CqsA-CqsS quorum-sensing signal-receptor specificity in *Photobacterium angustum*

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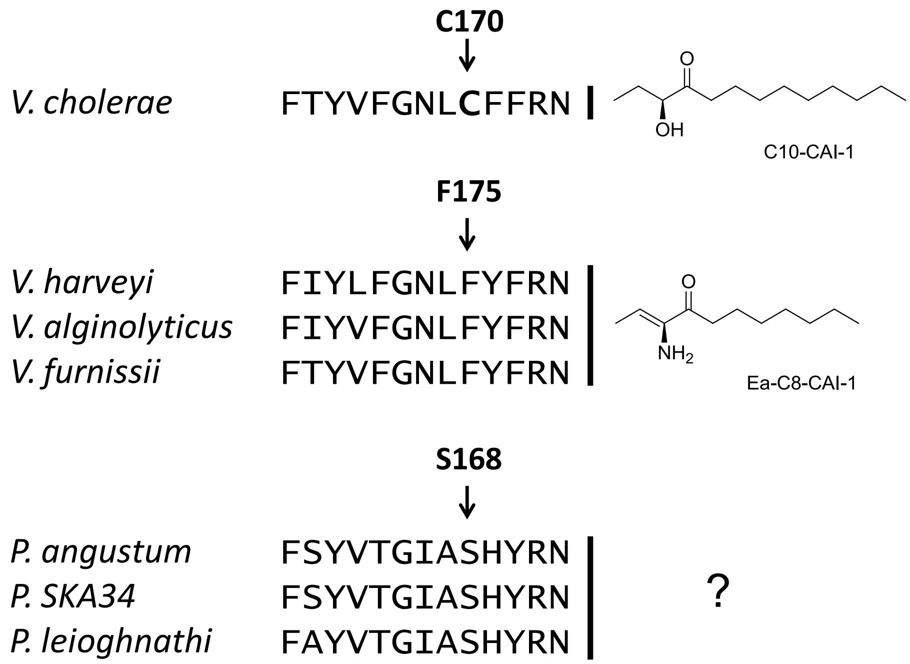

Fig. S1. Alignment of vibrio and photobacterial CqsS homologs. Comparison of the sixth TM helices of CqsS receptors. Cys^{170} and Phe^{175} are the crucial moieties for ligand detection specificity in $CqsS_{Vc}$ and $CqsS_{Vh}$, respectively. Ser^{168} is located at the corresponding position in $CqsS_{Pa}$.

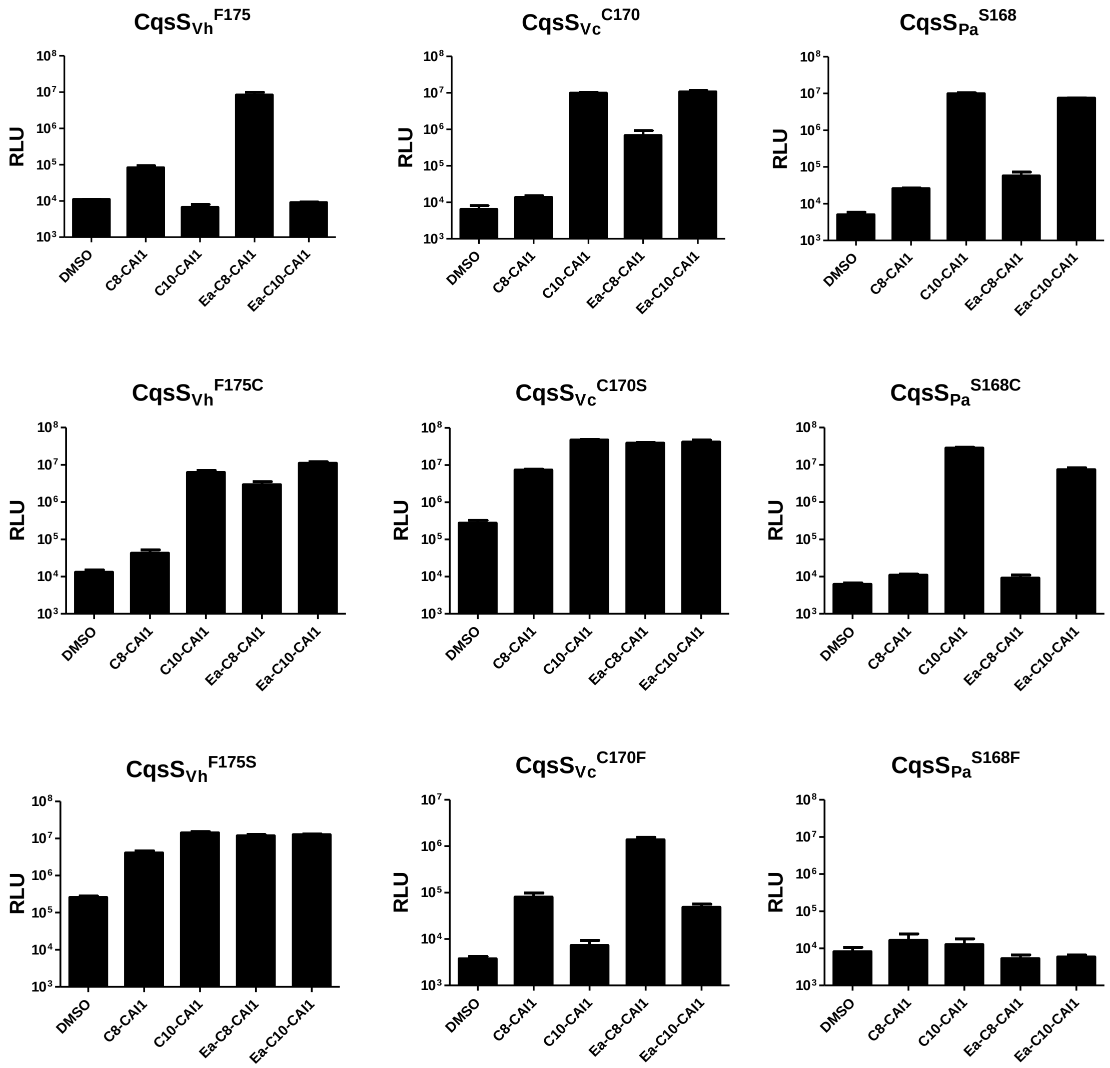

Fig. S2. CqsS ligand specificities. Synthetic CAI-1 compounds were added at 5 μM to a *V. harveyi* reporter strain expressing $CqsS_{Vh}$, $CqsS_{Vc}$, $CqsS_{Pa}$ or the designated CqsS mutants. Light production was measured. Error bars represent standard deviations for three replicates.

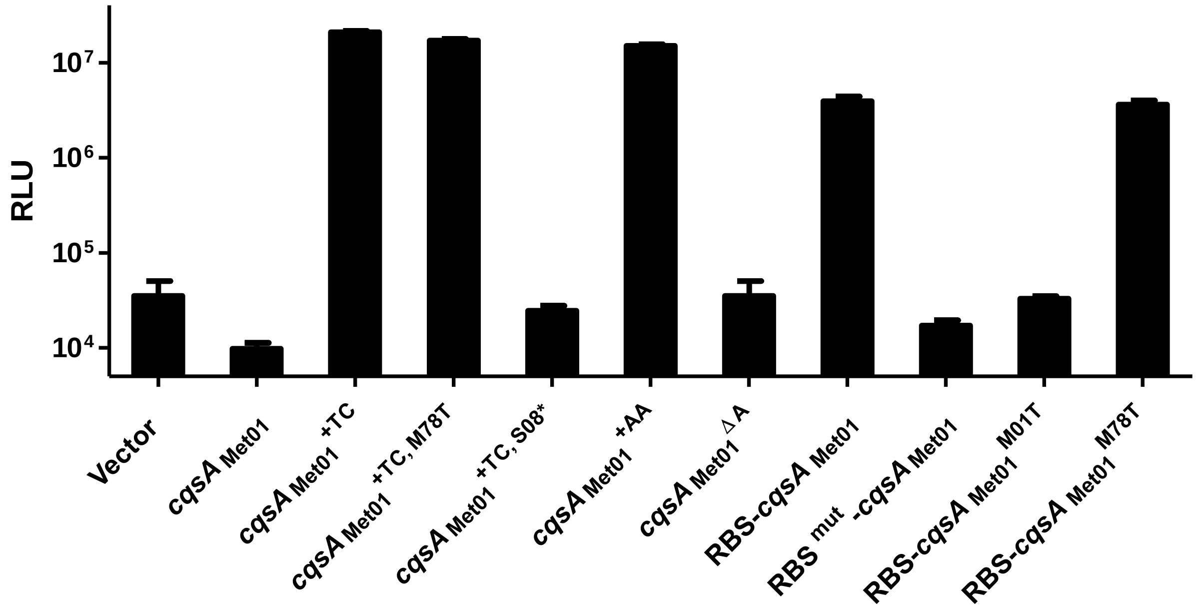

Fig. S3. CAI-1 activity depends on a correct *cqsA*$_{Pa}$ reading frame. *E. coli* harboring the designated *P. angustum cqsA* constructs were assayed for CAI-1 activity using the CqsS$_{Pa}$ bioluminescence reporter. Cell-free culture fluids were added at 1% v/v. Error bars represent standard deviations for three replicates.

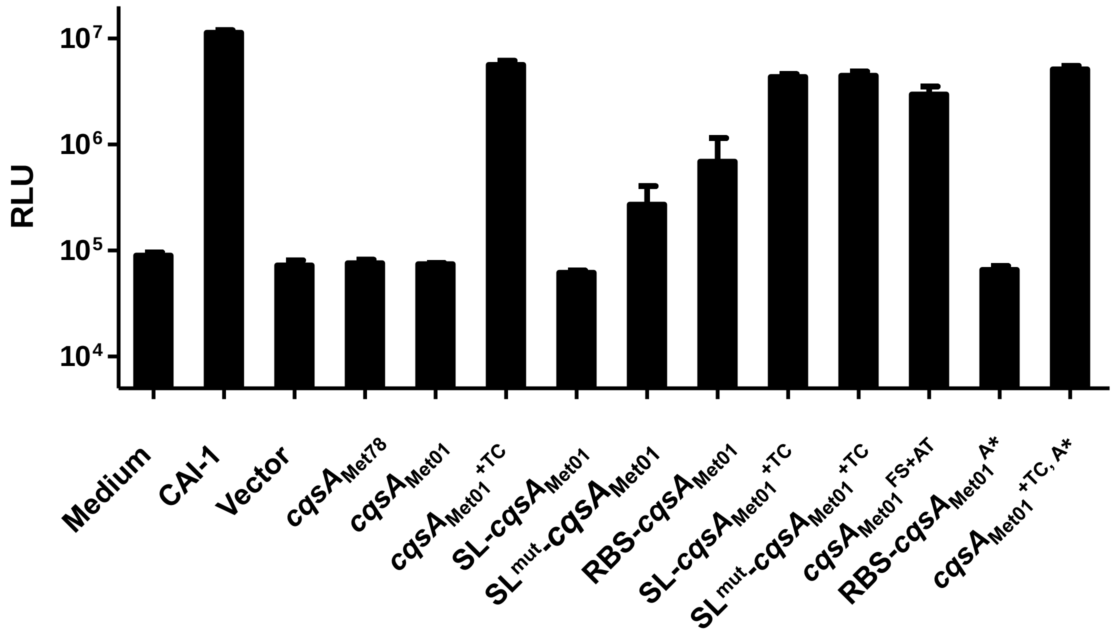

Fig. S4. Analysis of CAI-1 moieties produced by the *cqsA*$_{Pa}$ construct. *E. coli* harboring the *P. angustum cqsA* constructs in Fig. 5A were assayed for CAI-1 activity using the CqsS$_{Vc}$ bioluminescent reporter strain. Cell-free culture fluids were added at 5% v/v. Error bars represent standard deviations for three replicates.

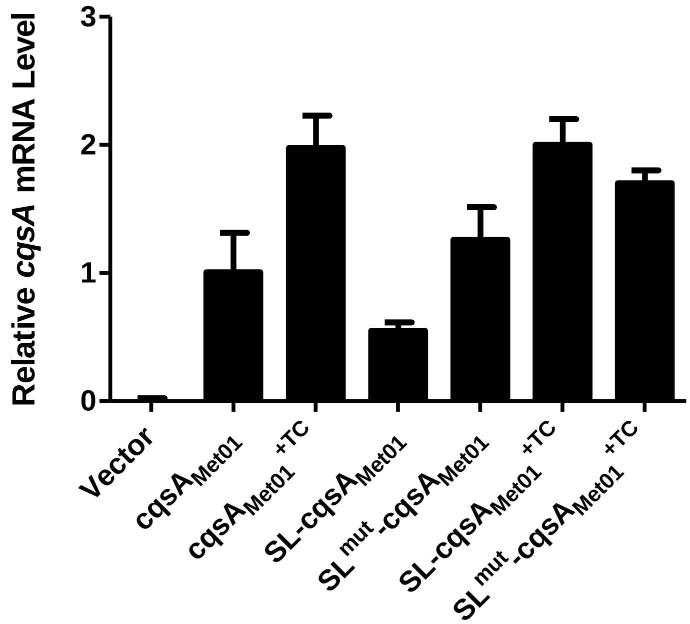

Fig S5. *cqsA* transcript levels from various *cqsA*$_{Pa}$ constructs. *E. coli* harboring the *P. angustum cqsA* constructs in Fig. 5A were grown as described for the bioluminescence assay. Cells were harvested at OD_{600} = 1.0. Total RNA was extracted using the Qiagen RNeasy Mini Kit, and *cqsA* levels were measured by qRT-PCR. The 5S rRNA transcript was used as the internal control and all *cqsA* levels were normalized to the transcript level of the *cqsA*$_{Met01}$ construct. Error bars represent standard deviations for four replicates.

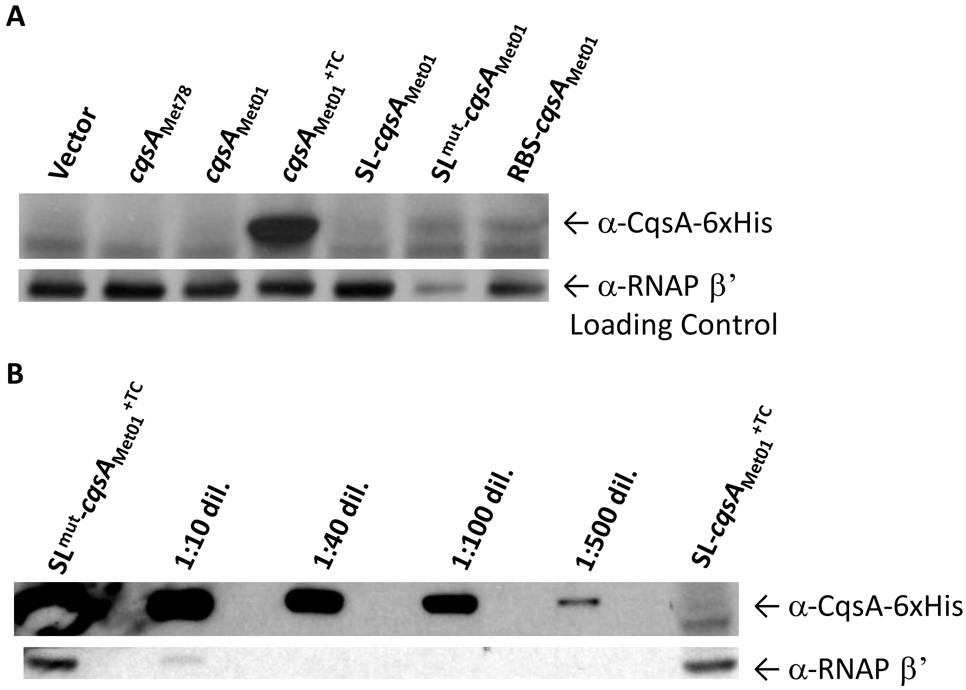

Fig. S6. Disruption of the $cqsA_{Pa}$ 5'UTR stem-loop activates protein production. A) Western blot analysis of 6x-His tagged CqsA protein detected in *E. coli* strains carrying the specified $cqsA_{Pa}$ constructs. **B)** CqsA protein detected from diluted lysates prepared from the *E. coli* strain carrying the SL^{mut}-$cqsA_{Met01}{}^{+TC}$ construct.

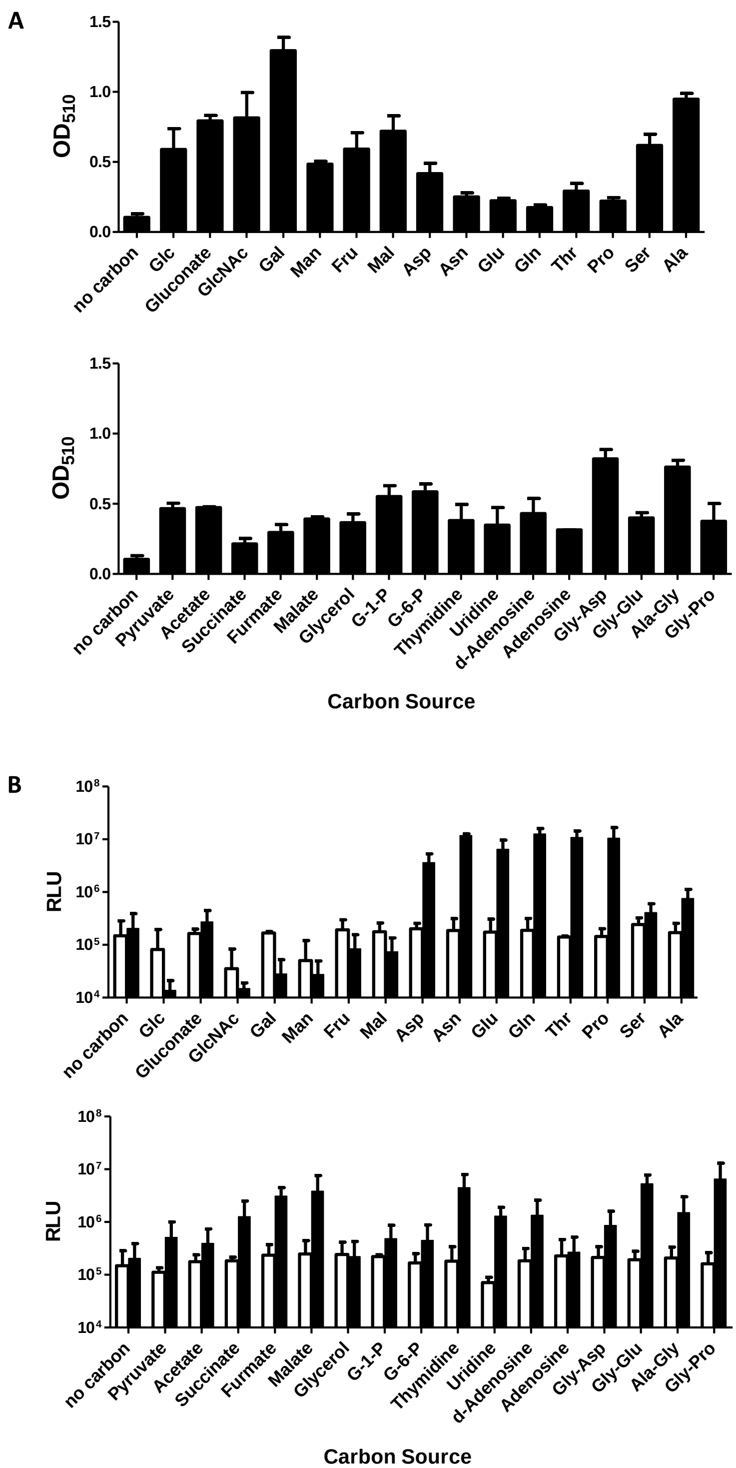

Fig. S7. *P. angustum* growth and CAI-1 activity on different carbon sources. A) *P. angustum* growth on various carbon sources was measured by absorbance (OD_{510}). **B)** CAI-1 activity in *P. angustum* culture fluids following growth on the carbon sources from panel **A** was assayed using the bioluminescent reporter strain carrying *cqsS*$_{Pa}$. Media controls (white) and *P. angustum* culture fluids (black) were added at 50% v/v. Error bars represent standard deviations for two replicates.

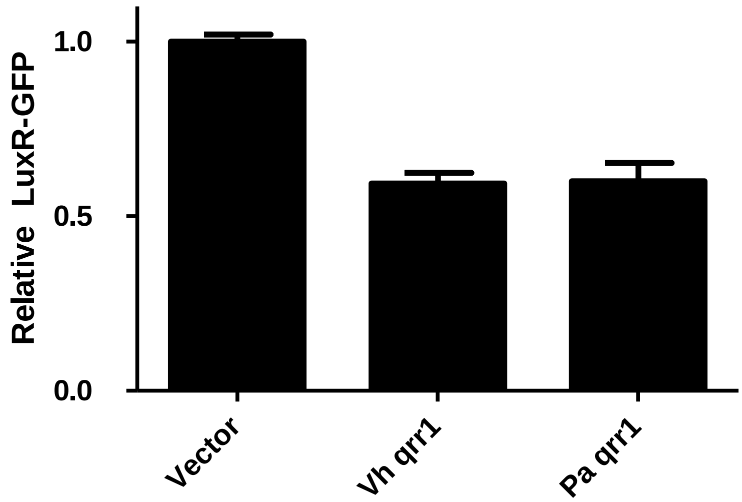

Fig. S8. *P. angustum qrr*1 represses *luxR* translation. *E. coli* carrying a LuxR-GFP fusion (pYS141) were transformed with constructs carrying pRHA109 (vector), *V. harveyi* (Vh) *qrr*1, or *P. angustum* (Pa) *qrr*1. *luxR* and *qrr*1 expression were induced by 10 μM IPTG and 10 mM L-Rhamnose, respectively. GFP levels were measured using an Envison Multilabel Reader following 16 h incubation at 37°C. GFP levels were normalized to the vector control. Error bars represent standard deviations for three replicates.

Table S1. Strains and plasmids

Plasmid or Strain	Relevant genotype or feature	Reference
Plasmid		
pLAFR2	Broad-host-range cosmid; mob, Tet^R	(Friedman *et al.*, 1982)
pJMH282	*V. harveyi cqsS* on pLAFR2, Tet^R	(Henke and Bassler, 2004)
pXKE220	*V. harveyi* $cqsS^{F175C}$ on pLAFR2, Tet^R	This study
pXKE165	*V. harveyi* $cqsS^{F175S}$ on pLAFR2, Tet^R	This study
pXKE071	*V. cholerae cqsS* on pLAFR2, Tet^R	This study
pXKE167	*V. cholerae* $cqsS^{C170S}$ on pLAFR2, Tet^R	This study
pXKE222	*V. cholerae* $cqsS^{C170F}$ on pLAFR2, Tet^R	This study
pXKE554	*P. angustum* S14 *cqsS* on pLAFR2, Tet^R	This study
pWN1671	*P. angustum* B70 *cqsS* on pLAFR2, Tet^R	This study
pXKE166	*P. angustum* B70 $cqsS^{S168C}$ on pLAFR2, Tet^R	This study
pXKE168	*P. angustum* B70 $cqsS^{S168F}$ on pLAFR2, Tet^R	This study
pEVS143	P15A *oriV*, $lacI^q$, P_{tac} expression cassette, Kan^R	(Bose *et al.*, 2008)
pXKE1054	*P. angustum* $cqsA_{Met01}$ on pEVS143, untagged, Kan^R	This study
pXKE903	*P. angustum* $cqsA_{Met01}^{+TC}$ on pEVS143, untagged, Kan^R	This study
pXKE909	*P. angustum* $cqsA_{Met01}^{+TC, S08*}$ on pEVS143, untagged, Kan^R	This study
pXKE907	*P. angustum* $cqsA_{Met01}^{+AA}$ on pEVS143, untagged, Kan^R	This study
pXKE1055	*P. angustum* RBS-$cqsA_{Met01}$ on pEVS143, untagged, Kan^R	This study
pXKE1325	*P. angustum* RBS^{mut}-$cqsA_{Met01}$ on pEVS143, untagged, Kan^R	This study
pXKE933	*P. angustum* RBS-$cqsA_{Met01}^{M01T}$ on pEVS143, untagged, Kan^R	This study
pXKE919	*P. angustum* RBS-$cqsA_{Met01}^{M78T}$ on pEVS143, untagged, Kan^R	This study
pXKE1360	*P. angustum* $cqsA_{Met78}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1128	*P. angustum* $cqsA_{Met01}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1331	*P. angustum* $cqsA_{Met01}^{+TC}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study

pXKE1459	*P. angustum cqsA*$_{Met01}$$^{+TC, M78T}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1334	*P. angustum* SL-*cqsA*$_{Met01}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1336	*P. angustum* SL^{mut}-*cqsA*$_{Met01}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1130	*P. angustum* RBS-*cqsA*$_{Met01}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1343	*P. angustum* SL-*cqsA*$_{Met01}$$^{+TC}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1345	*P. angustum* SL^{mut}-*cqsA*$_{Met01}$$^{+TC}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1132	*P. angustum cqsA*$_{Met01}$$^{FS+AT}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1366	*P. angustum* RBS-*cqsA*$_{Met01}$^{A*} on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
pXKE1368	*P. angustum cqsA*$_{Met01}$$^{+TC, A*}$ on pEVS143, C-terminal 6xHis-tagged, Kan^R	This study
P. angustum		
S14	Wildtype, surface water isolate (1 m, Botany Bay, Sydney Australia)	(Humphrey et al.</i>, 1983)
B70	Wildtype, seawater isolate (7.5 m, latitude 20°30', longitude 157°30')	(Baumann *et al.*, 1971; Reichelt *et al.*, 1976)
V. cholerae		
C6706str	Wild type	(Thelin and Taylor, 1996)
V. harveyi		
BB120	Wild type	(Bassler *et al.*, 1997)
JMH603	Δ*cqsA*	(Henke and Bassler, 2004)
WN1397	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ*	(Ng *et al.*, 2011)
WN1490	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pLAFR2 (Vector)	(Ng *et al.*, 2011)
WN1492	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pJMH282 (*cqsS*$_{Vh}$)	(Ng *et al.*, 2011)
XKE226	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pXKE220 (*cqsS*$_{Vh}$^{F175C})	This study
XKE169	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pXKE165 (*cqsS*$_{Vh}$^{F175S})	This study
XKE083	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pXKE071 (*cqsS*$_{Vc}$)	This study
XKE175	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pXKE167 (*cqsS*$_{Vc}$^{C170S})	This study

XKE228	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pXKE222 (*cqsS*$_{Vc}$^{C170F})	This study
XKE557	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pXKE554 (*cqsS*$_{Pa\ S14}$)	This study
WN1674	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pWN1671 (*cqsS*$_{Pa\ B70}$)	This study
pXKE173	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pXKE166 (*cqsS*$_{Pa\ B70}$^{S168C})	This study
pXKE177	Δ*cqsAS::Cm*^{r} , Δ*luxN*, Δ*luxPQ* / pXKE168 (*cqsS*$_{Pa\ B70}$^{S168F})	This study

Supplemental References

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