

Table S1. Strains and plasmids

Strains or plasmids	Genotype or description	Construction	Reference
<i>Pseudomonas aeruginosa</i>			
PAO1	Wild-type		(Stover et al., 2000)
PAO1 <i>hfq</i> -	<i>hfq</i> :: <i>aadA</i>		(Sonnleitner et al., 2003)
PAO1 Δ <i>ersA</i>	markerless Δ <i>ersA</i>		(Ferrara et al., 2015)
<i>P. aeruginosa</i> RP73	Wild-type		(Bianconi et al., 2015)
<i>P. aeruginosa</i> RP73 Δ <i>ersA</i>	markerless Δ <i>ersA</i>		(Ferrara et al., 2020)
<i>Escherichia coli</i>			
TOP10	mcrA Δ (mrr-hsdRMS-mcrBC) φ 80lacZDM15 Δ lacX74 deoR recA1 araD139 Δ (ara-leu)7697 galU galK rpsL endA1 nupG		Invitrogen
Plasmids			
pGM931	pHERD20T derivative, <i>araC/P_{BAD}</i> - t _O , Ap ^r		(Qiu et al., 2008; Delvillani et al., 2014; Ferrara et al., 2015)
pGM- <i>ersA</i>	pGM931 derivative, <i>ersA</i> under <i>P_{BAD}</i> , Ap ^r		(Ferrara et al., 2015)
pXG10-SF	sfGFP reporter plasmid; <i>lacZ</i> :: <i>gfp</i> under <i>P_{LtetO-1}</i> , Cm ^r		(Corcoran et al., 2012)
pXG10- <i>anr</i> ::sfGFP	pXG10-SF derivative; <i>P_{LtetO-1}</i> --> <i>anr</i> :: <i>gfp</i> , Cm ^r	contains PCR product using oligos 2 and 3	this work
pXG10- <i>dnr</i> ::sfGFP	pXG10-SF derivative; <i>P_{LtetO-1}</i> --> <i>dnr</i> :: <i>gfp</i> , Cm ^r	contains PCR product using oligos 4 and 5	this work
pBBR1-MCS5	<i>lacZ</i> α, Gm ^r		(Kovach et al., 1995)
pBBR1- sfGFP	pBBR1-MCS5 derivative; <i>P_{LtetO-1}</i> --> <i>gfp</i> , Gm ^r		(Ferrara et al., 2015)
pBBR1- <i>anr</i> ::sfGFP	pBBR1-MCS5 derivative; <i>P_{LtetO-1}</i> --> <i>anr</i> :: <i>gfp</i> , Gm ^r	contains PCR product using oligos 6 and 7	this work
pBBR1- <i>dnr</i> ::sfGFP	pBBR1-MCS5 derivative; <i>P_{LtetO-1}</i> --> <i>dnr</i> :: <i>gfp</i> , Gm ^r	contains PCR product using oligos 6 and 7	this work

References

- Bianconi, I., Jeukens, J., Freschi, L., Alcala-Franco, B., Facchini, M., Boyle, B., et al. (2015). Comparative genomics and biological characterization of sequential *Pseudomonas aeruginosa* isolates from persistent airways infection. *BMC Genomics* 16, 1105. doi: 10.1186/s12864-015-2276-8.
- Corcoran, C.P., Podkaminski, D., Papenfort, K., Urban, J.H., Hinton, J.C., and Vogel, J. (2012). Superfolder GFP reporters validate diverse new mRNA targets of the classic porin regulator, MicF RNA. *Mol Microbiol* 84(3), 428-445. doi: 10.1111/j.1365-2958.2012.08031.x.
- Delvillani, F., Sciandrone, B., Peano, C., Petiti, L., Berens, C., Georgi, C., et al. (2014). Tet-trap, a genetic approach to the identification of bacterial RNA thermometers: Application to *Pseudomonas aeruginosa*. *RNA* 20(12), 1963-1976. doi: 10.1261/rna.044354.114.
- Ferrara, S., Carloni, S., Fulco, R., Falcone, M., Macchi, R., and Bertoni, G. (2015). Post-transcriptional regulation of the virulence-associated enzyme AlgC by the σ22-dependent small RNA ErsA of *Pseudomonas aeruginosa*. *Environmental Microbiology* 17(1), 199-214. doi: 10.1111/1462-2920.12590.

- Ferrara, S., Rossi, A., Ranucci, S., De Fino, I., Bragonzi, A., Cigana, C., et al. (2020). The Small RNA ErsA Plays a Role in the Regulatory Network of *Pseudomonas aeruginosa* Pathogenicity in Airway Infections. *mSphere* 5(5). doi: 10.1128/mSphere.00909-20.
- Kovach, M.E., Elzer, P.H., Hill, D.S., Robertson, G.T., Farris, M.A., Roop, R.M., 2nd, et al. (1995). Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* 166(1), 175-176.
- Qiu, D., Damron, F.H., Mima, T., Schweizer, H.P., and Yu, H.D. (2008). PBAD-based shuttle vectors for functional analysis of toxic and highly regulated genes in *Pseudomonas* and *Burkholderia* spp. and other bacteria. *Appl Environ Microbiol* 74(23), 7422-7426. doi: AEM.01369-08 [pii]
- 10.1128/AEM.01369-08.
- Sonnleitner, E., Hagens, S., Rosenau, F., Wilhelm, S., Habel, A., Jager, K.E., et al. (2003). Reduced virulence of a hfq mutant of *Pseudomonas aeruginosa* O1. *Microp Pathog* 35(5), 217-228. doi: 10.1016/s0882-4010(03)00149-9.
- Stover, C.K., Pham, X.Q., Erwin, A.L., Mizoguchi, S.D., Warrener, P., Hickey, M.J., et al. (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* 406(6799), 959-964. doi: 10.1038/35023079.