

**S2 Table. Plasmids used in this study**

| Plasmid             | Relevant characteristic  | Source             |
|---------------------|--|--------------------|
| pAAS1544            | Cb <sup>R</sup> . Derived from pPR54 [1] encoding <i>A. vinelandii</i> NifL (147-519) and NifA-E356K.  | Adriano Stefanello |
| pPR34               | Cb <sup>R</sup> . <i>A. vinelandii</i> nifLA translated from the natural ribosome binding site of nifL in pT7-7  | [1]                |
| pPMA                | Cb <sup>R</sup> . Derived from pPR34, but encoding nifA-E356K  | [2]                |
| pRT22               | Cm <sup>R</sup> . pnifH::lacZ in pACYC184  | [3]                |
| pBlueScript SK II + | Cb <sup>R</sup> . Cloning vector   | [4]                |
| pUC19               | Cb <sup>R</sup> . Cloning vector   | [5]                |
| pK18mobsacB Km      | Km <sup>R</sup> , Mob. Suicide vector for gene replacement. sacB gene for counter selection  | [6]                |
| pALMAR3             | Tc <sup>R</sup> . Source of tetracycline resistance gene (tetA)  | [7]                |
| pUC18T-mini-Tn7T-Tp | Tp <sup>R</sup> . Source of the trimethoprim resistance gene (tmp)   | [8]                |
| pMB1724             | Cb <sup>R</sup> . 1893 bp PCR fragment corresponding to <i>A. vinelandii</i> nifHD region cloned into BamHI/HindIII sites of pBlueScript II +  | This study         |
| pMB1725             | Cb <sup>R</sup> . Tc <sup>R</sup> . tetA gene amplified by PCR from pALMAR3 inserted into BgIII and EcoRI sites of pMB1724   | This study         |
| pMB1804             | Cb <sup>R</sup> . Derived from pPR34 encoding <i>P. stutzeri</i> A1501 nifL-nifA   | This study         |
| pMB1805             | Cb <sup>R</sup> . Derived from pPR34 encoding <i>P. stutzeri</i> A1501 nifL-nifA-E356K   | This study         |
| pMB1806             | Cb <sup>R</sup> . Derived from pPR34 encoding <i>A. olearius</i> DQS-4 nifL-nifA   | This study         |
| pMB1807             | Cb <sup>R</sup> . Derived from pPR34 encoding <i>A. olearius</i> DQS-4 nifL-nifA-E351K   | This study         |
| pMB1816             | Cb <sup>R</sup> , Tp <sup>R</sup> . <i>A. vinelandii</i> nifH::lacZ fusion flanked by homology regions for integration into algU genome locus  | This study         |
| pMB1840             | Km <sup>R</sup> . Tp <sup>R</sup> . <i>A. vinelandii</i> glnE deletion. Fragments upstream (906 bp) and downstream (752 bp) of glnE were fused to the tmp gene, and inserted into pk18mobsacBKM cut with SmaI  | This study         |
| pMB2005             | Km <sup>R</sup> . The rmf-nifLA intergenic region (444 bp) from <i>A. vinelandii</i> was fused downstream to a fragment of the <i>P. stutzeri</i> rmfAB genes (1160 bp) and upstream to a fragment of the <i>P. stutzeri</i> nifL gene (1638 bp) and inserted into pk18mobsacBKM cut with SmaI | This study         |
| pMB2006             | Km <sup>R</sup> . Ps-nifLAE356K fragment (2364 bp) from pMB1805 was fused to an 847 bp fragment downstream Ps-nifA and inserted into pk18mobsacBKM cut with SmaI. Construct to recover tetA from Ps_EK <sup>C</sup> -tetA to generate Ps_EK <sup>C</sup>                                       | This study         |
| pMB2007             | Km <sup>R</sup> , Tc <sup>R</sup> . The plasmid pMB2006 was linearized by PCR and fused to a fragment encoding the tetA gene (1349 bp). Construct to generate Ps_EK <sup>C</sup> -tetA   | This study         |

Abbreviations: Cb: carbenicillin, Cm: chloramphenicol, Km: kanamycin, Tc: tetracycline, Tp: trimethiorim, <sup>R</sup>: resistance.

## S2 Table References

1. Söderbäck E, Reyes-Ramirez F, Eydmann T, Austin S, Hill S, Dixon R. The redox- and fixed nitrogen-responsive regulatory protein NIFL from *Azotobacter vinelandii* comprises discrete flavin and nucleotide-binding domains. *Mol Microbiol*. 1998;28: 179–192. doi:10.1046/j.1365-2958.1998.00788.x
2. Reyes-Ramirez F, Little R, Dixon R. Mutant Forms of the *Azotobacter vinelandii* Transcriptional Activator NifA Resistant to Inhibition by the NifL Regulatory Protein. *J Bacteriol*. 2002;184: 6777–6785. doi:10.1128/JB.184.24.6777-6785.2002
3. Tuli R, Merrick MJ. Over-production and characterization of the nifA gene product of *Klebsiella pneumoniae*--the transcriptional activator of nif gene expression. *J Gen Microbiol*. 1988;134: 425–432. doi:10.1099/00221287-134-2-425
4. Alting-Mees MA, Short JM. pBluescript II: gene mapping vectors. *Nucleic Acids Res*. 1989;17: 9494. Available: <http://www.ncbi.nlm.nih.gov/pubmed/2555794>
5. Norrander J, Kempe T, Messing J. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. *Gene*. 1983;26: 101–106. doi:10.1016/0378-1119(83)90040-9
6. Schäfer A, Tauch A, Jäger W, Kalinowski J, Thierbach G, Pühler A. Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: Selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene*. 1994;145: 69–73. doi:10.1016/0378-1119(94)90324-7
7. Malone JG, Jaeger T, Spangler C, Ritz D, Spang A, Arrieumerlou C, et al. YfiBNR mediates cyclic di-GMP dependent small colony variant formation and persistence in *Pseudomonas aeruginosa*. *PLoS Pathog*. 2010;6. doi:10.1371/journal.ppat.1000804
8. Choi K-H, Schweizer HP. mini-Tn7 insertion in bacteria with single attTn7 sites: example *Pseudomonas aeruginosa*. *Nat Protoc*. 2006;1: 153–161. doi:10.1038/nprot.2006.24