTGGTTATGGTATA | For construction of pBBR1-MCS5-*W909-14950* |
| *W909-14950*-FC-R | CGCTCTAGAACTAGTGGATCCTTAGTGGTGGTGGTGGTGGTGCAGCTCTGAACGGAGATCTT |



**Figure S1. Stable expression of FleS/FleR variants constructed in this study.** Variants of FleS and FleR used in this study are His-tagged and examined by western blot.



**Figure S2. Schematic diagram showing the aspartate residues in the FleR-REC domain**. The positions of each aspartate residue are shown.



**Figure S3. Alignment of the AAA domains from FleR and FleQ**. Residues shown with 100% identity are highlighted in red.



**Figure S4. Alanine substitution of leucine at the position of 143 in the FleS-PAS domain inactivates the signaling activity of FleS.**



**Figure S5. Alignment of the REC sequences from CheY and FleR**. D12 and D57 in CheY with corresponding to the D10 and D53 residues in FleR are marked with red boxes.



**Figure S6. Sequence alignment of FleQ and FleR**. Residues R144, R185, N186, E330 and R334 which are critical for c-di-GMP binding in FleQ are highlighted in red boxes.