Supplemental Information

Functional determinants of the quorum-sensing non-coding RNAs and their roles in target regulation

Yi Shao[1*], Lihui Feng[1*], Steven T. Rutherford[1], Kai Papenfort[1], Bonnie L. Bassler[1, 2**]

[1]Department of Molecular Biology, Princeton University, Princeton, NJ, USA

[2]Howard Hughes Medical Institute, Chevy Chase, MD, USA

This supplement contains:

Figures S1 to S9

Tables S1 to S4

Supplemental References

Figure S1 Gene expression changes in *qrr*1-5, *aphA*, and *luxR* following induction of each *qrr* gene. Qrr1-4 sRNA, *aphA* mRNA, and *luxR* mRNA following a pulse of induction of each *qrr* gene was measured by qRT-PCR. White: without arabinose, Black: with 0.2% arabinose. Qrr5 sRNA was measured by northern blot because qRT-PCR priming was unsuccessful for Qrr5. Qrr2 is shown as a control for the northern analysis. 5S rRNA was used as the loading control.

Figure S2 Schematic of plasmid-borne translational *gfp* fusions. Horizontal lines denote 5' UTRs; black bars indicate the target mRNA coding sequences present in the fusions; gray bars indicate the *gfp* coding sequence.

Figure S3 Predicted base-pairing between Qrr4 and its direct targets. The first forty-eight nucleotides of Qrr4 are shown from 5' to 3'. Sequences of the mRNA targets are shown from 3' to 5'. Nucleotides predicted to be involved in base-pairing are shown in red. Translational start sites are denoted as +1.

Figure S4 Regulation of mRNA targets by Qrr1-5. qRT-PCR was performed following induction of each q*rr* gene. White: without arabinose, Black: with 0.2% arabinose.

Figure S5 Qrr4 represses *vibhar_00505* through base-pairing. Fluorescence from *E. coli* carrying a plasmid with an IPTG inducible translational GFP fusion to *vibhar_00505* (pLF804) was measured in the presence of an empty vector (pLF253), a vector with wild-type Qrr4 (pLF127), a vector with Qrr4mut1 (pYS258), or a vector with Qrr4mut4 (pYS221). Mutations are highlighted by overlines. Means and SEMs of triplicate samples are shown. Base-pairing between *vibhar_00505* and Qrr4 was predicted by RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/). Nucleotides involved in base-pairing are shown in red. The translational start site is denoted as +1.

Figure S6 Regulation of sixteen Qrr sRNA targets by Qrr1, Qrr4, and Qrr4 mutants. Fluorescence from plasmid-encoded *V. harveyi* target-GFP translational fusions was measured in *E. coli*

carrying an empty vector (pLF253), a vector expressing a tetracycline inducible *qrr*1 (pLF396), *qrr*4 (pLF127), *qrr*4 stem-loop 1 deletion (ΔSL1, pYS225), *qrr*4 stem-loop 2 deletion (ΔSL2, pYS226), *qrr*4 stem-loop 3 deletion (ΔSL3, pYS227), *qrr*4 stem-loop 1 and stem-loop 3 double deletion (ΔSL1&3, pYS229), *qrr*4 stem-loop 1 inversion (SL1*, pYS230), *qrr*4 stem-loop 2 inversion (SL2*, pYS231), or *qrr*4 stem-loop 3 inversion (SL3*, pYS232). GFP from three independent cultures was measured for each strain. Means and SEMs are shown, with all measurements normalized to the mean of the vector controls.

Figure S7 Regulatory functions of Qrr4 chimeras. Fluorescence from plasmid-encoded *V. harveyi* AphA-GFP (pLF255) and LuxR-GFP (pLF128) translational fusions was measured in *E. coli* carrying an empty vector (pLF253), a vector expressing tetracycline inducible *qrr*4 (pLF127), WT chimeric *qrr*4 (WT-MicA/WT-OmrB, pYS281/pYS282), stem-loop 1 deletion chimeric *qrr*4 (ΔSL1-MicA/ΔSL1-OmrB, pYS278/pYS279), stem-loop 2 deletion chimeric *qrr*4 (ΔSL2-MicA/ΔSL2-OmrB, pYS275/pYS276), stem-loop 3 deletion chimeric *qrr*4 (ΔSL3-MicA/ΔSL3-OmrB, pYS272/pYS273), or the double stem-loop 1 and stem-loop 3 double deletion chimeric *qrr*4 (ΔSL1&3-MicA/ΔSL1&3-MicA, pYS269/pYS270). GFP from three independent cultures was measured for each strain and the means and SEMs are shown. All measurements were normalized to the mean of the vector controls.

Figure S8 SL1 confers stability to Qrr1. Half-lives of plasmid-encoded *V. harveyi* WT Qrr1 (pLF396), the SL1 disrupted Qrr1 mutant (Qrr1 SL1D, pYS299), and the SL1 restored Qrr1 mutant (Qrr1 SL1R, pYS300) were measured in *E. coli* by northern blot. Northern blots are shown with the data plotted. 5S rRNA was used as the control.

Figure S9 Control autoinducer responses of Qrr target mRNAs in the absence of Qrr sRNAs. *V. harveyi* Δ*luxM* Δ*luxPQ* Δ*cqsS* Δ*qrr*1-5 strain (LF1451) was grown in 1 μM AI-1 to mid-logarithmic phase. 100 μM 3-oxo-C12-HSL (black) or DMSO (white) was added to the culture for 15 minutes and the mRNA levels of the target genes were measured by qRT-PCR.

Table S1. Genes regulated by Qrr sRNA induction identified by microarray analysis.

		Qrr1	Qrr2	Qrr3	Qrr4[a]	Qrr4[b]	Qrr5
Known Qrr sRNA target genes							
VIBHAR_00046	AphA		5.46	7.41	7.36	5.74	10.93
VIBHAR_02765	LuxM	-3.41	-3.94	-2.77	-3.39	-3.81	-3.41
VIBHAR_02766	LuxN					-2.17	
VIBHAR_03459	LuxR	-7.89	-6.50	-10.27	-8.28	-9.25	-7.89
VIBHAR_03460	hypothetical protein				-2.43	-2.55	-2.46
Novel Qrr sRNA target genes							
VIBHAR_00417	prephrenate dehydratase		-2.36		-2.57	-2.41	
VIBHAR_00504	RNA polymerase ECF-type sigma factor		-2.77		-2.27	-3.43	-2.17
VIBHAR_00505	chromosome segregation ATPase			-2.58	-2.27	-3.32	-2.28
VIBHAR_02446	hypothetical protein			2.27	2.35		
VIBHAR_02474	virulence factor, aerolysin / hemolysin / leukocidin toxin						-2.51
VIBHAR_02509	hemagglutinin/protease				-3.10	-3.61	-3.63
VIBHAR_03626	deacetylase DA1	-4.76	-4.63	-5.58	-4.44	-4.11	-4.76
VIBHAR_04936	glutathione-dependent formaldehyde-activating like protein				-2.69		
VIBHAR_05020	hypothetical protein		-3.05	-3.71	-2.97	-4.82	
VIBHAR_05691	histidine kinase		-4.50	-4.50	-5.98	-5.28	-3.73
VIBHAR_05763	hypothetical protein		-2.30	-2.53	-3.46	-3.32	-2.93
VIBHAR_06448	hemolysin A		-2.41				
VIBHAR_06453	putative toxin transport protein					-2.31	
VIBHAR_06665	polysaccharide export outer membrane protein			-2.19			
VIBHAR_06666	phosphatase		-2.23				
VIBHAR_06667	tyrosine-protein kinase			-2.28		-2.58	
VIBHAR_06888	hypothetical protein	-4.23	-4.26	-4.03	-3.86	-4.26	-4.23
VIBHAR_06930	hypothetical protein		3.03	2.41	2.81	4.23	2.46
VIBHAR_06931	GGDEF family protein		2.41		2.39	3.16	
VIBHAR_p08221	hypothetical protein			-2.20			
VIBHAR_p08222	isoprenoid biosynthesis protein with amidotransferase-like domain		-2.58	-2.43	-2.62	-3.18	
VIBHAR_p08223	hypothetical protein		-2.83	-2.53	-2.77	-3.27	
Arabinose-induced genes							
VIBHAR_03326	aldose 1-epimerase	4.17	4.00	5.24	4.06	4.14	4.17
VIBHAR_03327	galactokinase	3.41	3.68	3.78	4.03	4.06	3.41
VIBHAR_03329	galactose-1-phosphate uridylyltransferase	4.53	4.08	4.32	3.94	3.89	4.53
VIBHAR_03331	UDP-glucose 4-epimerase	13.18	19.03	21.26	17.15	14.22	13.18
VIBHAR_03332	hypothetical protein	6.54	4.11	6.54			
False positive/Qrr sRNA indirect target genes							
VIBHAR_00986	hypothetical protein				-3.01		
VIBHAR_05213	maltose/maltodextrin transporter ATP-binding protein				-2.28		
VIBHAR_05384	peptidase T				-2.85		
VIBHAR_06097	hypothetical protein				-2.68		
VIBHAR_06299	hypothetical protein						3.05
VIBHAR_06895	predicted glycosyl hydrolase	4.44	6.96			3.68	4.44

Genes that changed expression two-fold or more in all four array replicates are listed and means are shown. Fold-change in gene expression is represented as the value from the Qrr sRNA induction strains divided by the value from the Qrr sRNA non-induction strains (OD_{600} ~ 0.5: Qrr1, Qrr2, Qrr3, Qrr4[a], Qrr5; OD_{600} ~ 1.0: Qrr4[b]). +, activation; -, repression.

Table S2. Strains used in this study.

Strain	Relevant Genotype	Source
E. coli		
S17λpir	wild type	(De Lorenzo & Timmis 1994)
BW-RI	wild type	(Levine et al. 2007)
LF1018	BW-RI *rne-50 zce-726*::Tn*10*	this study (Massé et al. 2003)
V. harveyi		
BB120	wild type	(Bassler et al. 1997)
TL25	Δ*luxM* Δ*luxPQ* Δ*cqsS*	(Long et al. 2009)
LF1451	Δ*luxM* Δ*luxPQ* Δ*cqsS* Δ*qrr1-5*	this study

Table S3. Plasmids Used in this Study.

Plasmid	Description	Source
pEVS143	vector	(Dunn et al. 2006)
pZA31-*luc*NB	vector	(Levine et al. 2007)
pZE12G	vector	(Levine et al. 2007)
pLF1097	pEVS143-Vh Qrr1	this study
pLF875	pEVS143-Vh Qrr2	this study
pLF898	pEVS143-Vh Qrr3	this study
pLF575	pEVS143-Vh Qrr4	this study
pLF878	pEVS143-Vh Qrr5	this study
pLF253	pZA31 empty vector	this study
pLF396	pZA31-Vh Qrr1	this study
pYS241	pZA31-Vh Qrr1^{9+}	this study
pYS299	pZA31-Vh Qrr1 SL1D	this study
pYS300	pZA31-Vh Qrr1 SL1R	this study
pLF186	pZA31-Vh Qrr2	this study
pLF126	pZA31-Vh Qrr3	this study
pLF127	pZA31-Vh Qrr4	this study
pYS258	pZA31-Vh Qrr4 mut1	this study
pYS259	pZA31-Vh Qrr4 mut2	this study
pLF770	pZA31-Vh Qrr4 mut3	this study
pYS221	pZA31-Vh Qrr4 mut4	this study
pYS239	pZA31-Vh Qrr4^{9-}	this study
pYS287	pZA31-Vh Qrr4 SL1D	this study
pYS296	pZA31-Vh Qrr4 SL1R	this study
pYS225	pZA31-Vh Qrr4 ΔSL1	this study
pYS226	pZA31-Vh Qrr4 ΔSL2	this study
pYS227	pZA31-Vh Qrr4 ΔSL3	this study
pYS229	pZA31-Vh Qrr4 ΔSL1&3	this study
pYS230	pZA31-Vh Qrr4 SL1*	this study
pYS231	pZA31-Vh Qrr4 SL2*	this study
pYS232	pZA31-Vh Qrr4 SL3*	this study
pYS280	pZA31-Vh Qrr4 WT-RybB	this study
pYS281	pZA31-Vh Qrr4 WT-MicA	this study
pYS282	pZA31-Vh Qrr4 WT-OmrB	this study
pYS277	pZA31-Vh Qrr4 ΔSL1-RybB	this study
pYS278	pZA31-Vh Qrr4 ΔSL1-MicA	this study
pYS279	pZA31-Vh Qrr4 ΔSL1-OmrB	this study
pYS274	pZA31-Vh Qrr4 ΔSL2-RybB	this study
pYS275	pZA31-Vh Qrr4 ΔSL2-MicA	this study
pYS276	pZA31-Vh Qrr4 ΔSL2-OmrB	this study
pYS271	pZA31-Vh Qrr4 ΔSL3-RybB	this study
pYS272	pZA31-Vh Qrr4 ΔSL3-MicA	this study
pYS273	pZA31-Vh Qrr4 ΔSL3-OmrB	this study
pYS268	pZA31-Vh Qrr4 ΔSL1&3-RybB	this study

pYS269	pZA31-Vh Qrr4 ΔSL1&3-MicA	this study
pYS270	pZA31-Vh Qrr4 ΔSL1&3-OmrB	this study
pLF187	pZA31-Vh Qrr5	this study
pLF255	pZE12G-AphA-GFP	this study
pLF128	pZE12G-LuxR-GFP	this study
pYS212	pZE12G-VIBHAR_00417-GFP	this study
pLF804	pZE12G-VIBHAR_00505-GFP	this study
pLF1092	pZE12G-VIBHAR_00986-GFP	this study
pLF1093	pZE12G-VIBHAR_02446-GFP	this study
pLF1382	pZE12G-VIBHAR_02474-GFP	this study
pYS214	pZE12G-VIBHAR_02509-GFP	this study
pLF1228	pZE12G-VIBHAR_03626-GFP	this study
pYS216	pZE12G-VIBHAR_04936-GFP	this study
pLF1222	pZE12G-VIBHAR_05020-GFP	this study
pLF1103	pZE12G-VIBHAR_05213-GFP	this study
pLF1231	pZE12G-VIBHAR_05384-GFP	this study
pLF767	pZE12G-VIBHAR_05691-GFP	this study
pYS256	pZE12G-VIBHAR_05691-GFP mutI	this study
pYS257	pZE12G-VIBHAR_05691-GFP mutII	this study
pYS218	pZE12G-VIBHAR_05763-GFP	this study
pLF1090	pZE12G-VIBHAR_06097-gfp	this study
pLF1727	pZE12G-VIBHAR_06299-gfp	this study
pLF1373	pZE12G-VIBHAR_06448-GFP	this study
pLF1237	pZE12G-VIBHAR_06455-GFP	this study
pLF806	pZE12G-VIBHAR_06665-GFP	this study
pLF1235	pZE12G-VIBHAR_06888-GFP	this study
pLF1285	pZE12G-VIBHAR_06930-GFP	this study
pLF840	pZE12G-VIBHAR_06930-GFP mutI	this study
pLF1730	pZE12G-VIBHAR_06930-GFP truncation	this study
pLF1225	pZE12G-VIBHAR_p08223-GFP	this study

Table S4. Primers used in this study.

Primer	Sequence	Use
YS701	GAAGTTGCATTAATAGTTAATAATTAAAGGGAATATC	pYS212
YS702	ATAGGTACCACACATTCCTAGCGCAAGCAACG	pYS212
YS704	GCATTTTTGCTACGAATATACACACATAAG	pYS214
YS705	ATAGGTACCGACCAGCGATAATAAAGTGACGTTTC	pYS214
YS707	ATTCCAATAAGAGAAGGACTAAAAAATGGAAG	pYS216
YS708	ATAGGTACCGCGGCATTCAGAGCAATGACAATT	pYS216
LF615	GCTAGGTTTTATATGTCAAGGAT	pYS218
LF616	AATAGGTACCGTTTTTGATTAGCTCTTCGAGGTAG	pYS218
YS715	ACCCTTATTAAGCCGAGGGTCACCTTCGGAACTGACGTTGTTAGTGAATACACA	pYS221
YS716	TGTGTATTCACTAACAACGTCAGTTCCGAAGGTGACCCTCGGCTTAATAAGGGT	pYS221
YS723	CAGTGATAGAGATACTGAGCACACCTAGCCAACTGACGTTGTTA	pYS225
YS724	TAACAACGTCAGTTGGCTAGGTGTGCTCAGTATCTCTATCACTG	pYS225
YS725	GACCCTTATTAAGCCGAGGGTCGTGAATACACATTGTTCACAAG	pYS226
YS726	CTTGTGAACAATGTGTATTCACGACCCTCGGCTTAATAAGGGTC	pYS226
YS727	ACCTAGCCAACTGACGTTGTTAAAGTATATACCGCCAATCAACT	pYS227/229
YS728	AGTTGATTGGCGGTATATACTTTAACAACGTCAGTTGGCTAGGT	pYS227/229
YS731	GATAGAGATACTGAGCACACTGGGAGATTAAGCCTTCCCAGACCTAGCCAACTGACGTT	pYS230
YS732	AACGTCAGTTGGCTAGGTCTGGGAAGGCTTAATCTCCCAGTGTGCTCAGTATCTCTATC	pYS230
YS733	CCTTATTAAGCCGAGGGTCATTGTTGCAGTCAACCGATCCAGTGAATACACATTGTTCAC	pYS231
YS734	GTGAACAATGTGTATTCACTGGATCGGTTGACTGCAACAATGACCCTCGGCTTAATAAGG	pYS231
YS735	CTAGCCAACTGACGTTGTTACACTTGTCACATATAAGTGAAGTATATACCGCCAATCAA	pYS232
YS736	TTGATTGGCGGTATATACTTCACTTATATGTGACAAGTGTAACAACGTCAGTTGGCTAG	pYS232
YS783	TAGAGATACTGAGCACAGACCCTTAGGGTCACCTAGCCAACTGACGTTGT	pYS239

YS784	ACAACGTCAGTTGGCTAGGTGACCCTAAGGGTCTGTGCTCAGTATCTCTA	pYS239
YS787	TAGAGATACTGAGCACGGACCCCTCTTAAGCCGAGGGTCACCTAGCCAACTGACGTTGT	pYS241
YS788	ACAACGTCAGTTGGCTAGGTGACCCTCGGCTTAAGAGGGGTCCGTGCTCAGTATCTCTA	pYS241
YS821	GTTGGAAAACACAATAAAAATCCGATAATTATGAAACTTAGTAAC	pYS256
YS822	GTTACTAAGTTTCATAATTATCGGATTTTTATTGTGTTTTCCAAC	pYS256
YS823	AACAAGATACTGAGCACTCCTCAACGAAAACACAATAAAAATGGC	pYS257
YS824	GCCATTTTTATTGTGTTTTCGTTGAGGAGTGCTCAGTATCTTGTT	pYS257
YS825	CTGAGCACAGACCCTTATTATCGGGAGGGTCACCTAGCCAACTG	pYS258
YS826	CAGTTGGCTAGGTGACCCTCCCGATAATAAGGGTCTGTGCTCAG	pYS258
YS651	TAAGCCGAGGGTCACCTAGCGTTGAGACGTTGTTAGTGAATACAC	pYS259
YS652	GTGTATTCACTAACAACGTCTCAACGCTAGGTGACCCTCGGCTTA	pYS259
YS757	ATAGGATCCATCAAATAAAACGAAAGGCTC	pYS268~282
YS933	GATGGATCCGAGAGGGTTGCAGGGTAGTAG	pYS268/271/274/277/280
YS934	GATGTCCCCATTTTGTGGAGCC	pYS268/271/274/277/280
YS936	GATGGATCCGCGGTGTGGCTGGAAAAACAC	pYS269/272/275/278/281
YS937	CATCCCTGAATTCAGAGATGAAATTTTGG	pYS269/272/275/278/281
YS939	GATGGATCCGTCGGTTACTGTTACAGATTGATGAC	pYS270/273/276/279/282
YS940	AGGTGAAGTCAACTTCGGGTTGAG	pYS270/273/276/279/282
YS941	TAACAACGTCAGTTGGCTAGGTGTG	pYS268/269/270
YS942	TAACAACGTCAGTTGGCTAGGTGAC	pYS271/272/273
YS943	GTGAACAATGTGTATTCACGACCCTC	pYS274/275/276
YS944	GTGAACAATGTGTATTCACTAACAACGTC	pYS277/278/279
YS945	GTGAACAATGTGTATTCACTAACAACGTC	pYS280/281/282
YS883	GTGATAGAGATACTGAGCACAGAGGGTTATTAAGCCGAGGGTCACCTAG	pYS287
YS884	CTAGGTGACCCTCGGCTTAATAACCCTCTGTGCTCAGTATCTCTATCAC	pYS287
YS956	GAGCACAGAGGGTTATTAAGCCGACCCTCACCTAGCCAACTGACGTTGTTAG	pYS296
YS957	CTAACAACGTCAGTTGGCTAGGTGAGGGTCGGCTTAATAACCCTCTGTGCTC	pYS296
YS946	GTGATAGAGATACTGAGCACGGAGGGCTCGGGTCACCTAGCCAACTGAC	pYS299
YS947	GTCAGTTGGCTAGGTGACCCGAGCCCTCCGTGCTCAGTATCTCTATCAC	pYS299
YS948	GAGATACTGAGCACGGAGGGCTCCCCTCACCTAGCCAACTGACGTTGTT	pYS300
YS949	AACAACGTCAGTTGGCTAGGTGAGGGGAGCCCTCCGTGCTCAGTATCTC	pYS300
LF145	AATAGGATCCGTGCTCAGTATCTCTATCACTGATAGG	pLF253
LF54	AATAGGATCCATCAAATAAAACGAAAGGCT	pLF253
LF53	GTGCTCAGTATCTCTATCACTGATAGG	pLF396/126/127
LF54	AATAGGATCCATCAAATAAAACGAAAGGCT	pLF396/126/127
LF55	GTGCTCAGTATCTTGTTATCCGCTC	pZE12G-target-GFP fusions
LF56	AATAGGTACCATGTCTAAAGGTGAAGAACTT	pZE12G-target-GFP fusions
LF610	AATAGGTACCTTATGTCTACGACCATAGCTCGGGC	pLF1097
LF611	GGACCCCTCGGGTCACCTA	pLF1097
LF98	CGACCCTTCTTAAGCCGAGG	pLF875
LF607	AATAGGTACCTGACAACGCTCTGAATCCTGCATCT	pLF875
LF57	TGACCCTTCTTAAGCCGAGGG	pLF898
LF608	AATAGGTACCTTGGTTCAACAGGGAGTAAGGATTT	pLF898
LF303	AATAGGTACCAGGATCCGGTGATTGATTGAGCAAG	pLF575
LF304	AATAGCATGCAAAAAGACCCTTCATAAAT	pLF575
LF305	AATAGCATGCATAATGTGCCTGTCAAATGG	pLF575
LF306	TGACCCTCGGCTTAATAAGGGTCTGGAGAAACAGTAGAGAGTTGCG	pLF575
LF307	CGCAACTCTCTACTGTTTCTCCAGACCCTTATTAAGCCGAGGGTCA	pLF575
LF308	AATAGGTACCCGGTGAATCAGCGGATTCAGGTGAC	pLF575
LF94	TGACCCTTTTAAGCCGAGGG	pLF878
LF609	AATAGGTACCCGGCATTATTCGACTGGAAACTGTA	pLF878
LF100	GGACCCCTCGGGTCACCTAT	pLF396
LF101	AATAGGATCCCACATCTGCAGACAAAAAAGAAGCC	pLF396
LF648	GGACCCCTCGGGTCACCTAGCCAACTGACGTTGTTAGTGA	pLF396
LF649	TCACTAACAACGTCAGTTGGCTAGGTGACCCGAGGGGTCC	pLF396
LF98	CGACCCTTCTTAAGCCGAGG	pLF186
LF99	AATAGGATCCTACAAACAAAAAAATAGCCAACCGC	pLF186
LF57	TGACCCTTCTTAAGCCGAGGG	pLF126

LF58	AATAGGATCCCCAGATAAAAAATAGCCAACCGCAA	pLF126
LF59	AGACCCTTATTAAGCCGAGGGTC	pLF127
LF60	AATAGGATCCCTAGAAAGAAAAAACGCCAATCACAA	pLF127
LF94	TGACCCTTTTAAGCCGAGGG	pLF187
LF95	AATAGGATCCTGATTGTGAAAAAAAAGCCAACCAC	pLF187
LF554	GCCGAGGGTCACCTACGGAACTGACGTTGTTAG	pLF770
LF555	CTAACAACGTCAGTTCCGTAGGTGACCCTCGGC	pLF770
LF61	TGCTTTAAGCAACTATTAAAATAATCAAT	pLF128
LF62	AATAGGTACCAGTACGAGGTCTCTTTGCAAT	pLF128
LF142	CCTGCTGGAAGCTCACAAAT	pLF255
LF113	AATAGGTACCTACAGTTAGAATTACGTGTGGTAATGAC	pLF255
LF562	AATAGGTACCTAAAGCAGACAGCCAGATCG	pLF804
LF544	CGAAGTCACGCGCCTTGTCTTTCAT	pLF804
LF637	GAGCTTAAAAAATTACCCTACCTAT	pLF1092
LF639	AATAGGTACCCTGAACAAGGTAAACACGTTTGGTT	pLF1092
LF644	GTCAGGTTGGGAGGGACGCCATGTC	pLF1093
LF645	AATAGGTACCAACCTCTCGAGATACGTTGAACATC	pLF1093
LF774	GAGTACGCCCGCTTTTAGGTCAAAA	pLF1382
LF775	AATAGGTACCCGTCGATAAGAAAGAAAGTGCAAGC	pLF1382
LF718	CAACCGGTTGCATTGTTCGTGAA	pLF1228
LF570	AATAGGTACCCGCAGTACCTAAAAGTGTAACCATA	pLF1228
LF725	AAACATTTTTATCAGTTCTACCTTTAAGAT	pLF1222
LF702	AATAGGTACCCCTTCTACGTGGTAAACAATTTGCA	pLF1222
LF679	TTCCTACTGTTACTGCTCGCTAGAT	pLF1103
LF680	AATAGGTACCCTTAGAAATCAGTACGTCGCCATAC	pLF1103
LF717	TGAGTGCTGATGGAGCCTGT	pLF1231
LF722	AATAGGTACCGCGAAGAAATCTTTCAACTAAATGC	pLF1231
LF567	TCCAGTTGGAAAACACAATAAAAAT	pLF767
LF397	AATAGGTACCGTTTCTAACGGATAGGTTACTAAGT	pLF767
LF640	AAAATGAGTTTTCATTAATGAAAGG	pLF1090
LF641	AATAGGTACCTGATGCGACACCTGCAGAAACAGAA	pLF1090
LF1020	AATAGGATCCATGTCTAAAGGTGAAGAATT	pLF1727
LF1021	AATAGGATCCTGAATACTTAAAACGAACGTGAGTG	pLF1727
LF749	TTGGAATTTGCACCAAATTGTTAAT	pLF1727
LF776	TACACCAACCAATTTTTGTTCACCT	pLF1373
LF777	AATAGGTACCTTCTTTTTGTAAGTCATTCGTCAGA	pLF1373
LF716	CGTGTCTCACATATGAGAATGGAGG	pLF1237
LF724	AATAGGTACCCGCATCCCGTTTTGGGTAAGCCCTA	pLF1237
LF549	ATGCCCAATTAAATTATGGCGCGTT	pLF806
LF563	AATAGGTACCACTACCTAGCTTTGTATAGTTGAAA	pLF806
LF715	CCCCCTTTCCTTATTGTTTGTTTTG	pLF1235
LF723	AATAGGTACCAAGGGTCACGAAGCCAAGAACGTTTTTCAT	pLF1235
LF564	ATAACGTATTATATAAATTCATCAA	pLF1285
LF565	AATAGGTACCAAGTAAAGCGAGTACTACTGTTTTT	pLF1285/1730
LF720	GCCACTAAATTGCAATGTAGTTAGC	pLF1225
LF727	AATAGGTACCAAATGATAGGAAGAGTATTTTTAAC	pLF1225
LF556	TAAATTCATCAAGTTCCGTCTATTATTAATAAC	pLF840
LF557	GTTATTAATAATAGACGGAACTTGATGAATTTA	pLF840
LF558	ATATAACCGAGATTTCAGCAGATCG	pLF1730
LF583	ATAAGGCCTATCGTATTTGTTGGCAGTAATGCCA	*vibhar_00417* 5' RACE
LF584	ATAAGGCCTACAAACGCAAGGTAGAAATACGGTT	*vibhar_00417* 5' RACE
LF589	ATAAGGCCTATCTATCGATAACTGACGCTCTACT	*vibhar_02446* 5' RACE
LF590	ATAAGGCCTCTTTCTTAAGTTCCTTCTCGAGGAA	*vibhar_02446* 5' RACE
LF591	ATAAGGCCTCATCTTTTCTATGTTGAGTCCGCCA	*vibhar_02446* 5' RACE
LF594	ATAAGGCCTAATCGATTGAGCAAAGCCTTTTGCG	*vibhar_02509* 5' RACE
LF595	ATAAGGCCTCCGTTGCGATCTCAATCGCTTGG	*vibhar_02509* 5' RACE
YS679	ATAAGGCCTAGCCGCAGTCACGGCAGAAT	*vibhar_04936* 5' RACE
YS680	GCTGGGTTTGTCGCAAGGGGT	*vibhar_04936* 5' RACE

LF624	ATAAGGCCTACTGGAACACCATACCAACACCACG	*vibhar_05213* 5' RACE
LF625	ATAAGGCCTCCTGAAGTAATGTCTTCAAGACCGGC	*vibhar_05213* 5' RACE
LF418	CAAGCTGCTTGCGCTTGTTAT	*vibhar_05691* 5' RACE
LF419	ATAAGGCCTTGGATCGCATTCAGCGCTTGTTGT	*vibhar_05691* 5' RACE
LF420	ATAAGGCCTGGCTTAGTCGGATGAAGCAAGACT	*vibhar_05691* 5' RACE
YS675	ATAAGGCCTGCACGAGCTTTCGTTCATGTAACAC	*vibhar_05763* 5' RACE
YS676	ACGGATGATACGCTGTGCTTCGT	*vibhar_05763* 5' RACE
LF424	CGATCTTGCCGAAACAAGGGCAAT	*vibhar_06930* 5' RACE
LF425	ATAAGGCCTCCATTAAACTCGGCGGGCTTTTCA	*vibhar_06930* 5' RACE
LF426	ATAAGGCCTCCTTACTTGGACGTGCTGAGGAA	*vibhar_06930* 5' RACE
YS740	ACCCTTATTAAGCCGAGGGTC	Qrr northern blot riboprobe
YS789	GTTTTTTTAATACGACTCACTATAGGGAGGCGCCAATCACAATAAAGTTG	Qrr northern blot riboprobe
KPO-0243	TTCGTTTCACTTCTGAGTTCGG	5S rRNA northern blot probe
STR0381	ATCCATCAACTCTAGGTGATAAACG	*aphA* qRT-PCR
STR0382	CGTCGCGAGTGCTAAGTACA	*aphA* qRT-PCR
STR0383	ACATCAACTCAAATGGCAAGG	*luxR* qRT-PCR
STR0384	GCAAACACTTCAAGAGCGATTT	*luxR* qRT-PCR
STR0038	CCTTATTAAGCCGAGGGTCAC	Qrr4 qRT-PCR
STR0039	GTTGATTGGCGGTATATACTTGTG	Qrr4 qRT-PCR
LF22	CCATTCCGAACTCAGAAGTGAA	5S rRNA qRT-PCR
LF23	TACTCTCACATGGGGAAGCC	5S rRNA qRT-PCR
LF_RT13	CGACCGATTGGAAAACGCTA	*vibhar_00417* qRT-PCR
LF_RT14	ACGGTTGGCTATAACCTGCT	*vibhar_00417* qRT-PCR
YS603	TGATGATGAAGGCGACTGGA	*vibhar_00505* qRT-PCR
YS604	GTTCATTTGGCCCCAAACCT	*vibhar_00505* qRT-PCR
LF_RT47	CGGTACACTTGACGGTCTTG	*vibhar_00986* qRT-PCR
LF_RT48	GAAAGCAATCACACCACCGA	*vibhar_00986* qRT-PCR
LF_RT19	AGTCCAAAGTATCGCTGAACA	*vibhar_02446* qRT-PCR
LF_RT20	TACGTTGAACATCAGCCCCT	*vibhar_02446* qRT-PCR
LF770	TCATGGGCGAAGGCTATCAT	*vibhar_02474* qRT-PCR
LF771	GCATTGAGACGTTACGAGGG	*vibhar_02474* qRT-PCR
LF487	CATGAAGTCAGCCACGGTTT	*vibhar_02509* qRT-PCR
LF488	ACGTATTGGCTAAGTGCAGC	*vibhar_02509* qRT-PCR
LF495	AAACTGGCGCTTGATACAGG	*vibhar_03626* qRT-PCR
LF496	ACATTCTGCACCACTCGTTG	*vibhar_03626* qRT-PCR
LF_RT55	TTGCTCTGAATGCCGCAAAT	*vibhar_04936* qRT-PCR
LF_RT56	TGGCGCTCTTCTGATAGGTT	*vibhar_04936* qRT-PCR
LF497	TTTTGCTTCAACAGGCGCTA	*vibhar_05020* qRT-PCR
LF498	TGTCTATCTACGCATCGGCT	*vibhar_05020* qRT-PCR
LF_RT9	TGTTGAACACGCAGCAGAAA	*vibhar_05213* qRT-PCR
LF_RT10	TAGCGTACGACCGATAGCAA	*vibhar_05213* qRT-PCR
LF_RT59	TGGCGGCTAAATTCCAAGTG	*vibhar_05384* qRT-PCR
LF_RT60	AGCCTAACTCACTACGAGCC	*vibhar_05384* qRT-PCR
LF331	TACTTCTGGGTACTGACGCC	*vibhar_05691* qRT-PCR
LF332	CATTTCACGCCAGTGGAACT	*vibhar_05691* qRT-PCR
YS611	ACGAAGCACAGCGTATCATC	*vibhar_05763* qRT-PCR
YS612	TAGCAGCTGGCTCACTTCTT	*vibhar_05763* qRT-PCR
LF_RT51	ATTCCGTAGTTCGCGTTTCC	*vibhar_06097* qRT-PCR
LF_RT52	CTCTGCTGACATACCCCACA	*vibhar_06097* qRT-PCR
YS631	GACTAAGTCGGCACTTGAGC	*vibhar_06299* qRT-PCR
YS632	ACACCCGGAGCATATCTGAG	*vibhar_06299* qRT-PCR
LF772	CAAAACTGGCGACTGTCCAA	*vibhar_06448* qRT-PCR
LF773	TGCTTGCCAGATTCCCCTTA	*vibhar_06448* qRT-PCR
LF_RT27	GACAGTGAAAAGTCTGGCCC	*vibhar_06455* qRT-PCR
LF_RT28	TGACTTGCGCTTGGAAACTT	*vibhar_06455* qRT-PCR
LF465	CGAGGTTAACAAGCCTGGTC	*vibhar_06665* qRT-PCR
LF466	TTACGCCAATCCGCATCTTC	*vibhar_06665* qRT-PCR
LF1053	CTGCAAGCTATGGTTCTTAT	*vibhar_06741* qRT-PCR

LF1054	CCTGAGATTTGATCTGAGTC	*vibhar_06741* qRT-PCR
LF_RT43	ACCCTTCTTGCTGCTTCTCT	*vibhar_06888* qRT-PCR
LF_RT44	ACCAACACAATGGGATGCTG	*vibhar_06888* qRT-PCR
LF342	GAGTCGATGCCTCAAACCAC	*vibhar_06930* qRT-PCR
LF343	AGGAACTTCACCGAGTGTGT	*vibhar_06930* qRT-PCR
LF_RT1	CGTGAAGTCAGTCGTTTGGT	*vibhar_p08223* qRT-PCR
LF_RT2	GCATGTTCTGGATTTTGCGT	*vibhar_p08223* qRT-PCR
LF1059	TGTTCATTTAACTCAGATGGTGACT	*luxC* qRT-PCR
LF1060	TTCTTCTTGAATACTCTTCGCTCTT	*luxC* qRT-PCR

Supplemental References:

Bassler BL, Greenberg EP, Stevens a M. 1997. Cross-species induction of luminescence in the quorum-sensing bacterium Vibrio harveyi. *Journal of bacteriology* **179**: 4043–5.

Dunn AK, Millikan DS, Adin DM, Jeffrey L, Stabb E V, Bose JL. 2006. New rfp- and pES213-Derived Tools for Analyzing Symbiotic Vibrio fischeri Reveal Patterns of Infection and lux Expression In Situ. *Applied and environmental micriobiology* **72**: 802–10.

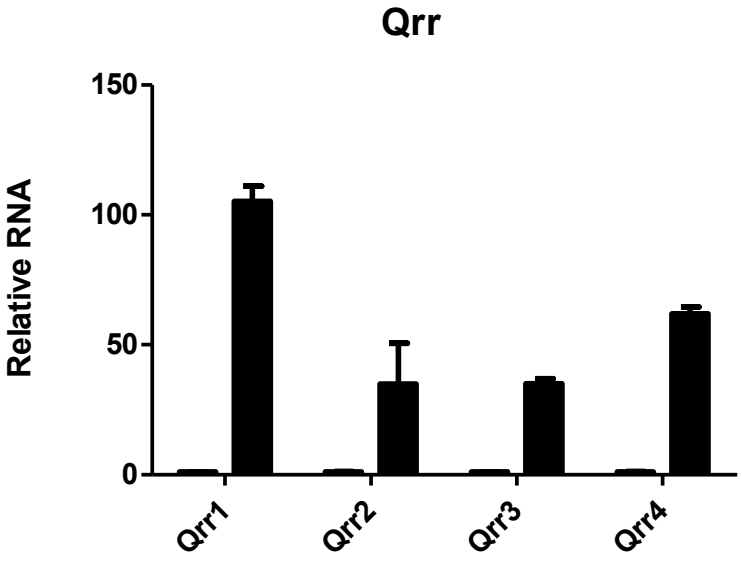
Levine E, Zhang Z, Kuhlman T, Hwa T. 2007. Quantitative characteristics of gene regulation by small RNA. *PLoS biology* **5**: e229.

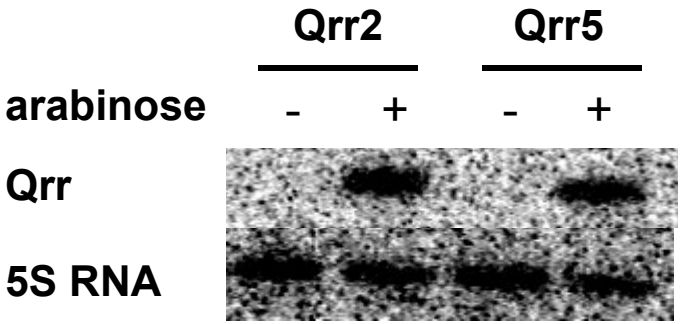
Long T, Tu KC, Wang Y, Mehta P, Ong NP, Bassler BL, Wingreen NS. 2009. Quantifying the integration of quorum-sensing signals with single-cell resolution. *PLoS biology* **7**: e68.

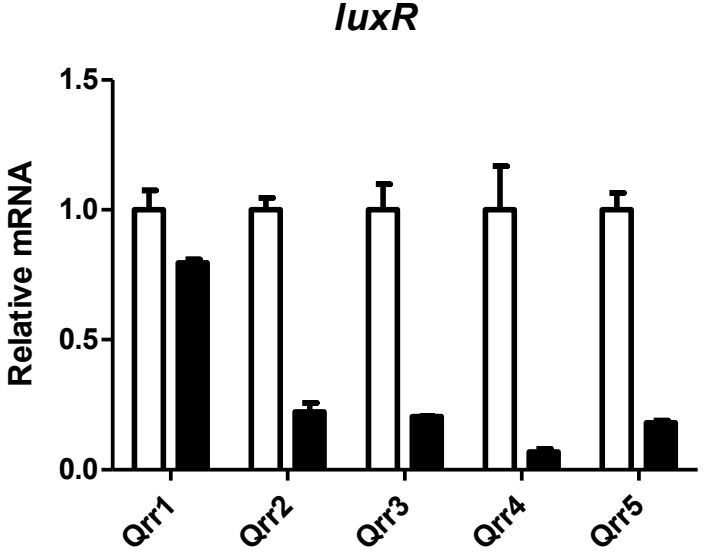
De Lorenzo V, Timmis KN. 1994. Analysis and construction of stable phenotypes in gram-negative bacteria with Tn5- and Tn10-derived minitransposons. *Methods in Enzymology* **235**: 386–405.

Massé E, Escorcia FE, Gottesman S. 2003. Coupled degradation of a small regulatory RNA and its mRNA targets in Escherichia coli. *Genes & development* **17**: 2374–83.

Figure S1


Qrr
Relative RNA
150
100
50
0
Qrr1
Qrr2
Qrr3
Qrr4


Qrr2
Qrr5
arabinose
-
+
-
+
Qrr
5S RNA


luxR
Relative mRNA
1.5
1.0
0.5
0.0
Qrr1
Qrr2
Qrr3
Qrr4
Qrr5

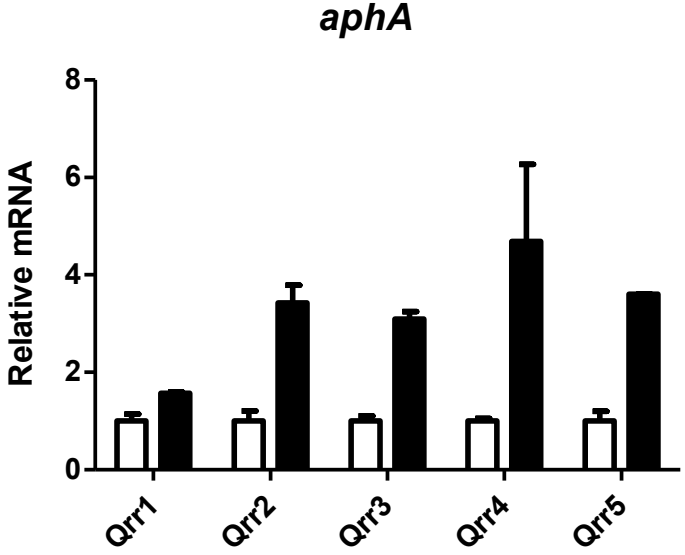

aphA
Relative mRNA
8
6
4
2
0
Qrr1
Qrr2
Qrr3
Qrr4
Qrr5

Figure S2

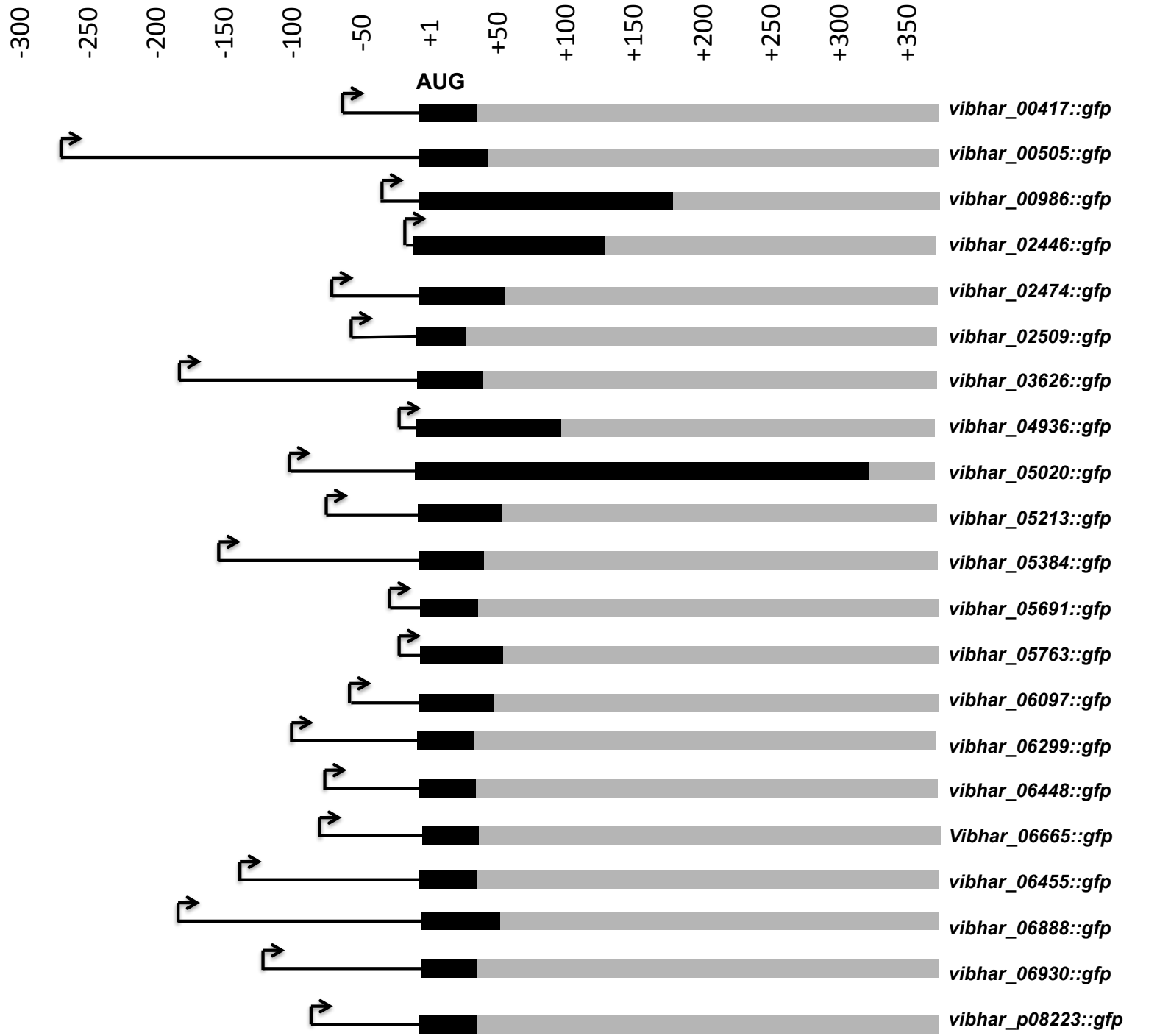

-300
-250
-200
-150
-100
-50
+1
+50
+100
+150
+200
+250
+300
+350
AUG
vibhar_00417::gfp
vibhar_00505::gfp
vibhar_00986::gfp
vibhar_02446::gfp
vibhar_02474::gfp
vibhar_02509::gfp
vibhar_03626::gfp
vibhar_04936::gfp
vibhar_05020::gfp
vibhar_05213::gfp
vibhar_05384::gfp
vibhar_05691::gfp
vibhar_05763::gfp
vibhar_06097::gfp
vibhar_06299::gfp
vibhar_06448::gfp
Vibhar_06665::gfp
vibhar_06455::gfp
vibhar_06888::gfp
vibhar_06930::gfp
vibhar_p08223::gfp

Figure S3

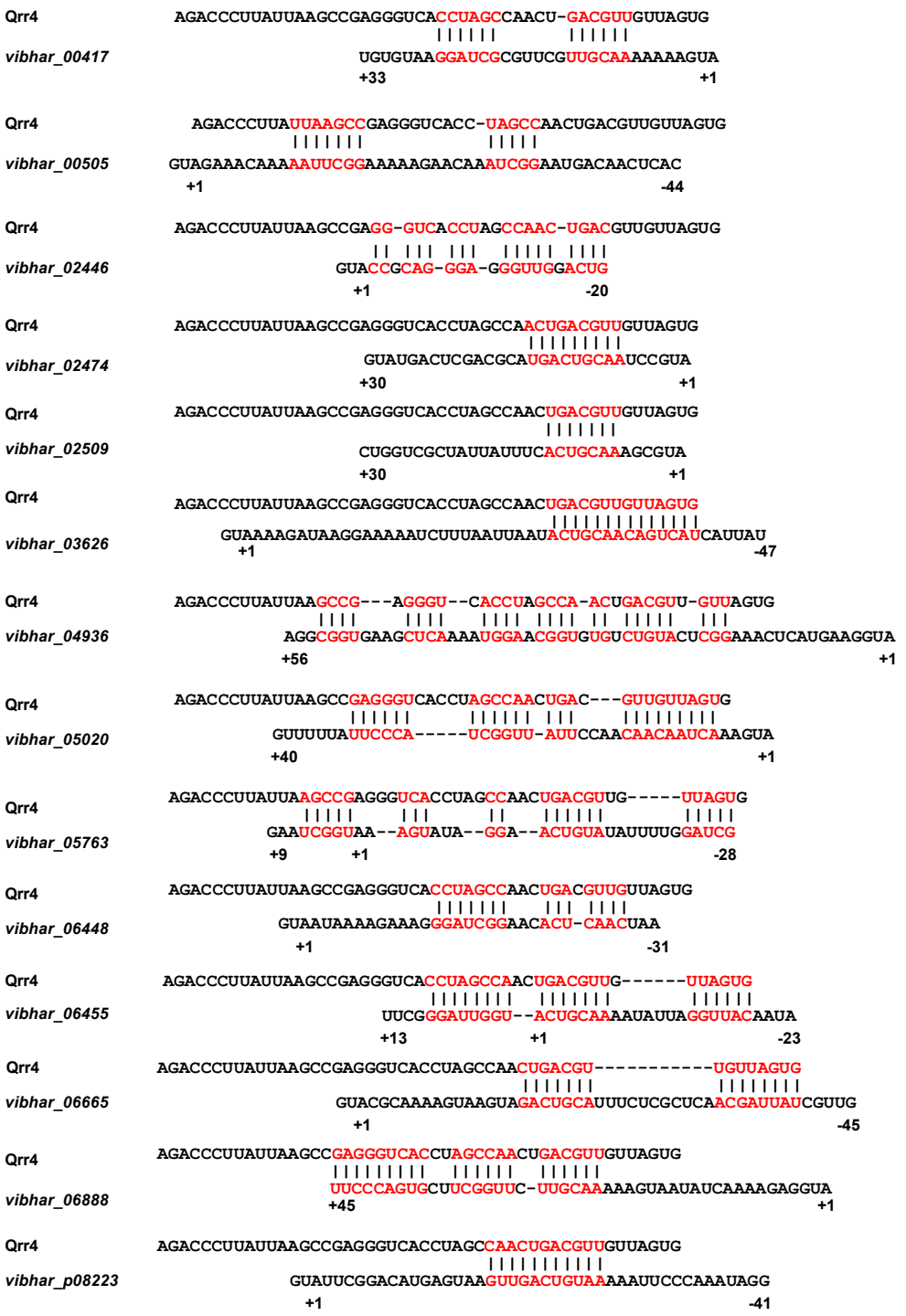

Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACU-GACGUUGUUAGUG
vibhar_00417
UGUGUAAGGAUCGCGUUCGUUGCAAAAAAGUA
+33
+1
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACC-UAGCCAACUGACGUUGUUAGUG
vibhar_00505
GUAGAAACAAAAAUUCGGAAAAAGAACAAAUCGGAAUGACAACUCAC
+1
-44
Qrr4
AGACCCUUAUUAAGCCGAGG-GUCACCUAGCCAAC-UGACGUUGUUAGUG
vibhar_02446
GUACCGCAG-GGA-GGGUUGGACUG
+1
-20
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGUUGUUAGUG
vibhar_02474
GUAUGACUCGACGCAUGACUGCAAUCCGUA
+30
+1
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGUUGUUAGUG
vibhar_02509
CUGGUCGCUAUUAUUUCACUGCAAAGCGUA
+30
+1
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGUUGUUAGUG
vibhar_03626
GUAAAAGAUAAGGAAAAAUCUUUAAUUAAUACUGCAACAGUCAUCAUUAU
+1
-47
Qrr4
AGACCCUUAUUAAGCCG---AGGGU--CACCUAGCCA-ACUGACGUU-GUUAGUG
vibhar_04936
AGGCGGUGAAGCUCAAAAUGGAACGGUGUGUCUGUACUCGGAAACUCAUGAAGGUA
+56
+1
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGAC---GUUGUUAGUG
vibhar_05020
GUUUUUAUUCCCA-----UCGGUU-AUUCCAACAACAAUCAAAGUA
+40
+1
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGUUG-----UUAGUG
vibhar_05763
GAAUCGGUAA--AGUAUA--GGA--ACUGUAUAUUUUGGAUCG
+9
+1
-28
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGUUGUUAGUG
vibhar_06448
GUAAUAAAAGAAAGGGAUCGGAACACU-CAACUAA
+1
-31
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGUUG------UUAGUG
vibhar_06455
UUCGGGAUUGGU--ACUGCAAAAUAUUAGGUUACAAUA
+13
+1
-23
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGU----------UGUUAGUG
vibhar_06665
GUACGCAAAAGUAAGUAGACUGCAUUUCUCGCUCAACGAUUAUCGUUG
+1
-45
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGUUGUUAGUG
vibhar_06888
UUCCCAGUGCUUCGGUUC-UUGCAAAAGUAAUAUCAAAAGAGGUA
+45
+1
Qrr4
AGACCCUUAUUAAGCCGAGGGUCACCUAGCCAACUGACGUUGUUAGUG
vibhar_p08223
GUAUUCGGACAUGAGUAAGUUGACUGUAAAAAUUCCCAAAUAGG
+1
-41

Figure S4

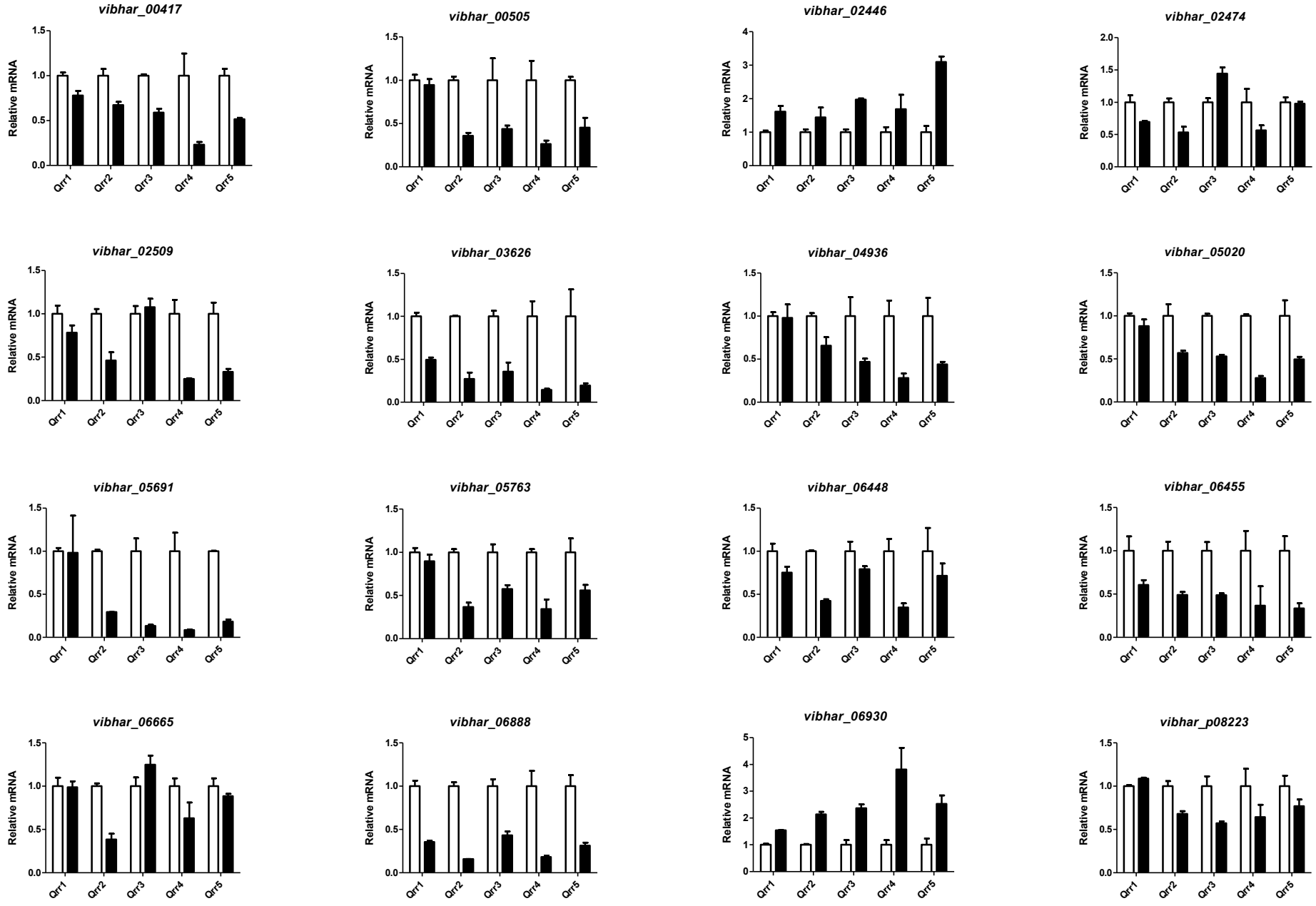

vibhar_00417
vibhar_00505
vibhar_02446
vibhar_02474
vibhar_02509
vibhar_03626
vibhar_04936
vibhar_05020
vibhar_05691
vibhar_05763
vibhar_06448
vibhar_06455
vibhar_06665
vibhar_06888
vibhar_06930
vibhar_p08223
Relative mRNA
Qrr1
Qrr2
Qrr3
Qrr4
Qrr5

Figure S5


mut1
mut4
1
45
Qrr4
5' AGACCCUUAUUAAGCCGAGGGUCACC-UAGCCAACUGACGUUGUUA 3'
vibhar_00505
3' GUAGAAACAAAAAUUCGGAAAAAGAACAAAUCGGAAUGACAACUCAC 5'
+1
-44

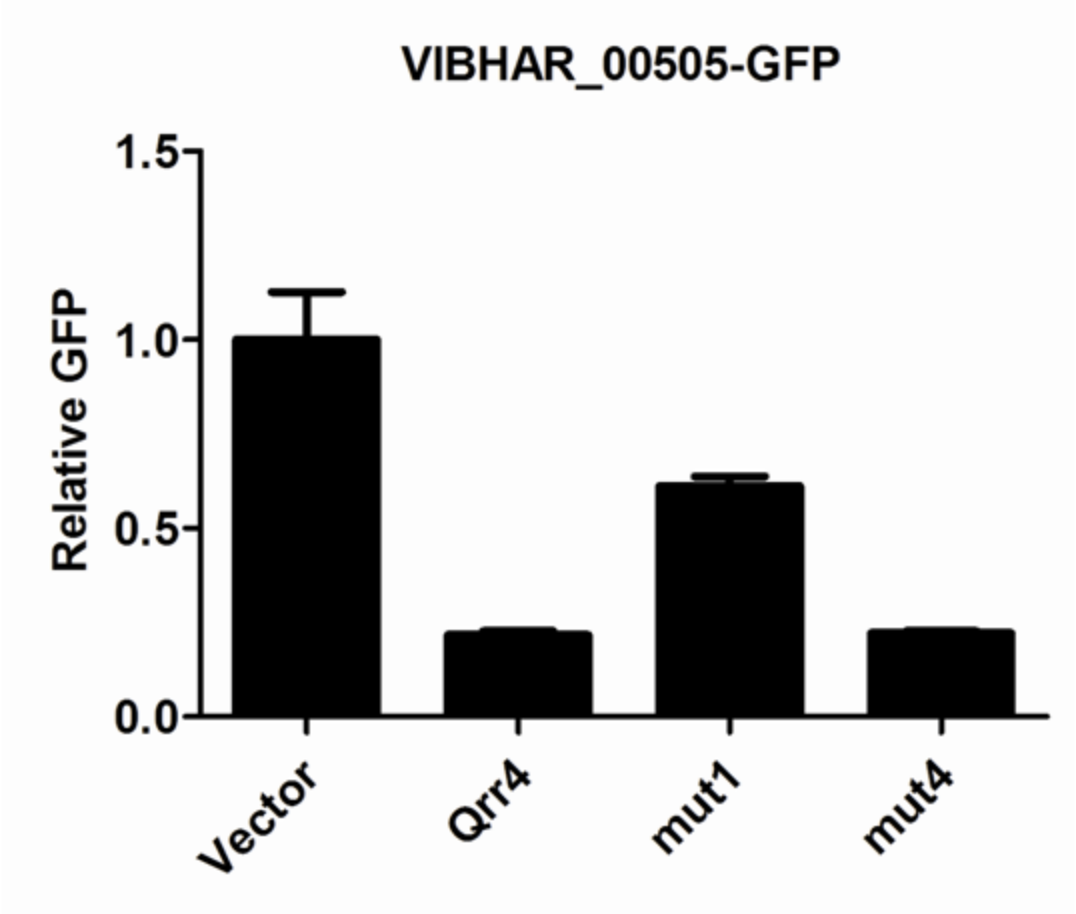

VIBHAR_00505-GFP
Relative GFP
1.5
1.0
0.5
0.0
Vector
Qrr4
mut1
mut4

Figure S6

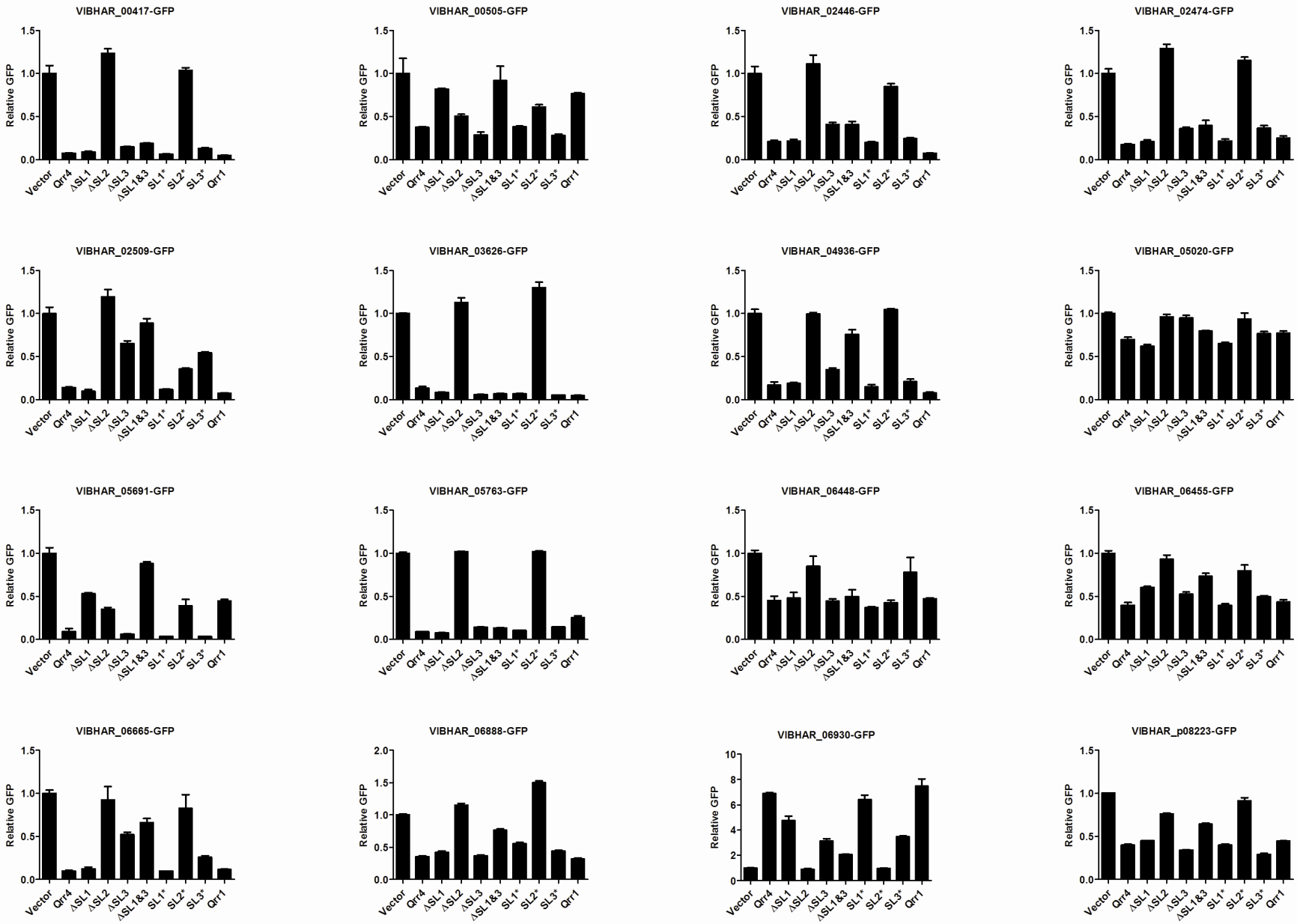
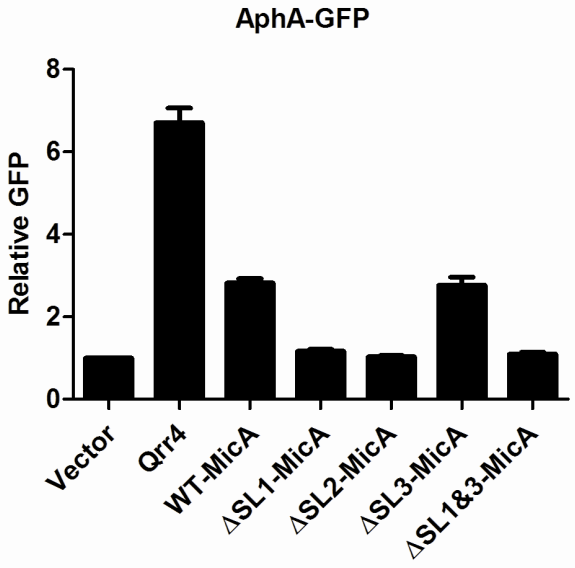
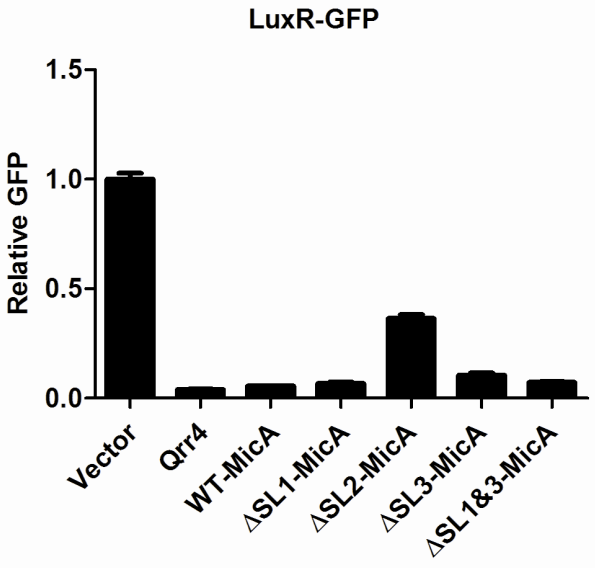
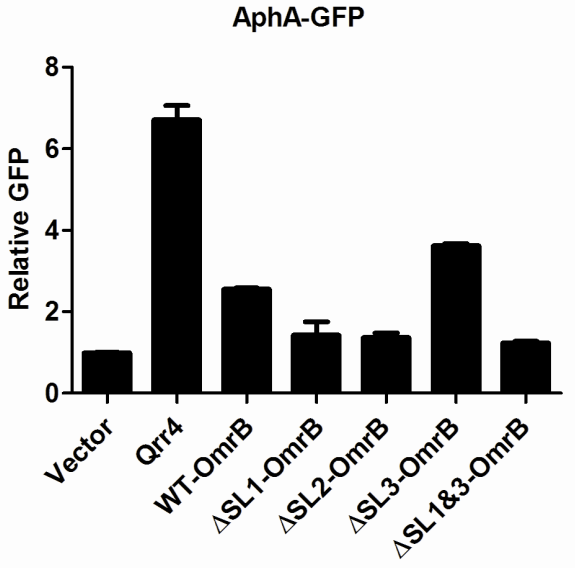

VIBHAR_00417-GFP
VIBHAR_00505-GFP
VIBHAR_02446-GFP
VIBHAR_02474-GFP
VIBHAR_02509-GFP
VIBHAR_03626-GFP
VIBHAR_04936-GFP
VIBHAR_05020-GFP
VIBHAR_05691-GFP
VIBHAR_05763-GFP
VIBHAR_06448-GFP
VIBHAR_06455-GFP
VIBHAR_06665-GFP
VIBHAR_06888-GFP
VIBHAR_06930-GFP
VIBHAR_p08223-GFP
Relative GFP
Vector
Qrr4
ΔSL1
ΔSL2
ΔSL3
ΔSL1&3
SL1*
SL2*
SL3*
Qrr1

Figure S7


AphA-GFP
Relative GFP
8
6
4
2
0
Vector
Qrr4
WT-MicA
ΔSL1-MicA
ΔSL2-MicA
ΔSL3-MicA
ΔSL1&3-MicA


LuxR-GFP
Relative GFP
1.5
1.0
0.5
0.0
Vector
Qrr4
WT-MicA
ΔSL1-MicA
ΔSL2-MicA
ΔSL3-MicA
ΔSL1&3-MicA


AphA-GFP
Relative GFP
8
6
4
2
0
Vector
Qrr4
WT-OmrB
ΔSL1-OmrB
ΔSL2-OmrB
ΔSL3-OmrB
ΔSL1&3-OmrB

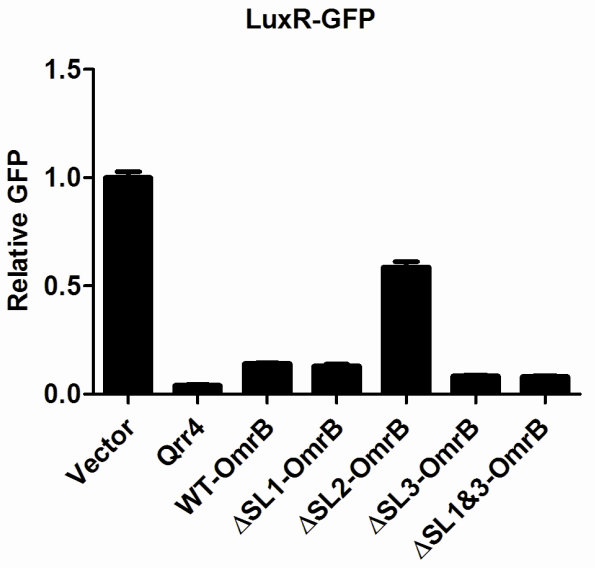
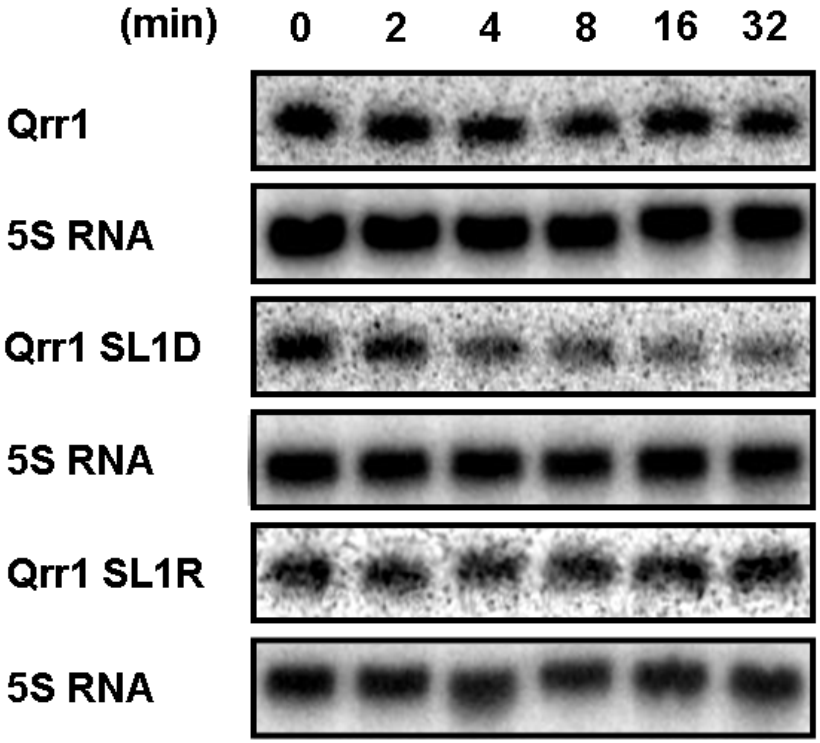

LuxR-GFP
Relative GFP
1.5
1.0
0.5
0.0
Vector
Qrr4
WT-OmrB
ΔSL1-OmrB
ΔSL2-OmrB
ΔSL3-OmrB
ΔSL1&3-OmrB

Figure S8


(min)
0
2
4
8
16
32
Qrr1
5S RNA
Qrr1 SL1D
5S RNA
Qrr1 SL1R
5S RNA

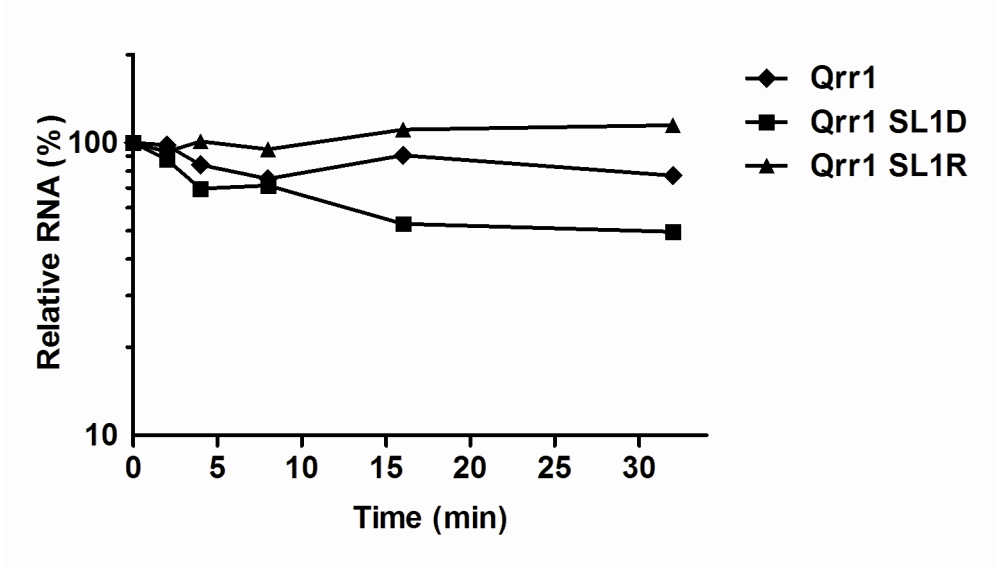
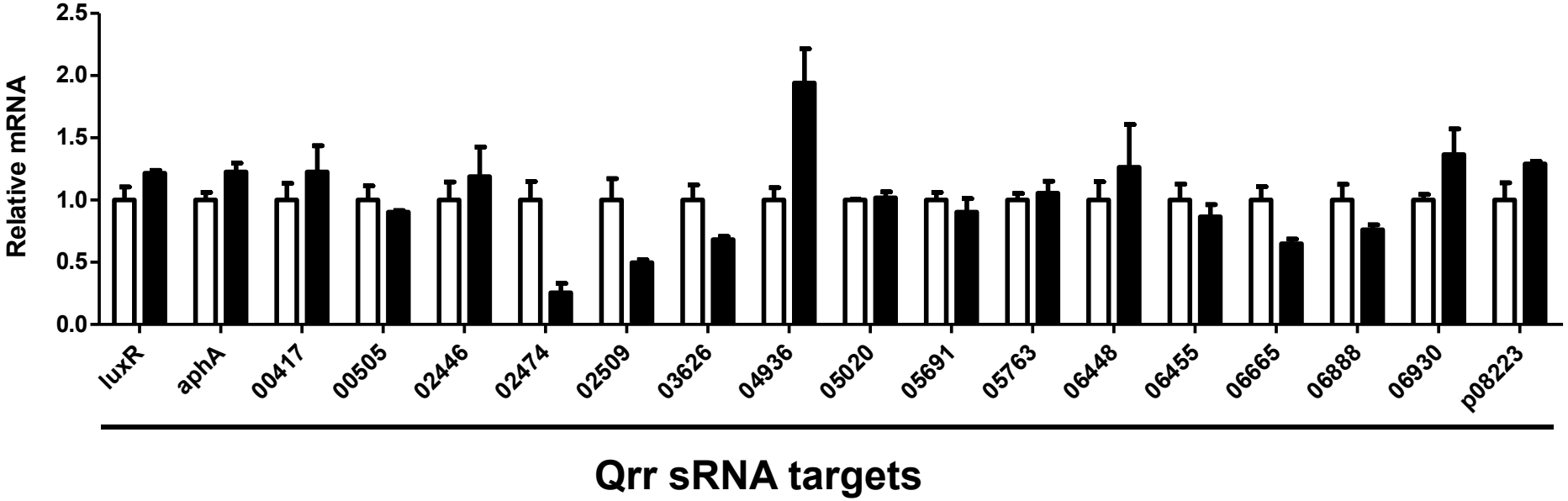

Relative RNA (%)
100
10
0
5
10
15
20
25
30
Time (min)
Qrr1
Qrr1 SL1D
Qrr1 SL1R

Figure S9


Relative mRNA
2.5
2.0
1.5
1.0
0.5
0.0
luxR
aphA
00417
00505
02446
02474
02509
03626
04936
05020
05691
05763
06448
06455
06665
06888
06930
p08223
Qrr sRNA targets