

**Table S3. Bacterial strains and plasmids used in this study.**

Strain or plasmid	Relevant Genotype	Source
<i>E. coli</i> strain		
SM10 ( $\lambda$ pir)	<i>Thi, thr, leu, tonA, lacY, supE, recA,</i> RP4-2-Tc::Mu <i>lpir</i> Km <sup>r</sup> $\pi^+$	(1)
<i>V. cholerae</i> strains		
Fy_Vc_1915	C6706, St <sup>r</sup>	Ronald K. Taylor
Fy_Vc_10247	C6706 $\Delta$ <i>fliA</i> , St <sup>r</sup>	This work
Fy_Vc_11134	C6706 $\Delta$ <i>fliR</i> , St <sup>r</sup>	This work
Fy_Vc_10616	C6706 $\Delta$ <i>fliB</i> , St <sup>r</sup>	This work
Fy_Vc_11136	C6706 $\Delta$ <i>fliC</i> , St <sup>r</sup>	This work
Fy_Vc_11132	C6706 $\Delta$ <i>fliA</i> , St <sup>r</sup>	This work
Fy_Vc_10632	C6706 $\Delta$ <i>fliG</i> , St <sup>r</sup>	This work
Fy_Vc_10250	C6706 $\Delta$ <i>fliM</i> , St <sup>r</sup>	This work
Fy_Vc_10634	C6706 $\Delta$ <i>fliN</i> , St <sup>r</sup>	This work
Fy_Vc_10254	C6706 $\Delta$ <i>fliH</i> , St <sup>r</sup>	This work
Fy_Vc_11124	C6706 $\Delta$ <i>fliB</i> , St <sup>r</sup>	This work
Fy_Vc_11126	C6706 $\Delta$ <i>fliH</i> , St <sup>r</sup>	This work
Fy_Vc_11128	C6706 $\Delta$ <i>fliI</i> , St <sup>r</sup>	This work
Fy_Vc_11130	C6706 $\Delta$ <i>fliJ</i> , St <sup>r</sup>	This work
Fy_Vc_13823	C6706 $\Delta$ <i>fliE</i> , St <sup>r</sup>	This work
Fy_Vc_11142	C6706 $\Delta$ <i>fliD</i> , St <sup>r</sup>	This work
Fy_Vc_12821	C6706 $\Delta$ <i>pomA</i> , St <sup>r</sup>	This work
Fy_Vc_12825	C6706 $\Delta$ <i>pomB</i> , St <sup>r</sup>	This work
Fy_Vc_10256	C6706 $\Delta$ <i>motX</i> , St <sup>r</sup>	This work
Fy_Vc_13771	C6706 $\Delta$ <i>motY</i> , St <sup>r</sup>	This work
Fy_Vc_10247	C6706 $\Delta$ <i>fliA</i> , St <sup>r</sup>	This work
Fy_Vc_12827	C6706 $\Delta$ <i>fliA</i> $\Delta$ <i>pomA</i> , St <sup>r</sup>	This work

Fy_Vc_12831	C6706 $\Delta flaA \Delta pomB$ , St <sup>r</sup>	This work
Fy_Vc_10249	C6706 $\Delta flaA \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_13773	C6706 $\Delta flaA \Delta motY$ , St <sup>r</sup>	This work
Fy_Vc_13799	C6706:: <i>pomB</i> -D23E, St <sup>r</sup>	This work
Fy_Vc_13801	C6706:: <i>pomB</i> -D23N, St <sup>r</sup>	This work
Fy_Vc_13803	C6706 $\Delta flaA$ :: <i>pomB</i> -D23E, St <sup>r</sup>	This work
Fy_Vc_13805	C6706 $\Delta flaA$ :: <i>pomB</i> -D23N, St <sup>r</sup>	This work
Fy_Vc_14289	C6706 $\Delta nqrB$ , St <sup>r</sup>	This work
Fy_Vc_14291	C6706 $\Delta nqrC$ , St <sup>r</sup>	This work
Fy_Vc_13479	C6706 $\Delta flaA \Delta nqrB$ , St <sup>r</sup>	This work
Fy_Vc_13481	C6706 $\Delta flaA \Delta nqrC$ , St <sup>r</sup>	This work
Fy_Vc_14287	C6706 $\Delta sssA$ , St <sup>r</sup>	This work
Fy_Vc_13785	C6706 $\Delta flaA \Delta sssA$ , St <sup>r</sup>	This work
Fy_Vc_15289	C6706 $\Delta mshA$ , St <sup>r</sup>	Yildiz lab collection
Fy_Vc_11157	C6706 $\Delta flrA \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_13809	C6706 $\Delta flrB \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_11159	C6706 $\Delta flrC \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_13807	C6706 $\Delta fliA \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_11153	C6706 $\Delta fliG \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_10261	C6706 $\Delta fliM \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_11155	C6706 $\Delta fliN \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_10638	C6706 $\Delta flhA \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_11168	C6706 $\Delta flhB \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_11170	C6706 $\Delta fliH \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_11172	C6706 $\Delta flil \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_11174	C6706 $\Delta fliJ \Delta motX$ , St <sup>r</sup>	This work
Fy_Vc_13827	C6706 $\Delta flgE \Delta motX$ , St <sup>r</sup>	This work
FY_Vc_11190	C6706 $\Delta fliD \Delta motX$ , St <sup>r</sup>	This work
FY_Vc_10252	C6706 $\Delta rocS$ , St <sup>r</sup>	This work
FY_Vc_13457	C6706 $\Delta flaA \Delta rocS$ , St <sup>r</sup>	This work

Fy_Vc_12833	C6706 $\Delta flaA \Delta VC1029$ ( <i>cdgB</i> ) , St <sup>r</sup>	This work
Fy_Vc_11151	C6706 $\Delta flaA \Delta VC1067$ ( <i>cdgH</i> ) , St <sup>r</sup>	This work
Fy_Vc_11149	C6706 $\Delta flaA \Delta VC1104$ ( <i>cdgK</i> ) , St <sup>r</sup>	This work
Fy_Vc_12835	C6706 $\Delta flaA \Delta VC1185$ , St <sup>r</sup>	This work
Fy_Vc_12837	C6706 $\Delta flaA \Delta VC1216$ , St <sup>r</sup>	This work
Fy_Vc_12840	C6706 $\Delta flaA \Delta VC1353$ , St <sup>r</sup>	This work
Fy_Vc_12843	C6706 $\Delta flaA \Delta VC1370$ , St <sup>r</sup>	This work
Fy_Vc_12845	C6706 $\Delta flaA \Delta VC1372$ , St <sup>r</sup>	This work
Fy_Vc_10659	C6706 $\Delta flaA \Delta VC1376$ ( <i>cdgM</i> ) , St <sup>r</sup>	This work
Fy_Vc_12847	C6706 $\Delta flaA \Delta VC1593$ , St <sup>r</sup>	This work
Fy_Vc_13487	C6706 $\Delta flaA \Delta VC1599$ , St <sup>r</sup>	This work
Fy_Vc_12849	C6706 $\Delta flaA \Delta VC2224$ , St <sup>r</sup>	This work
Fy_Vc_10657	C6706 $\Delta flaA \Delta VC2285$ ( <i>cdgL</i> ) , St <sup>r</sup>	This work
Fy_Vc_12851	C6706 $\Delta flaA \Delta VC2370$ , St <sup>r</sup>	This work
Fy_Vc_12853	C6706 $\Delta flaA \Delta VC2454$ ( <i>vpvC</i> ) , St <sup>r</sup>	This work
Fy_Vc_12855	C6706 $\Delta flaA \Delta VC2697$ , St <sup>r</sup>	This work
Fy_Vc_12209	C6706 $\Delta flaA \Delta VCA0050-49$ ( <i>cdgO</i> ) , St <sup>r</sup>	This work
Fy_Vc_10655	C6706 $\Delta flaA \Delta VCA0074$ ( <i>cdgA</i> ) , St <sup>r</sup>	This work
Fy_Vc_12857	C6706 $\Delta flaA \Delta VCA0165$ , St <sup>r</sup>	This work
Fy_Vc_12859	C6706 $\Delta flaA \Delta VCA0217$ , St <sup>r</sup>	This work
Fy_Vc_12861	C6706 $\Delta flaA \Delta VCA0557$ , St <sup>r</sup>	This work
Fy_Vc_12863	C6706 $\Delta flaA \Delta VCA0560$ , St <sup>r</sup>	This work
Fy_Vc_12865	C6706 $\Delta flaA \Delta VCA0697$ ( <i>cdgD</i> ) , St <sup>r</sup>	This work
Fy_Vc_12867	C6706 $\Delta flaA \Delta VCA0848$ , St <sup>r</sup>	This work
Fy_Vc_12869	C6706 $\Delta flaA \Delta VCA0939$ ( <i>cdgP</i> ) , St <sup>r</sup>	This work
Fy_Vc_13119	C6706 $\Delta flaA \Delta VCA0956$ ( <i>cdgF</i> ) , St <sup>r</sup>	This work
Fy_Vc_12871	C6706 $\Delta flaA \Delta VCA0960$ , St <sup>r</sup>	This work
Fy_Vc_13115	C6706 $\Delta flaA \Delta VCA0965$ , St <sup>r</sup>	This work
Fy_Vc_10628	C6706 $\Delta cdgA$ , St <sup>r</sup>	This work
Fy_Vc_10624	C6706 $\Delta cdgL$ , St <sup>r</sup>	This work

Fy_Vc_12209	C6706 $\Delta$ cdgO, St <sup>r</sup>	This work
Fy_Vc_14259	C6706 $\Delta$ cdgLO, St <sup>r</sup>	This work
Fy_Vc_13467	C6706 $\Delta$ cdgALO, St <sup>r</sup>	This work
Fy_Vc_13751	C6706 $\Delta$ fliA $\Delta$ cdgALO, St <sup>r</sup>	This work
Fy_Vc_14261	C6706 $\Delta$ fliA $\Delta$ cdgLO	This work
Fy_Vc_14216	C6706 $\Delta$ fliR $\Delta$ cdgALO, St <sup>r</sup>	This work
Fy_Vc_14218	C6706 $\Delta$ fliC $\Delta$ cdgALO, St <sup>r</sup>	This work
Fy_Vc_14220	C6706 $\Delta$ fliA $\Delta$ cdgALO, St <sup>r</sup>	This work
Fy_Vc_14222	C6706 $\Delta$ fliG $\Delta$ cdgALO, St <sup>r</sup>	This work
Fy_Vc_14224	C6706 $\Delta$ fliH $\Delta$ cdgALO, St <sup>r</sup>	This work
Fy_Vc_14226	C6706 $\Delta$ fliH $\Delta$ cdgALO, St <sup>r</sup>	This work
Fy_Vc_14043	C6706:: <i>hubP</i> -sfGFP, St <sup>r</sup>	This work
Fy_Vc_14047	C6706:: <i>cdgA</i> -sfGFP, St <sup>r</sup>	This work
Fy_Vc_14071	C6706:: <i>cdgL</i> -sfGFP, St <sup>r</sup>	This work
Fy_Vc_14049	C6706:: <i>cdgO</i> -sfGFP, St <sup>r</sup>	This work
Fy_Vc_10640	C6706 $\Delta$ fliA $\Delta$ vpsR, St <sup>r</sup>	
Fy_Vc_15545	C6706 $\Delta$ fliA $\Delta$ vpsR:: <i>vpsR</i> -D59E, St <sup>r</sup>	
Fy_Vc_15645	C6706 $\Delta$ fliA $\Delta$ vpsR:: <i>vpsR</i> -D59A, St <sup>r</sup>	
Fy_Vc_14039	C6706 $\Delta$ fliA $\Delta$ motX $\Delta$ vpsR, St <sup>r</sup>	This work
Fy_Vc_14067	C6706 $\Delta$ fliA $\Delta$ motX $\Delta$ vpsR:: <i>vpsR</i> -D59A, St <sup>r</sup>	This work
Fy_Vc_14069	C6706 $\Delta$ fliA $\Delta$ motX $\Delta$ vpsR:: <i>vpsR</i> -D59E, St <sup>r</sup>	This work
Fy_Vc_13829	C6706 $\Delta$ fliG, St <sup>r</sup>	This work
Fy_Vc_11138	C6706 $\Delta$ hapR, St <sup>r</sup>	This work
Fy_Vc_12697	C6706 $\Delta$ fliA $\Delta$ hapR	This work
Fy_Vc_13453	C6706 $\Delta$ fliA $\Delta$ motX $\Delta$ hapR	This work
Plasmids		

pFY_23 (pGP704sac28)	pGP704 derivative; mob-oriT, sacB, Ap <sup>r</sup>	G. Schoolnik
pFY_4394	pGP704sac28- <i>flaA</i> , Ap <sup>r</sup>	This work
pFY_4393	pGP704sac28- <i>flrA</i> , Ap <sup>r</sup>	This work
pFY_4298	pGP704sac28- <i>flrB</i> , Ap <sup>r</sup>	This work
pFY_4392	pGP704sac28- <i>flrC</i> , Ap <sup>r</sup>	This work
pFY_4391	pGP704sac28- <i>fliA</i> , Ap <sup>r</sup>	This work
pFY_4288	pGP704sac28- <i>fliG</i> , Ap <sup>r</sup>	This work
pFY_4396	pGP704sac28- <i>fliM</i> , Ap <sup>r</sup>	This work
pFY_4287	pGP704sac28- <i>fliN</i> , Ap <sup>r</sup>	This work
pFY_4305	pGP704sac28- <i>flhA</i> , Ap <sup>r</sup>	This work
pFY_4397	pGP704sac28- <i>flhB</i> , Ap <sup>r</sup>	This work
pFY_4399	pGP704sac28- <i>fliH</i> , Ap <sup>r</sup>	This work
pFY_4401	pGP704sac28- <i>fliI</i> , Ap <sup>r</sup>	This work
pFY_4403	pGP704sac28- <i>fliJ</i> , Ap <sup>r</sup>	This work
pFY_5915	pGP704sac28- <i>flgE</i> , Ap <sup>r</sup>	This work
pFY_4408	pGP704sac28- <i>fliD</i> , Ap <sup>r</sup>	This work
pFY_5309	pGP704sac28- <i>pomA</i> , Ap <sup>r</sup>	This work
pFY_5310	pGP704sac28- <i>pomB</i> , Ap <sup>r</sup>	This work
pFY_5883	pGP704sac28- <i>motX</i> , Ap <sup>r</sup>	This work
pFY_5550	pGP704sac28- <i>motY</i> , Ap <sup>r</sup>	This work
pFY_5778	pGP704sac28- <i>pomB</i> -D23E, Ap <sup>r</sup>	This work
pFY_5779	pGP704sac28- <i>pomB</i> -D23N, Ap <sup>r</sup>	This work
pFY_5574	pGP704sac28-VC2294 ( <i>nqrB</i> ), Ap <sup>r</sup>	This work
pFY_5573	pGP704sac28-VC2293 ( <i>nqrC</i> ), Ap <sup>r</sup>	This work
pFY_5869	pGP704sac28- <i>sssA</i>	This work
pFY_5868	pGP704sac28-VC0303	This work
pFY_4405	pGP704sac28- <i>rocS</i> , Ap <sup>r</sup>	(2)

pFY_4805	pGP704sac28-VC1029 ( <i>cdgB</i> ), Ap <sup>r</sup>	(2)
pFY_4300	pGP704sac28- <i>cdgH</i> , Ap <sup>r</sup>	(3)
pFY_4301	pGP704sac28- <i>cdgK</i> , Ap <sup>r</sup>	(3,4)
pFY_4804	pGP704sac28-VC1185, Ap <sup>r</sup>	(3,4)
pFY_4802	pGP704sac28-VC1216, Ap <sup>r</sup>	(3,4)
pFY_4810	pGP704sac28-VC1353, Ap <sup>r</sup>	(3,4)
pFY_4806	pGP704sac28-VC1370, Ap <sup>r</sup>	(3,4)
pFY_4803	pGP704sac28-VC1372, Ap <sup>r</sup>	(3,4)
pFY_4303	pGP704sac28- <i>cdgM</i>	(3,4)
pFY_4811	pGP704sac28-VC1593, Ap <sup>r</sup>	(3,4)
pFY_4812	pGP704sac28-VC1599, Ap <sup>r</sup>	(3,4)
pFY_4813	pGP704sac28-VC2224, Ap <sup>r</sup>	(3,4)
pFY_4302	pGP704sac28- <i>cdgL</i> , Ap <sup>r</sup>	(3,4)
pFY_4814	pGP704sac28-VC2370, , Ap <sup>r</sup>	(3,4)
pFY_4807	pGP704sac28-VC2454, , Ap <sup>r</sup>	(3,4)
pFY_4815	pGP704sac28-VC2697, Ap <sup>r</sup>	(3,4)
pFY_4734	pGP704sac28-VCA0050-49, Ap <sup>r</sup>	This work
pFY_4299	pGP704sac28- <i>cdgA</i> , Ap <sup>r</sup>	(2)
pFY_4817	pGP704sac28-VCA0165, Ap <sup>r</sup>	(3,4)
pFY_4808	pGP704sac28-VCA0217, Ap <sup>r</sup>	(3,4)
pFY_4818	pGP704sac28-VCA0557, Ap <sup>r</sup>	(3,4)
pFY_4819	pGP704sac28-VCA0560, Ap <sup>r</sup>	(3,4)
pFY_4820	pGP704sac28-VCA0697 ( <i>cdgD</i> ), Ap <sup>r</sup>	(2)
pFY_4821	pGP704sac28-VCA0848, Ap <sup>r</sup>	(3,4)
pFY_4822	pGP704sac28-VCA0939, Ap <sup>r</sup>	(3,4)
pFY_4823	pGP704sac28-VCA0956, Ap <sup>r</sup>	(3,4)
pFY_4809	pGP704sac28-VCA0960, Ap <sup>r</sup>	(3,4)
pFY_5546	pGP704sac28-VCA0965, Ap <sup>r</sup>	(3,4)
pFY_5676	pGP704sac28- <i>vipA-sfGFP</i> , Ap <sup>r</sup>	Yildiz lab collection
pFY_5790	pGP704sac28- <i>hubP-sfGFP</i> , Ap <sup>r</sup>	This work

pFY_5792	pGP704sac28-cdgA-sfGFP, Ap <sup>r</sup>	This work
pFY_5799	pGP704sac28-cdgL-sfGFP, Ap <sup>r</sup>	This work
pFY_5793	pGP704sac28-cdgO-sfGFP, Ap <sup>r</sup>	This work
pFY_4395	pGP704sac28-hapR, Ap <sup>r</sup>	(3)
pFY_4390	pGP704sac28-vpsR, Ap <sup>r</sup>	(5)
pFY_5797	pGP704sac28-vpsR-D59A, Ap <sup>r</sup>	This work
pFY_5798	pGP704sac28-vpsR-D59E, Ap <sup>r</sup>	This work
pFY_5752	Plasmid expressing GST- <i>fliI</i>	This work
pFY_691 (pBBRlux)	<i>luxCDABE</i> -based promoter fusion vector; Cm <sup>r</sup>	(6)
pFY_4290	pBBRlux-P <i>vpsL</i> ; Cm <sup>r</sup>	This work
pFY_1122	pBBRlux-P <i>flaA</i> ; Cm <sup>r</sup>	(7)
pFY_6409	pBBRlux-P <i>motX</i> ; Cm <sup>r</sup>	This work
pFY_1303 (pMMB67EH)	Expression vector harboring <i>lacI</i> and containing a Ptac promoter, Ap <sup>r</sup> .	Yildiz lab collection
pFY_4289	C-di-GMP biosensor cloned in pMMB67EH, Ap <sup>r</sup> .	This work
pFY_4535	C-di-GMP biosensor containing the <i>hok/sok</i> region from pXB300, Gm <sup>r</sup>	(7)

## References

1. Simon R, Priefer U, Pühler A. A Broad Host Range Mobilization System for In Vivo Genetic Engineering: Transposon Mutagenesis in Gram Negative Bacteria. *Bio/Technology* [Internet]. 1983 Nov 1 [cited 2017 Jul 5];1(9):784–91. Available from: <http://www.nature.com/doi/10.1038/nbt1183-784>
2. Lim B, Beyhan S, Meir J, Yildiz FH. Cyclic-diGMP signal transduction systems in *Vibrio cholerae*: modulation of rugosity and biofilm formation. *Mol Microbiol* [Internet]. 2006 Apr [cited 2016 Sep 25];60(2):331–48. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16573684>
3. Beyhan S, Odell LS, Yildiz FH. Identification and characterization of cyclic diguanylate signaling systems controlling rugosity in *Vibrio cholerae*. *J Bacteriol* [Internet]. 2008 Nov [cited 2016 Sep 25];190(22):7392–405. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18790873>
4. Liu X, Beyhan S, Lim B, Linington RG, Yildiz FH. Identification and characterization of a phosphodiesterase that inversely regulates motility and biofilm formation in *Vibrio cholerae*. *J Bacteriol* [Internet]. 2010 Sep [cited 2016 Sep 25];192(18):4541–52. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20622061>
5. Yildiz FH, Dolganov NA, Schoolnik GK. VpsR , a Member of the Response Regulators of the Two-Component Regulatory Systems , Is Required for Expression of vps Biosynthesis Genes and EPS ETr -Associated Phenotypes in *Vibrio cholerae* O1 El Tor. *J Bacteriol*. 2001 Mar;183(5):1716–26.
6. Lenz DH, Mok KC, Lilley BN, Kulkarni R V, Wingreen NS, Bassler BL. The Small RNA Chaperone Hfq and Multiple Small RNAs Control Quorum Sensing in *Vibrio harveyi* and *Vibrio cholerae*. *Cell* [Internet]. 2004 Jul 9 [cited 2017 Mar 2];118(1):69–82. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15242645>
7. Zamorano-Sánchez D, Xian W, Lee CK, Salinas M, Thongsomboon W, Cegelski L, et al. Functional Specialization in *Vibrio cholerae* Diguanylate Cyclases: Distinct Modes of Motility Suppression and c-di-GMP Production. Peter Greenberg E, editor. *MBio* [Internet]. 2019 Apr 23 [cited 2019 Jul 7];10(2). Available from: <http://www.ncbi.nlm.nih.gov/pubmed/31015332>