**TABLE S7. Primers used in this study**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence (5' to 3')** | **Use** |
| F\_E\_ PA14\_03490 | GTCCGAATTCGTGGAGGATCGCGTCGC | gel shift assay |
| R\_B\_PA14\_03490 | GTCCGGATCCGGCTGATTGGCAGCC | gel shift assay |
| F\_E\_ PA14\_09480 | GTCCGAATTCTTTCCTGCGTACCGAAAG | gel shift assay |
| R\_B\_PA14\_09480 | GTCCGGATCCTTCCGTGAAGTGTTTCAAATAG | gel shift assay |
| F\_E\_ PA14\_09490 | GTCCGAATTCCGTCATTCCGTGAAGTG | gel shift assay |
| R\_B\_PA14\_09490 | GTCCGGATCCGAAAGAATAAAATTACAACT TGGC | gel shift assay |
| F\_E\_ PA14\_23220 | GTCCGAATTCGCGAAGATCGCCGCCAG | gel shift assay |
| R\_B\_PA14\_23220 | GTCCGGATCCGTTGAAGAGTGAAGCCTTTG | gel shift assay |
| F\_E\_ PA14\_33830 | GTCCGAATTCGCAATGGCAGACCTTGC | gel shift assay |
| R\_B\_PA14\_33830 | GTCCGGATCCTGCACGTGTTCCCTGG | gel shift assay |
| F\_H\_ PA14\_33890 | GTCCAAGCTTAGCGGCGAGGAGCGG | gel shift assay |
| R\_B\_PA14\_33890 | GTCCGGATCCAGTCGCACGGCGACTG | gel shift assay |
| F\_E\_ PA14\_34870 | GTCCGAATTCCTGCTGTCCCCGGCAC | gel shift assay |
| R\_B\_ PA14\_34870 | GTCCGGATCCGTCTACCGCAGGGCATTC | gel shift assay |
| F\_E\_ PA14\_37745 | GTCCGAATTCCTTCTGATGCCGGCGCG | gel shift assay |
| R\_B\_ PA14\_37745 | GTCCGGATCCTGCGCGAACGGGTGG | gel shift assay |
| F\_E\_ PA14\_40310 | GTCCGAATTCCCGGGAAGCGCCCTGTG | gel shift assay |
| R\_B\_ PA14\_40310 | GTCCGGATCCACTGCCCGGCCCTGG | gel shift assay |
| F\_E\_ PA14\_41500 | GTCCGAATTCGCGGCTGAATCCGGCTG | gel shift assay |
| R\_B\_ PA14\_41500 | GTCCGGATCCGCCTGCGGCTCCTAG | gel shift assay |
| F\_E\_ PA14\_48530 | GTCCGAATTCCCGTGCTACCCCCGG | gel shift assay |
| R\_B\_ PA14\_48530 | GTCCGGATCCGGTTTCCGAAGTGTTTCGC | gel shift assay |
| F\_E\_ PA14\_53250 | GTCCGAATTCGGCGGCCCTTTGCCTG | gel shift assay |
| R\_B\_ PA14\_53250 | GTCCGGATCCTTTCCAGAACCGGATTC | gel shift assay |
| F\_E\_ PA14\_07430 | GTCCGAATTCTGGATCGTTTCGTCACAGG | gel shift assay |
| R\_B\_ PA14\_07430 | GTCCGGATCCAATGCCGGACATACGCATG | gel shift assay |
| F\_E\_ PA14\_16660 | GTCCGAATTCTTCGCGCTCGTAGTAGC | gel shift assay |
| R\_B\_ PA14\_16660 | GTCCGGATCCGTTTGACGTGTTGGGTG | gel shift assay |
| F\_E\_ PA14\_18800 | GTCCGAATTCTTGTTCTATATGTTCGAACTTGGC | gel shift assay |
| R\_B\_ PA14\_18800 | GTCCGGATCCTGGTTTGTTGACTCCTG | gel shift assay |
| F\_E\_ PA14\_69090 | GTCCGAATTCTCCAGCAGACCCTCGAG | gel shift assay |
| R\_B\_ PA14\_69090 | GTCCGGATCCGCAGGTGTTGATGCAC | gel shift assay |
| F\_E\_ lrs2 | GTCCGAATTCCAATGAAAGATTTTCAGCCAAACG | gel shift assay, lrs2-lacZ |
| R\_B\_ lrs2 | GTCCGGATCCCGAGGCTCTCCAGAG | gel shift assay, lrs2-lacZ |
| F\_E\_ rsaL | GTCCGAATTCTTGTGCATCTCGCCCAGC | gel shift assay |
| R\_B\_ rsaL | GTCCGGATCCTCGGACGTTTCTTCGAG | gel shift assay |
| F\_E\_lrs1 | GTCCGAATTCTGGCAGGCGAGACGGG | gel shift assay, lrs1-lacZ |
| R\_B\_lrs1 | GTCCGGATCCGTCCGAACCCATGAGATG | gel shift assay, lrs1-lacZ |
| F\_E\_hrpA | GTCCGAATTCCGGGAAGCGCCCTTCCTTG | gel shift assay |
| R\_B\_hrpA | GTCCGGATCCCGGCCCTGGCGATACCC | gel shift assay |
| R\_lrs1+126 | CTGCGCGGGATCCGAGAACGGCAAC | 5' and 3', Northern blot mapping |
| R\_lrs1+151 | CGAGAAATTTGGCACACCCACC | 5' and 3', mapping |
| F\_lrs1+152 | CGGTTTGGATCGCGCCGATTGTCGC | 5' and 3' mapping |
| F\_T7lrs1+1 | GCGTAATACGACTCACTATAGGCCATCTCAT GGGTTCGG | *in vitro* transcription |
| R\_lrs1+191 | CGGGCTTCGTAGGCCGCGACAATC | *in vitro* transcription |
| F\_T7prrf1+1 | GCGTAATACGACTCACTATAGGAACTGGTCGCAGATCAG | *in vitro* transcription |
| R\_prrf1+116 | AAAAAAAGACCCGGCAAAGTGCCGGGTCAAAAACCGTGATTAGCC | *in vitro* transcription |
| F\_NdI\_hfqO1+1 | GTCC CATATGTCAAAAGGGCATTCGCTAC | pET29a+Hfq |
| R\_XhI\_hfqO1+stp | GTCC CTCGAG AGCGTTGCCCGGCTCGG | pET29a+Hfq |
| F\_XhI\_phzA1 | GTCCGGTACCTTCCGTGAAGTGTTTCAAATAG | pZE21-phzA1 |
|  |  |  |
| R\_kpnI\_phzA1 | GTCCGGTACCCCTCCT TTCCGTGAAGTGTTTCAAATAG | pZE21-phzA1 |
| F\_XhI\_phzM-63 | GTCCCTCGAGCCGGCTCAACTACAAGATC | pZE21-phzM |
| R\_kpnI\_phzM | GTCCGGTACCCCTCCTGAAAGAATAAAATTACAACTTGGC | pZE21-phzM |
| F\_XhI\_rhlA | GTCCCTCGAG CTTATGCGCAGGCGACC | pZE21-rhlA |
| R\_kpnI\_rhlA | GTCCGGTACCCCTCCTCGAACAGGCAAACAGCTATC | pZE21-rhlA |
| F\_BHI\_rhlR-483 | GTCCGGATCCCACTGGGAGCCTTGCTG | pEXG2-RhlR |
| R\_Hnd3\_rhlR+497 | GTCCAAGCTTGCGTAGCGCGAAAGCTC | pEXG2-RhlR |
| F\_rhlR\_fusN | CGGCGCGTCGTGCAGTAAGCCCTGATCG | pEXG2-RhlR |
| R\_rhlR\_fusN | CGGCGCGTCGTGCAGTAAGCCCTGATCG | pEXG2-RhlR |
| F\_q\_lrs1 | GCCGCCCTTCTTGCTTG | Real time PCR |
| R\_q\_lrs1 | TTTGGCACACCCACCG | Real time PCR |
| F\_q\_lrs2 | CTGGAGAGCCTCGAAACATAGG | Real time PCR |
| R\_q\_lrs2 | GGCTGTTATTGGGGATGTTT CTTGG | Real time PCR |
| F\_q\_prrf1 | CGCGAGATCAGCCGGTAAGC | Real time PCR |
| R\_q\_prrf1 | AAACCGTGATTAGCCTGATGAGGAG | Real time PCR |
| F\_q\_rsmZ | GAACACGCAACCCCGAAGGATC | Real time PCR |
| R\_q\_rsmZ | CACTCTTCAGTCCCTCGTCATCATC | Real time PCR |
| R\_5S +67 | CGTTTCACTTCTGAGTTCGGGAAGG | Northern blot |
| F\_Hnd3\_pqsA  -246 | GTCCGTCCAAGCTTGGCCTCGAGCAAGGGTTG | pCTX-*pqsA*-*lacZ* cloning |
| R\_XmaI\_pqsA  +231 | GTCCCCCGGGCGCCGGGCTTGAGCAG | pCTX-*pqsA*-*lacZ* cloning |

Table S8. Strains and plasmids used in this study

|  |  |  |
| --- | --- | --- |
| Strain or plasmid | Relevant genotype or description | Source or reference |
| *P. aeruginosa* |  |  |
| PA14 | Wild type |  |
| PA14*lasR* | Isogenic deletion strain constructed with pJTT4 | This study |
| PA14*lrs1* | Isogenic deletion strain constructed with pEXG2-lrs1 | This study |
| PA14*rhlR* | Isogenic deletion strain constructed with pEXG-rhlR | This study |
| PA14 *phzM* | PA14 phzM::MAR2xT7 (mutant 40343) | (1) |
| PA14 *pqsA* | Deletion of *pqsA* gene, | Gift of Dr. Laurence Rahme |
| *pqsA-lacZ* PAO1Δ*pqsA* | Chromosomal *pqsA-lacZ* transcriptional fusion in PAO1 Δ*pqsA* | This study |
|  |  |  |
| *E.coli* |  |  |
| DH5α | φ80d*lacZ*∆M15 ∆(*lacZYA-argF*)*U169 recA1 endA1 hsdR17*(rK- m K-) *supE44 thi-1 gyrA relA1* | (2) |
| BL21(DE3)/pLysS | F- *ompT hsdSB(r-Bm-B ) gal dcm* (DE3) pLysS(Cmr) | Novagen |
| SM10 | *thi-1 leuB6 supE44 tonA21 lacY1 recA*::RP4-2-Tc::Mu Kmr | (3) |
|  |  |  |
| Plasmids |  |  |
| pEXG2 | ColE1 suicide vector; mob sacB Gmr | (4) |
| pMMB67EH | Broad-host-range expression vector from Ptac; lacIq Apr | (5) |
| pEXG2-lrs1 | pEXG2 with lrs1 fragment from +1 to +191 | This study |
| pEXG2-rhlR | pEXG2 with rhlR allele constructed by SOE | This study |
| pLasR | pJTT201, pMMB67EH with lasR gene | (6) |
| pRhlR | pJTT202, pMMB67EH with rhlR gene | (6) |
| pET29a+Hfq | pET29a(+) with hfq-His6 in NdeI/XhoI site | This study |
| pCTX-pqsA-lacZ | Mini-CTX-lacZ with pqsA fragment (-246 to +231) in HindIII/XmaI site | This study |

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