Supplementary Information

Differential RNA-seq of *Vibrio cholerae* identifies the VqmR sRNA as a regulator of biofilm formation

Kai Papenfort, Konrad U. Förstner, Jian-Ping Cong, Cynthia M. Sharma and Bonnie L. Bassler

This supplement contains:

Figures S1 to S8

Tables S1 to S5

Datasets S1 to S4

Supplementary Figure Legends

Supplementary Materials and Methods

Supplemental References

TABLE OF CONTENTS

Supplementary Figure Legends

Figure S1: Accuracy of TSS predictions. Histogram indicating distances between 35 representative TSS identified by dRNA-seq vs. annotated TSS. 94% matched within ±1 nt tolerance.

Figure S2: Intergenic and 5' UTR-derived sRNAs. Total RNA was obtained following growth for the indicated times from wild-type, *luxO* D47E, and Δ*hfq V. cholerae* strains and probed for the designated sRNAs by Northern Blot. The genomic locations of the sRNAs are shown above the gels. Genes are shown in black, sRNAs are shown in red. Arrows indicate TSS. Filled triangles indicate TSS, open triangles indicate processing sites. 5S rRNA served as the loading control. **(A)** sRNAs from intergenic regions **(B)** sRNAs from 5' UTRs of mRNAs.

Figure S3: 3' UTR-derived and *cis*-encoded sRNAs. Total RNA was obtained following growth for the indicated times from wild-type, *luxO* D47E, and Δ*hfq V. cholerae* strains and probed for the designated sRNAs by Northern Blot. The genomic locations of the sRNAs are shown above the gels. Genes are shown in black, sRNAs are shown in red. Arrows and scissors indicate TSS and processing sites, respectively. Filled triangles indicate TSS, open triangles indicate processing sites. 5S rRNA served as the loading control. **(A)** sRNAs from 3' UTRs of mRNAs **(B)** *cis*-encoded sRNAs. VqmR (Vcr107) is transcribed separately from the *vca1078* mRNA (see main text). The genomic locations of the sRNAs are shown above the gels.

Figure S4: Annotation of the VqmA protein and its start site. (A) Alignment of VqmA (Vca1078) protein sequences from eleven vibrio species. The first amino acid is boxed. When the residue is valine (V) the start codon is a GTG. **(B)** Left: schematic drawing of the *vca1078::gfp* translational reporter construct. The relative positions of the annotated ATG start codon and the predicted alternative GTG start codon are indicated. Arrows indicate TSS identified by dRNA-seq (see Figure 3A). Right: Western Blot analysis of Vca1078::GFP. Mutation of the annotated start codon is designated ATG-ATC, mutation of the conserved start codon is designated GTG-GTC, ctr designates the control plasmid. RNAPα served as loading control.

Figure S5: Stability, copy number, and conservation of *vqmR*. (A) Left: *V. cholerae* wild-type and Δ*hfq* strains were grown to OD_{600} of 1.0 and treated with rifampicin (250 µg/ ml) to terminate transcription. Total RNA was collected at the indicated time-points followed by Northern Blotting and analysis of VqmR. 5S rRNA served as loading control. Right: Quantification of data obtained from three independent biological replicates performed for (A). Diamonds; wild-type, squares; Δ*hfq* strain. The dashed line indicates the sRNA half-life (50% of the initial abundance). **(B)**

Alignment of *vqmR* sequences from eight vibrio species. Nomenclature is according to Figure 3B with the addition of *Vibrio proteolyticus* (Vpr). The highly conserved R1 and R2 regions are boxed and marked. **C)** Electrophoretic mobility shift assay (EMSA) showing that VqmA protein does not bind a mutated variant of the *vqmR* promoter sequence. The mutated sequence is indicated in Figure 4C (-47 to -49 relative to TSS). Migration of the [P32] end-labeled DNA fragments in the absence and presence of different concentrations of purified VqmA::3XFLAG protein was determined by native polyacrylamide gel electrophoresis and autoradiography.

Figure S6: Secondary structure of VqmR. Left: Enzymatic probing of the VqmR secondary structure. *In vitro* synthesized and radio-labeled VqmR was treated with RNase T1, RNase V1, and RNase A, designated T1, V1, and A, respectively. C indicates the untreated control, A and T1 indicate RNase ladders for VqmR treated with RNase A and RNase T1, respectively, under denaturing conditions, OH indicates the alkaline ladder. Conserved regions R1 and R2 are marked in red. Right: Schematic representation of the VqmR secondary structure. Conserved regions R1 and R2 are marked in red. Cleavage by RNase T1 (red), RNase V1 (green), and RNase A (blue) is indicated by arrows.

Figure S7: Target gene regulation by VqmR. (A) Translational GFP-fusions to the VqmR-controlled target genes depicted on the x-axis were tested for repression by VqmR in an *E. coli* Δ*hfq* strain. GFP levels were determined in triplicate using a plate reader. Gray bars show GFP production in the presence of the control plasmid (pctr), black bars show GFP production when VqmR is expressed from the plasmid (pVqmR). **(B)** Northern Blot analysis of VqmR, VqmRΔR1, and VqmRΔR2. *E. coli* cells carrying the indicated plasmids were grown to OD_{600}=1.0 and assessed for VqmR levels. **(C)** Predicted base-pairing interaction of VqmR with target mRNAs using RNA hybrid (1). Conserved sequences of VqmR are shown in red. The Shine-Dalgarno sequences and start codons of the mRNAs are boxed. The proposed strength of interaction is indicated below each RNA duplex.

Figure S8: Gene synteny analysis of *vqmR* and *vqmA*. The sequences upstream of *vqmA* genes from vibrios were examined for the presence of *vqmR*. *vqmA* is shown in red and *vqmR* is shown in black. The genes upstream of *vqmR* are shown in gray, and their conservation and orientation vary among vibrios. Nomenclature as in Figure 3B. **(B)** Copy number of VqmR. Total RNA from wild-type *V. cholerae* was collected at the time points indicated followed by Northern Blot. The amounts of RNA were compared to serial dilutions of *in vitro* transcribed VqmR (lanes 4-8). Copy numbers per cell are indicated below the blot. 5S rRNA served as loading control.

Supplementary Materials and Methods

Plasmid construction

A complete list of all plasmids used in this study is in Table S7. Plasmid pKP-331 was constructed by amplification of the *V. cholerae vqmR* gene using oligonucleotides KPO-0456/0457 and ligation into pLF575 (2). Plasmid pKP-333 was made by PCR amplification of the *vqmR* gene using oligonucleotides KPO-0456/0465 followed by ligation into pEVS143 (3). This plasmid served as the template for plasmids pKP-410, pKP-442, pKP344 and pKP-345 using oligonucleotides KPO-0750/0751, KPO-0949/0950, KPO-0493/0494 and KPO-0491/0492, respectively. Cloning of GFP fusions (pKP-337, pKP-338, pKP-444, pKP-346, pKP-347, pKP-353, pKP-354, pKP-358) to target genes has been described previously (4) and oligonucleotides for cloning are listed in Table S8. Plasmids pKP-337 and pKP-347 were used to obtain plasmids pKP-398 and pKP-441 using oligonucleotides KPO-0725/0726 and KPO-0947/0948, respectively. For the GFP-based transcriptional reporter plasmids pKP-361 and pKP386, promoter sequences of *vqmR* and *vpsL* were amplified with oligonucleotides KPO-0460/0461 and KPO-0671/0696 and cloned into pCMW-1. The translational reporter P*vqmA*::GFP (pKP-357) was constructed by amplification of the *vqmR-vqmA* sequence using oligonucleotides KPO460/467 and the product ligated into pEVS143 amplified with oligonucleotides KPO-0092/0285. This plasmid pKP-357 served as th template to obtain plasmids pKP-359 and pKP-366 using oligonucleotides KPO-0468/0469 and KPO-0470/0471, respectively. For plasmid pKP-418, the *vqmA* sequence was amplified with oligonucleotides KPO-0475/0547 and cloned into pEVS143-CAM. Plasmid pKP-367 was constructed by amplifying the *vqmA* sequence using oligonucleotides KPO-0545/0546, and the product was cloned into pKP8-35 (5). Plasmid pKP-437 was constructed by PCR amplification of the *vqmA*::3XFLAG gene (oligonucleotides KPO-0934/0935) from strain KPS-0518 and cloning into pET15b (Novagen) via restriction sites NcoI and BamHI.

Construction of *V. cholerae* mutant strains

A list of all strains used in this study is provided in Table S6. *V. cholerae* C6706 was the wild-type strain used throughout this study. All *V. cholerae* mutants were generated using the pKAS32 suicide vector (6) and established cloning strategies (7). To generate strain KPS-0429, the flanking regions of *vqmR* were amplified with KPO-0495/0496 and KPO-0497/0498 and the fused PCR product was amplified using KPO-0499/0500. The PCR products were ligated into pKAS32 using AvrII/KpnI restriction sites. The plasmid was conjugated into *V. cholerae* and cells were selected for Ampicillin resistance. Single colonies were transferred to new plates selecting for Streptomycin resistance. Finally, cells were tested for the correct muatation usinfg PCR. The

same strategy was used to construct KPS-0431 (oligonucleotides: KPO-0495/0502, KPO-0503/0504, KPO-0499/0505). For strain KPS-0518, the 3XFLAG epitope was introduced downstream of *vpsT* by PCR amplification using oligonucleotides KPO-0527/0528 and KPO-0524/0529. Oligonucleotides KPO-0526 and KPO-0531 were used to amplify the fused PCR product, which was subsequently cloned into pKAS32. A similar strategy was used to make strain KPS-0647 except the *vqmR* promoter was fused upstream of *lacZ* to generate the transcriptional reporter.

RNA structure probing

Secondary structure probing of *in vitro* synthesized and 5' end-labeled RNA was conducted as described previously (8). In brief, VqmR RNA (~0.2 pmol) was denatured, chilled on ice and incubated in 1X structure buffer (Ambion) and 1 µg yeast RNA at 37°C for 10 min. Subsequently, samples were treated with RNase T1, RNase A or RNase V1 (all Ambion) and reactions were terminated at indicated time-points by addition of 2 vol. of precipitation buffer (Ambion). Precipitated samples were washed with 70% ethanol, and resuspended in loading buffer (Ambion). To prepare RNase T1 sequencing ladders, 0.4 pmol RNA was denatured (95°C, 2 min) in 1X sequencing buffer (Ambion) and chilled on ice. RNase T1 was added, and RNA was digested for 5 min at 37°C. Alkaline (OH) sequencing ladders were prepared by incubating 0.4 pmol RNA at 95°C for 5 min in the presence of alkaline hydrolysis buffer (Ambion). Reactions were stopped by addition of 1 vol. of loading buffer. Samples were denatured prior to loading (95°C, 2 min) and separated on 8% PAA/7M urea sequencing gels. Gels were dried and signals were analysed on a Typhoon phosphorimager.

Library construction and sequencing

Libraries for Illumina sequencing (HiSeq) of cDNA were constructed by vertis Biotechnology AG, Germany (http://www.vertis-biotech.com/), as described previously for eukaryotic microRNAs (9), except that the RNA size-fractionation step prior to cDNA synthesis was omitted. Total RNA samples were used for the preparation of two libraries each, either covering all transcripts or being specifically enriched for primary transcripts by treatment with terminator exonuclease (TEX, Epicentre). Next, RNA was treated with Poly(A) polymerase to add poly-A-tails and 5'-triphosphates were removed using tobacco acid pyrophosphatase (TAP). This step resulted in 5'-monophosphates at the ends of transcripts. Subsequently, an RNA adapter was ligated to the 5'-phosphate. First-strand cDNA was synthesized using an oligo(dT)-adapter primer, and M-MLV reverse transcriptase. A PCR-based amplification step was performed to increase the cDNA

concentration to ~20-30 ng/µl. A library-specific barcode for multiplex sequencing was incorporated into the 3'-sequencing adapters. The following adapter sequences flanked the cDNA inserts:

TrueSeq Sense primer

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'

TrueSeq Antisense NNNNNN primer (NNNNNN = 6n barcode for multiplexing)

5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNN-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC(dT25)-3'

The resulting cDNA libraries were sequenced using a HiSeq 2500 instrument (Illumina) in single-read mode for 100 cycles. The raw, de-multiplexed reads as well as the normalized coverage files have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) (10) and are accessible via the GEO accession number GSE62084.

Read mapping and coverage plot construction

Detailed information on data analyses are available at: https://zenodo.org/record/13136. To assure a high sequence quality, the Illumina reads in FASTQ format were trimmed with a cut-off phred score of 20 using the program fastq_quality_trimmer from FASTX toolkit version 0.10.1 (http://hannonlab.cshl.edu/fastx_toolkit/). The following steps were performed using subcommand "create", "align" and "coverage" in the tool READemption (11) version 0.3.0: The poly(A)-tail sequences were computationally removed, and a size filtering step was applied in which sequences shorter than 12 nt were eliminated. The remaining reads were mapped to the reference genome sequence using segemehl version 0.1.7 (12). As reference sequence, two replicons from *V. cholerae* with the accession numbers NC_002505.1 and NC_002506.1 were downloaded from the NCBI ftp server ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Vibrio_cholerae_O1_biovar_El_Tor_N16961_uid57623. As there was a small fraction of fungal contamination in the sample, the following additional replicons NC_001133.9, NC_001134.8, NC_001135.5, NC_001136.10, NC_001137.3, NC_001138.5, NC_001139.9, NC_001140.6, NC_001141.2, NC_001142.9, NC_001143.9, NC_001144.5, NC_001145.3, NC_001146.8, NC_001147.6, NC_001148.4, NC_001224.1 were downloaded from ftp://ftp.ncbi.nih.gov/genomes/Fungi/Saccharomyces_cerevisiae_uid128/.

READemtion's subcommand align was called with the following parameters:
--split -r -p 24 -a 95 -l 12 --poly_a_clipping –progress –x
"Vibrio:NC_002505.1,NC_002506.1;Fungus:NC_001133.9,NC_001134.8,NC_001135.5,NC_001136.10,NC_001137.3,NC_001138.5,NC_001139.9,NC_001140.6,NC_001141.2,NC_001142.9,N

C_001143.9,NC_001144.5,NC_001145.3,NC_001146.8,NC_001147.6,NC_001148.4,NC_00122
4.1".

Mapping statistics (input, aligned, uniquely aligned reads, etc.) can be found in Table S9. Coverage plots in wiggle format that represent the number of aligned reads per nucleotide were generated. The data were visualized using the Integrated Genome Viewer (13). Each graph was normalized using the total number of reads that could be aligned from the corresponding library. To restore the original data range and to prevent rounding of small errors to zero by genome browsers, each graph was subsequently multiplied by the minimum number of mapped reads calculated over all libraries.

Transcription start site prediction

Transcription start sites were predicted based on the normalized wiggle files using TSSpredator (14) with the “more strict” parameter setting.

Meme analysis

Sequences of the TSS and the 50 nts upstream were extracted from the TSSpredator output master table. MEME version 4.9.1 (15) was used to detect motifs of lengths of 45 nt.

Differential gene expression analysis

The predicted TSS were used to extend the gene annotations of the existing *V. cholerae* genome database. Gene expression quantification and expression comparisons were performed based on these extended annotations using the non-TEX treated libraries and READemption in combination with DESeq2 version 1.4.5 (11). Genes changing >1.5-fold (p-value < 0.05) were defined as differentially expressed.

Table S1: Microarray following VqmR pulse expression

Gene[a]	Fold-change[b]	Annotation
vc0200	-2.8	iron(III) compound receptor
vc0201	-2.2	iron(III) ABC transporter, ATP-binding protein
vc1063	-3.2	tesB;acyl-CoA thioesterase II
vc1186	+2.2	sanA protein
vc1187	+2.4	hypothetical protein
vc1188	+3.0	sfcA;malate dehydrogenase
vc1449	-2.2	hypothetical protein
vc1450	-2.4	rtxC;RTX toxin activating protein
vc1865	-2.5	hypothetical protein
vca0068	-17.2	methyl-accepting chemotaxis protein
vca0590	-2.4	peptide ABC transporter, permease protein
vca0591	-2.2	peptide ABC transporter, periplasmic peptide-binding protein
vca0676	-3.6	ferredoxin-type protein NapF
vca0677	-4.2	napD protein
vca0679	-5.9	napB;periplasmic nitrate reductase, cytochrome c-type protein
vca0917	-2.3	transcriptional regulator, TetR family
vca0952	-2.8	transcriptional regulator, LuxR family (VpsT)

a. according to *V. cholerae* N16961 gene annotation
b. Fold-change as obtained by transcriptome analysis using *V. cholerae* specific whole genome microarrays.

Table S2: Bacterial strains used in this study

Strain	Relevant markers/ genotype	Reference/ source
V. cholerae		
KPS-0014	Wild-type C6706	(16)
KPS-0023	*luxO* D47E C6706	(17)
KPS-0053	Δ*hapR* C6706	(17)
KPS-0054	Δ*hfq* C6706	(17)
KPS-0429	Δ*vqmR* C6706	This study
KPS-0431	Δ*vqmA* C6706	This study
KPS-0518	VpsT::3XFLAG C6706	This study
KPS-0519	Δ*hapR*/vpsT::3XFLAG C6706	This study
KPS-0520	Δ*luxO/vpsT*::3XFLAG C6706	This study
KPS-0521	Δ*vqmR/vpsT*::3XFLAG C6706	This study
KPS-0522	Δ*vqmA/vpsT*::3XFLAG C6706	This study
KPS-0647	P*vqmR*::*lacZ*	This study
KPS-0678	Δ*hapR*/ptac-*mKate2*::*lacZ* C6706	(18)
KPS-0932	Δ*hapR* /Δ*vqmR/vpsT*::3XFLAG C6706	This study
KPS-0933	Δ*luxO* /Δ*vqmR/vpsT*::3XFLAG C6706	This study
ZLV-101	Δ*vqmR/vqmA* C6706	(19)
other vibrios		
Vha	*Vibrio harveyi* BB120	(20)
Van	*Vibrio anguillarum 775*	Bassler strain collection
Val	*Vibrio alignolyticus 12G01*	Bassler strain collection
Vfu	*Vibrio furnissii CDC B3215*	Bassler strain collection
Vpa	*Vibrio parahaemolyticus EB101*	Bassler strain collection
Vpr	*Vibrio proteolyticus NCMB 1326*	Bassler strain collection
E. coli		
TOP10	*mcr*AΔ*(mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15Δ*lac*X74*deo*R*rec*A1 *ara*D139Δ*(ara-leu)*7697 *gal*U *gal*K *rps*L *end*A1 *nup*G	Invitrogen
S17λpir	Δ*lacU*169 (Φ*lacZ*ΔM15), *recA*1, *endA*1, *hsdR*17, *thi*-1, *gyrA*96, *relA*1, λ*pir*	(21)
STR1353	F-, DE(*araD-araB*)567, *lacZ*4787(del)::*rrn*B-3, LAM-, *rph*-1, DE(*rhaD-rhaB*)568, *hsdR*514, Δ*hfq*	Bassler strain collection

Table S3: Plasmids used in this study

Plasmid trivial name	Plasmid stock name	Relevant fragment	Comment	Origin, marker	Reference
pctr	pCMW-1		Control plasmid	P15A, Kan^R	(22)
pBAD-VqmR	pKP-331	VqmR	pBAD-VqmR expression plasmid	P15A, Kan^R	This study
pVqmR	pKP-333	VqmR	VqmR expression plasmid	P15A, Kan^R	This study
pVqmRΔR1	pKP-344	VqmRΔR1	VqmRΔR1	P15A, Kan^R	This study
pVqmRΔR2	pKP-345	VqmRΔR2	VqmRΔR2	P15A, Kan^R	This study
p*vpsL::gfp*	pKP-386	*vpsL::gfp*	transcriptional reporter *vpsL*	P15A, Kan^R	This study
p*vpsT::gfp*	pKP-337	*vpsT::gfp*	translational reporter *vpsT::gfp*	PSC101*, Cm^R	This study
P*vca0068::gfp*	pKP-338	*vca0068::gfp*	translational reporter *vca0068::gfp*	PSC101*, Cm^R	This study
P*vca0917::gfp*	pKP-444	*vca0917::gfp*	translational reporter *vca0917::gfp*	PSC101*, Cm^R	This study
P*vc0200::gfp*	pKP-346	*vc0200::gfp*	translational reporter *vc0200::gfp*	PSC101*, Cm^R	This study
P*vc1063::gfp*	pKP-347	*vc1063::gfp*	translational reporter *vc1063::gfp*	PSC101*, Cm^R	This study
P*vc1449::gfp*	pKP-353	*vc1449::gfp*	translational reporter *vc1449::gfp*	PSC101*, Cm^R	This study
P*vca0591::gfp*	pKP-354	*vca0591::gfp*	translational reporter *vca0591::gfp*	PSC101*, Cm^R	This study
P*vc1865::gfp*	pKP-358	*vc1865::gfp*	translational reporter *vc1865::gfp*	PSC101*, Cm^R	This study
P*vqmA::gfp*	pKP-357	*vqmA::gfp*	translational reporter *vqmA::gfp*	P15A, Kan^R	This study
P*vqmA::gfp* (ATC)	pKP-359	*vqmA::gfp*	translational reporter *vqmA::gfp*	P15A, Kan^R	This study
P*vqmA::gfp* (GTG)	pKP-366	*vqmA::gfp*	translational reporter *vqmA::gfp*	P15A, Kan^R	This study
pBAD-ctr	pKP8-35			PBR322, Amp^R	(5)
pBAD-*vqmA*	pKP-367	VqmA	pBAD-*vqmA* expression plasmid	PBR322, Amp^R	This study
p*vqmR::gfp*	pKP-361	*vqmR::gfp*	transcriptional reporter *vqmR*	P15A, Kan^R	This study
pVqmA	pKP-418	VqmA	*vqmA* complementation plasmid	P15A, Cm^R	This study
p*vpsT::gfp* G-17C	pKP-398	*vpsT::gfp*	translational reporter *vpsT::gfp* G-17C	PSC101*, Cm^R	This study
pVqmR C94G	pKP-410	VqmR	VqmR C94G expression plasmid	P15A, Kan^R	This study
P*vc1063::gfp* G-10C	pKP-441	*vc1063::gfp*	translational reporter *vc1063::gfp* G-10C	PSC101*, Cm^R	This study
pVqmR C63G	pKP-442	VqmR	VqmR C63G expression plasmid	P15A, Kan^R	This study
pET-vqmA::3xFLAG	pKP-437	VqmA	Over-expression construct for VqmA::3XFLAG purification	PBR322, Amp^R	This study
pKAS32	-		suicide plasmid for allelic exchange	R6K	(6)

Table S4: DNA oligonucleotides used in this study

Sequences are given in 5' → 3' direction; 5'P denotes a 5' monophosphate.

	Sequence	Description
KPO-009	CTACGGCGTTTCACTTCTGAGTTC	5S rRNA probing E. coli
KPO-0092	CCACACATTATACGAGCCGA	plasmid construction
KPO-0285	GTTTTTTCTAGAGCTAGCAAAGGAGAAGAACTCT	plasmid construction
KPO-0456	P-CAGAGCATGAGTTGCATGAC	plasmid construction
KPO-0457	GTTTTTGGTACCAGCTTATCTTGCCTATTCGG	plasmid construction
KPO-0460	GTTTTTTGTCGACTGGTAGGTAATGTTTGGGAATG	plasmid construction
KPO-0461	GTTTTTTGCATGCATGCTCTGGTTTGTACTTTACC	plasmid construction
KPO-0465	GTTTTTGGATCCAGCTTATCTTGCCTATTCGG	plasmid construction
KPO-0468	CTGATCCTTGGTATCAATATGAT	plasmid construction
KPO-0469	CCAAGGATCAGTCATGCAACTC	plasmid construction
KPO-0470	GGCGTCCCTAACCATCTGA	plasmid construction
KPO-0471	TTAGGGACGCCATATCCTC	plasmid construction
KPO-0475	GTTTTTTGTCGACCAGAGCATGAGTTGCATGAC	plasmid construction
KPO-0477	GTTTTTTATGCATGTTAGCCTTGATGTGTTAGGAG	cloning vpsT::gfp
KPO-0478	GTTTTTTGCTAGCTCTGGATTGCATGCAAACAT	cloning vpsT::gfp
KPO-0479	GTTTTTTATGCATGCATTTATTTTAAATAACGATAATT	cloning vca0068::gfp
KPO-0480	GTTTTTTGCTAGCGAAAAATACCGGTACTACAACCTG	cloning vca0068::gfp
KPO-0484	GTTTTTTGCTAGCAACCGCATCCATAATGGTCT	cloning vca0917::gfp
KPO-0487	GTTTTTTATGCATACTACAAAAGAGGGTCCTAAGTC	cloning vc1063::gfp
KPO-0488	GTTTTTTGCTAGCCCCCTCTTCCAGTTTCTCC	cloning vc1063::gfp
KPO-0489	GTTTTTTATGCATATTAATACTAATAAAAACGATTCTCAA	cloning vc0200::gfp
KPO-0490	GTTTTTTGCTAGCGTGCAAGCGAAGCGCACGA	cloning vc0200::gfp
KPO-0491	P-TATGTTTCTTGTATTTATGCCAG	plasmid construction
KPO-0492	CATGAATCCAGCGAGGGTC	plasmid construction
KPO-0493	P-TGGATTCATGGAACCTCTTC	plasmid construction
KPO-0494	CTGCTGAACACACAGAGGTAT	plasmid construction
KPO-0495	GCCAAACAATATAACCTTGA	strain construction
KPO-0496	GTTTGTACTTTACCGAACGC	strain construction
KPO-0497	GCGTTCGGTAAAGTACAAACATCCAAACAGTCCCCGAATA	strain construction
KPO-0498	CAATCAGTTGGTCTTTGCTCAA	strain construction
KPO-0499	GTTTTTGGTACCTCATGCTTTCGCACTCAGCT	strain construction
KPO-0500	GTTTTTCCTAGGAGTTTGGCCTCATACCCTTC	strain construction
KPO-0502	ATGCGGCGATATGATACCAA	strain construction
KPO-0503	TTGGTATCATATCGCCGCATGCAACAACGTCAAGCTGATTG	strain construction
KPO-0504	TATCTGCGGCGAAAGGCCTA	strain construction
KPO-0505	GTTTTTCCTAGGTTGTGATCTTATTTTGTCGGCC	strain construction
KPO-0521	TGATATCTTGGCTCAACTCA	strain construction
KPO-0522	ACGTTAAGAAGAGGGAAGTC	strain construction
KPO-0523	GACTTCCCTCTTCTTAACGTTTCGTTGTGTAATGTCTCTTC	strain construction
KPO-0524	CACACATACCAACCTCGGT	strain construction
KPO-0525	GTTTTTGGTACCGCGAAGTTTCACGTACTCGC	strain construction
KPO-0526	GTTTTTCCTAGGTCCATTAATCCATCTAACGCCAT	strain construction
KPO-0527	TCCGCTTGATTAAACGTTTGTC	strain construction
KPO-0528	GTCGATATCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCAGAATTGACTTCCTCAATTCCA	strain construction
KPO-0529	ATAAAGATCATGATATCGACTACAAAGATGACGATAAATAGTAATTCGTTGTGTAATGTCTCTTC	strain construction
KPO-0531	GTTTTTTATGCATTTAAAATAAATAATACAATAATAACCC	cloning vc1449::gfp
KPO-0531	GTTTTTTATGCATTTAAAATAAATAATACAATAATAACCC	strain construction
KPO-0532	GTTTTTTGCTAGCAGAAAACACACCTTTATTGTAGT	cloning vc1449::gfp
KPO-0533	GTTTTTTATGCATAAATATAAGGTCATCATAATGATCA	cloning vc1865::gfp
KPO-0534	GTTTTTTGCTAGCAAGCTCCCGGTAGCTTTTC	cloning vc1865::gfp
KPO-0537	GTTTTTTATGCATGTTAACTGAATTATTAATAATTTCA	cloning vca0591::gfp
KPO-0538	GTTTTTTGCTAGCACTGAATTGGCTCGATAAC	cloning vca0591::gfp
KPO-0545	P-GCATTTCCAGTGGAGGATAT	plasmid construction
KPO-0546	GTTTTTTCTAGAATCAGCTTGACGTTGTTGC	plasmid construction
KPO-0547	GTTTTTGGTACCAATCAGCTTGACGTTGTTGC	plasmid construction
KPO-0554	GAGAATAAACCAGAAAGTCGCAG	sense oligo qRT-PCR vpsT
KPO-0555	TCTTTCGCATCAGGACAACTG	antisense oligo qRT-PCR vpsT
KPO-0556	TCATTCGATCAGTGCCTTGG	sense oligo qRT-PCR vca0068
KPO-0557	GGTTGCGGTTGATGTCTTTG	antisense oligo qRT-PCR vca0068
KPO-0558	CCGCTTCGATTTTGTATGGTG	sense oligo qRT-PCR vc0200
KPO-0559	ATCATTACCCACTTGTAGGCC	antisense oligo qRT-PCR vc0200
KPO-0560	TGCCAAACCGACTGTACTG	sense oligo qRT-PCR vc1188
KPO-0561	GATAGCGGGAAGACAATAGGG	antisense oligo qRT-PCR vc1188
KPO-0562	AGAGGCGATTCATTTTGCTTTG	sense oligo qRT-PCR vca0676
KPO-0563	CATTGCGTTACTGGGACAAAC	antisense oligo qRT-PCR vca0676
KPO-0566	CAAGATGTTCGAGTCCGTGAG	sense oligo qRT-PCR vca0591
KPO-0567	GTGTTTTGGAAATAGCTGCGG	antisense oligo qRT-PCR vca0591
KPO-0570	GGCTGAAAGCGATAATGATCTTG	sense oligo qRT-PCR vc1449
KPO-0571	CGGCTTCCATTCTAGGATCTTC	antisense oligo qRT-PCR vc1449
KPO-0572	TTACGAAGATGAGCATGGGC	sense oligo qRT-PCR vc1450
KPO-0573	AGTCGGATGGAGATATTTCGC	antisense oligo qRT-PCR vc1450
KPO-0574	AGGTTGGATGTTGGGTATGC	sense oligo qRT-PCR vc1865
KPO-0575	CAGCTCTTGGAAGATACGGTG	antisense oligo qRT-PCR vc1865
KPO-0578	ATACCTCTGTGTGTTCAGCAG	sense oligo qRT-PCR vqmR
KPO-0579	AGACGGGCTGGCATAAATAC	antisense oligo qRT-PCR vqmR
KPO-0580	TGGATGACTGGCTACTGTTTG	sense oligo qRT-PCR vc1063

KPO-0581	GGCTGTTGCGACCAAATTG	antisense oligo qRT-PCR vc1063
KPO-0582	CCTGCTGTTTCACCACATTG	sense oligo qRT-PCR vca0917
KPO-0583	TGTCCGAATTTCTCTTCCGTC	antisense oligo qRT-PCR vca0917
KPO-0584	GCAAGGTCAGATCGAATCATTTG	sense oligo qRT-PCR hfq
KPO-0585	GGTGGCTAACTGGACGAG	antisense oligo qRT-PCR hfq
KPO-0671	GTTTTTTGTCGACTTCAATGTAAATCCAAAATGTAATACA	plasmid construction
KPO-0696	GTTTTTGCGGCCGCAATATCTGCATCAATTCTAAGACT	plasmid construction
KPO-0725	TTGATCTGTTAGGAGGGGTG	plasmid construction
KPO-0726	TAACAGATCAAGGCTAACATGCA	plasmid construction
KPO-0750	CTACAGGTCGACAAGATATGTTT	plasmid construction
KPO-0751	TCGACCTGTAGAAGAGGTTCC	plasmid construction
KPO-0803	GTTTTTTATGCATACCAAAATATGATTTATTGCGGTT	cloning vca0917::gfp
KPO-0947	AGAGGCTCCTAAGTCATGAGTA	plasmid construction
KPO-0948	TAGGAGCCTCTTTTGTAGTATGC	plasmid construction
KPO-0949	CAGAGCCTCGCTGGATTCAT	plasmid construction
KPO-0950	GAGGCTCTGCTGAACACACA	plasmid construction
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KPO-0243	TTCGTTTCACTTCTGAGTTCGG	5S rRNA probing vibrios
KPO-0331	GAGCCAATCTACAATTCATCAGA	sRNA probing
KPO-0346	TTTGTGTCCGAAATGTACTGACG	sRNA probing
KPO-0379	TTAATAACCCTAGCCATGAGTTGTC	sRNA probing
KPO-0452	ATCTTGTCGACGTGTAGAAGAGGTT	sRNA probing (*vqmR* region R2)
KPO-0539	ACACAGAGGTATCATATTGATACC	sRNA probing (5' end *vqmR*)
KPO-0813	GTGTATCTCAAACCAACCTAATAAG	sRNA probing
KPO-0814	GTGTTTCGTAGTCTTCACTTTCTC	sRNA probing
KPO-0818	GTTCAAGTTCATAGTAAAGTCGTTT	sRNA probing
KPO-0819	TAGGGGAATGGCTCCAAAGAG	sRNA probing
KPO-0820	GGCCTTCTTAGAGTCTTCTAAGAA	sRNA probing
KPO-0821	AGGTTGTCAGAGAGGCCTTGA	sRNA probing
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KPO-0824	CTATCAACTCGAACTAGCCTAGA	sRNA probing
KPO-0826	TCGGACTCATCCTTTGAGCGT	sRNA probing
KPO-0828	GGACCTTGCTCCACCTGATAA	sRNA probing
KPO-0833	CATCAATGCATATTTATCCAAGTAAG	sRNA probing
KPO-0835	GAGCTAGTTTGCTCTACCCTTT	sRNA probing
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KPO-0838	GGTTGCGATCAAAGTACCGATA	sRNA probing
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KPO-00857	GATGGCTCTAAGAAAGTCGCTT	sRNA probing
KPO-0858	CCACTTGAACTCTTATGTACCTC	sRNA probing
KPO-0867	TCATCGCAGGATTAGCAACGATT	sRNA probing
KPO-0873	CTCTCCATGGGACAGAGTCT	sRNA probing
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KPO-0881	CATTGTCATCTGTAACAATCCACA	sRNA probing
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KPO-0918	AGTTCCACTTATCCTCCTAAGC	sRNA probing
KPO-0923	ACAACAGGACCCAGTTGTTTGAT	sRNA probing
KPO-0924	TAACAACGTCAGTTGGCTAGGT	sRNA probing
KPO-0934	CTTGCTTCCCCAATAACCGG	plasmid construction
KPO-0935	GTTTTTCCATGGCTAACCATCTGACATTAGAGCAG	plasmid construction

Table S5: Mapping statistics for *V. cholerae* dRNA-seq

Libraries	D47ES1_0.1 minus_TEX	D47E_S1_0. 1_plus_TEX	D47E_S1_2.0 _minus_TEX	D47E_S1_2. 0_plus_TEX	D47E_S2_0.1 _minus_TEX	D47E_S2_0. 1_plus_TEX	D47E_S2_2.0 _minus_TEX	D47E_S2_2. 0_plus_TEX	WT_S1_0.1_ minus_TEX	WT_S1_0.1 _plus_TEX	WT_S1_2.0_ minus_TEX	WT_S1_2.0 _plus_TEX	WT_S2_0.1_ minus_TEX	WT_S2_0.1 _plus_TEX	WT_S2_2.0_ minus_TEX	WT_S2_2.0 _plus_TEX
No. of input reads	12020323	10134004	6041793	7312970	15984818	15403926	7972525	9330337	13012710	6423924	6719857	6679325	10717828	20739202	7331811	7759407
No. of reads - PolyA detected and removed	8674534	7536490	4189449	3102702	12298501	11040700	5494342	4409395	8622196	3962800	5160930	3220767	8706350	16302487	5249579	4046300
No. of reads - Single 3' A removed	407920	497820	282028	656295	575781	1037249	387562	771477	576353	481959	253071	669546	256527	1068032	332091	659685
No. of reads - Unmodified	2937869	2099694	1570316	3553973	3110536	3325977	2090621	4149465	3814161	1979165	1305856	2789012	1754951	3368683	1750141	3053422
No. of reads - Removed as too short	733608	1198196	299168	221690	1306388	1898173	473496	388582	637372	830044	400704	325092	802474	3117308	348585	486877
No. of reads - Long enough and used for alignment	11286715	8935808	5742625	7091280	14678430	13505753	7499029	8941755	12375338	5593880	6319153	6354233	9915354	17621894	6983226	7272530
Total no. of aligned reads	10536538	8186187	5472276	6890068	13259927	12157299	7159818	8627012	11591377	5191739	5942884	6173775	9120565	15516660	6643865	7036898
Total no. of unaligned reads	602356	710740	257038	196671	1323488	1304246	327270	308792	614663	380711	360059	175089	699857	2030722	325040	230462
Total no. of uniquely aligned reads	2838220	2525662	4018715	4970468	3506745	3646857	5321223	5848685	3641476	2091139	4110536	3996992	1968041	4156019	4369033	4179564
Total no. of alignments	35069849	25876009	12036938	17467196	41408199	36949222	15484789	24190661	38080442	16187184	13369414	18172308	28413639	47196025	17211719	22726156
Total no. of split alignments	35539	20279	23993	9857	23417	37026	11812	10555	38348	10394	10721	9642	29612	49468	15720	8604
Percentage of aligned reads (compared to total input reads)	87.66	80.78	90.57	94.22	82.95	78.92	89.81	92.46	89.08	80.82	88.44	92.43	85.1	74.82	90.62	90.69
Percentage of uniquely aligned reads (in relation to all aligned reads)	26.94	30.85	73.44	72.14	26.45	30	74.32	67.8	31.42	40.28	69.17	64.74	21.58	26.78	65.76	59.39

Supplementary References

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Figure S1

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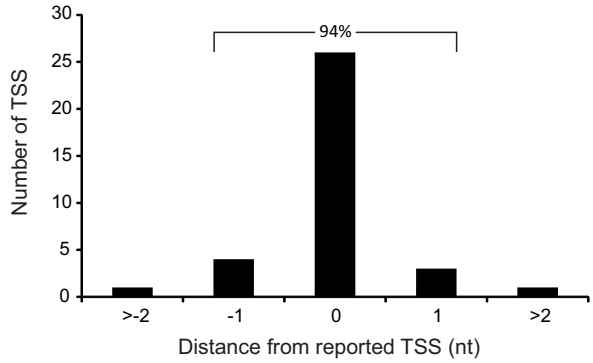

Figure S2

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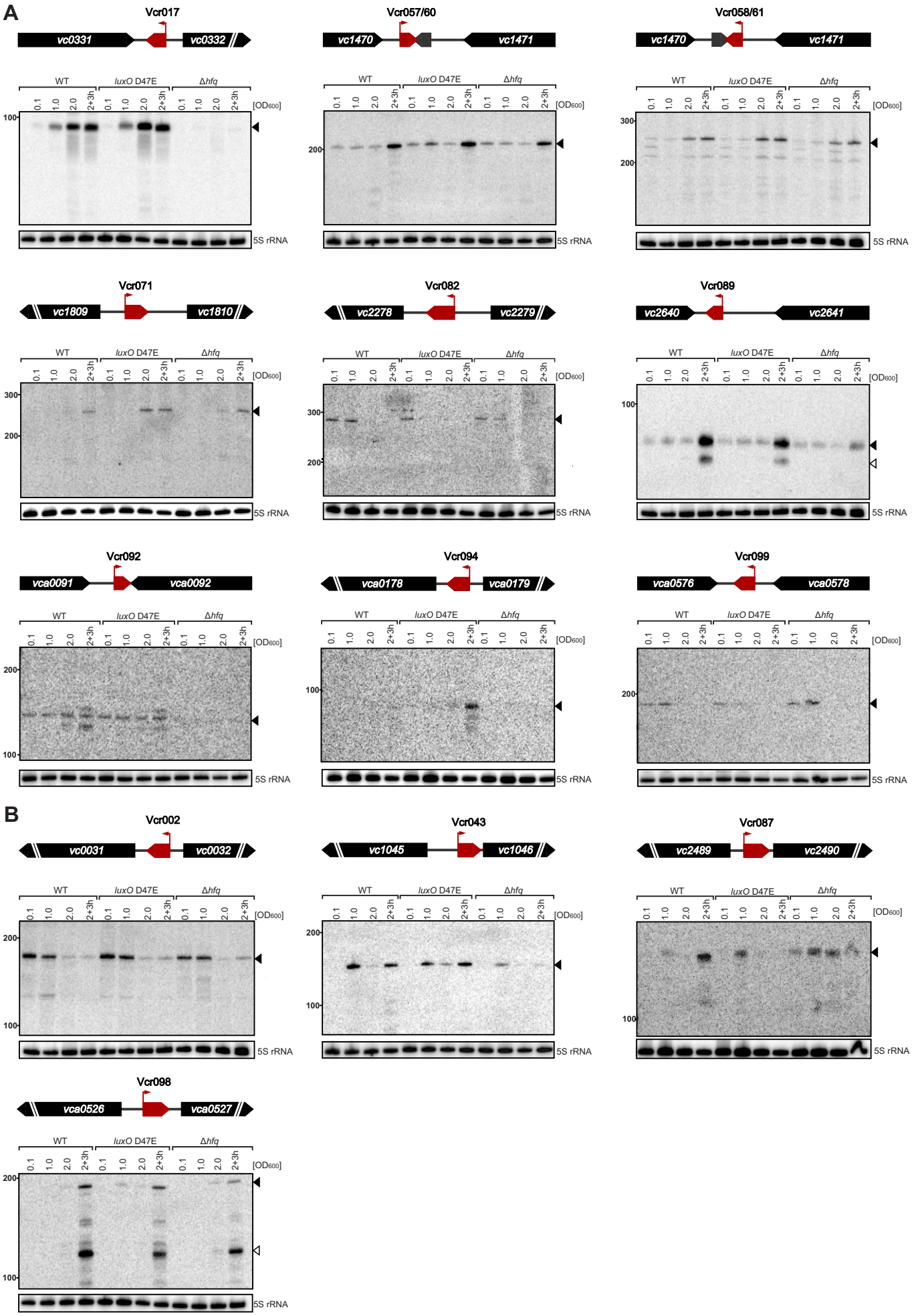

Figure S3

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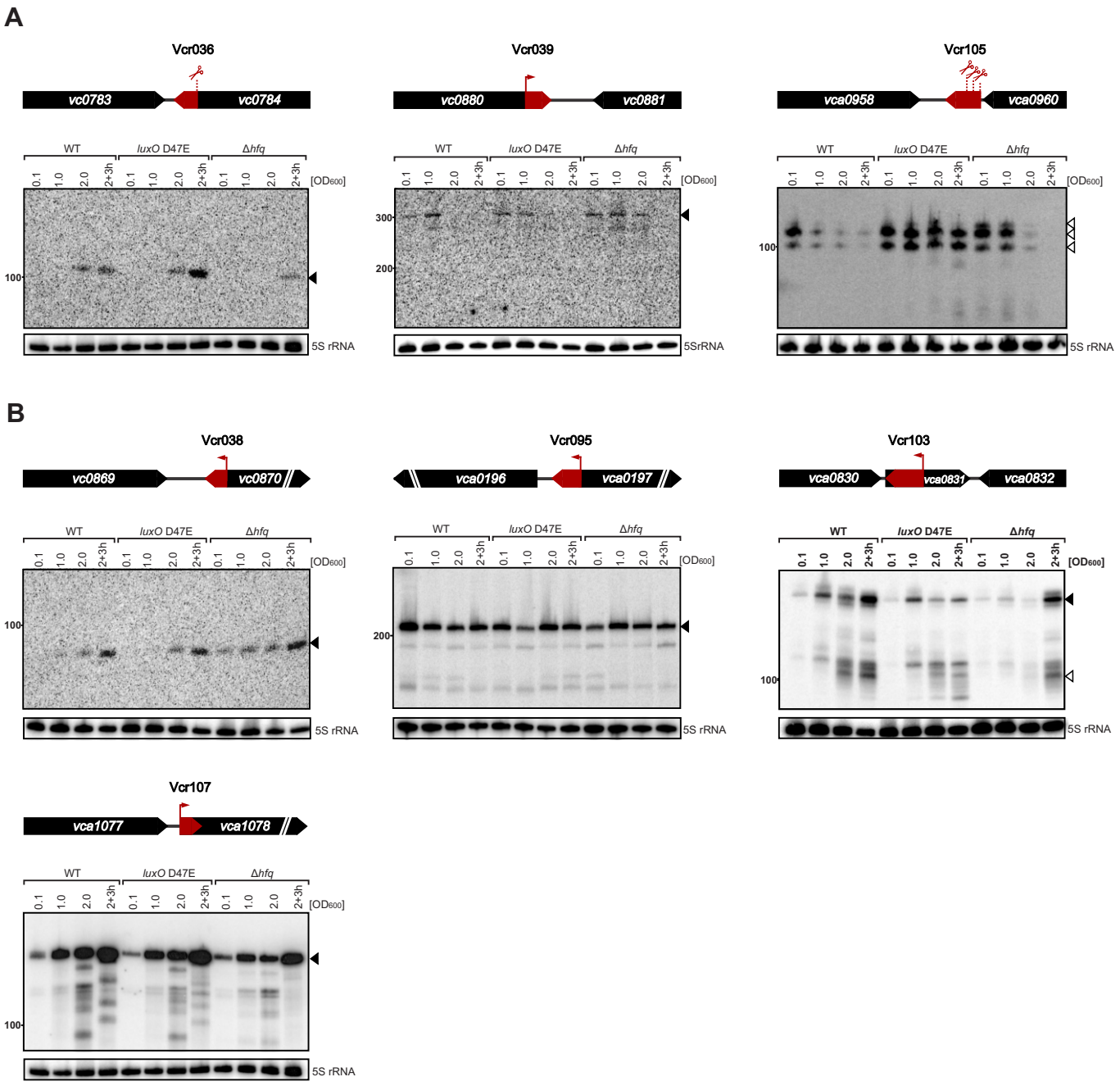

Figure S4

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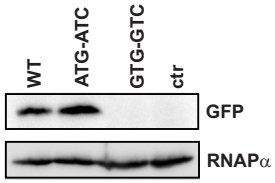

Figure S5

Papenfort *et al*., 2015

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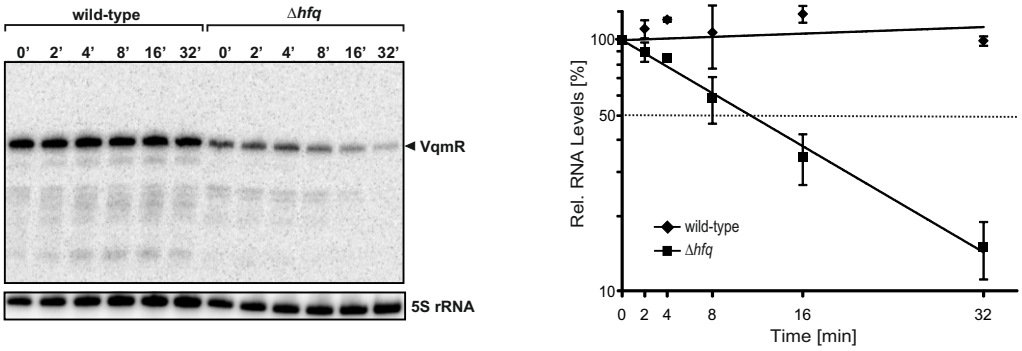

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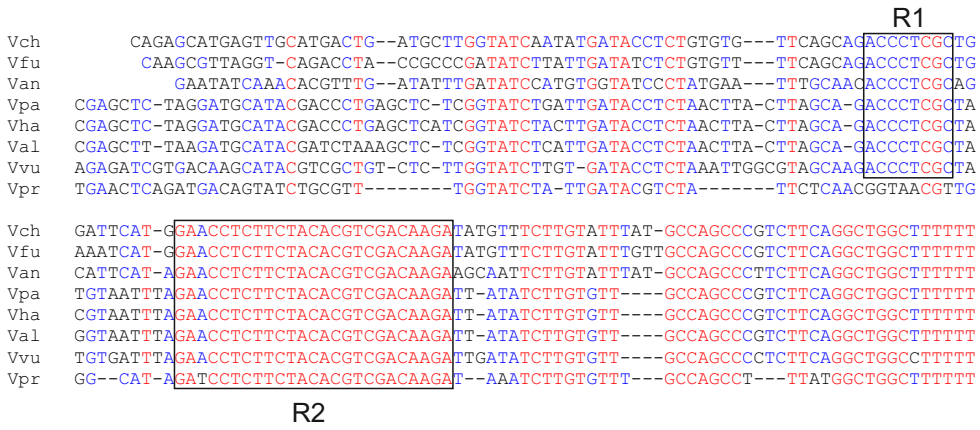

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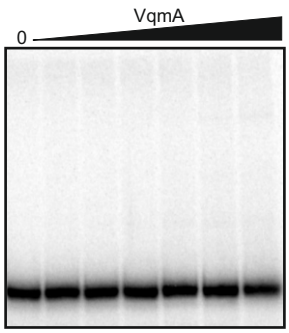

P*vqmR*mut

Figure S6

Papenfort *et al*., 2015

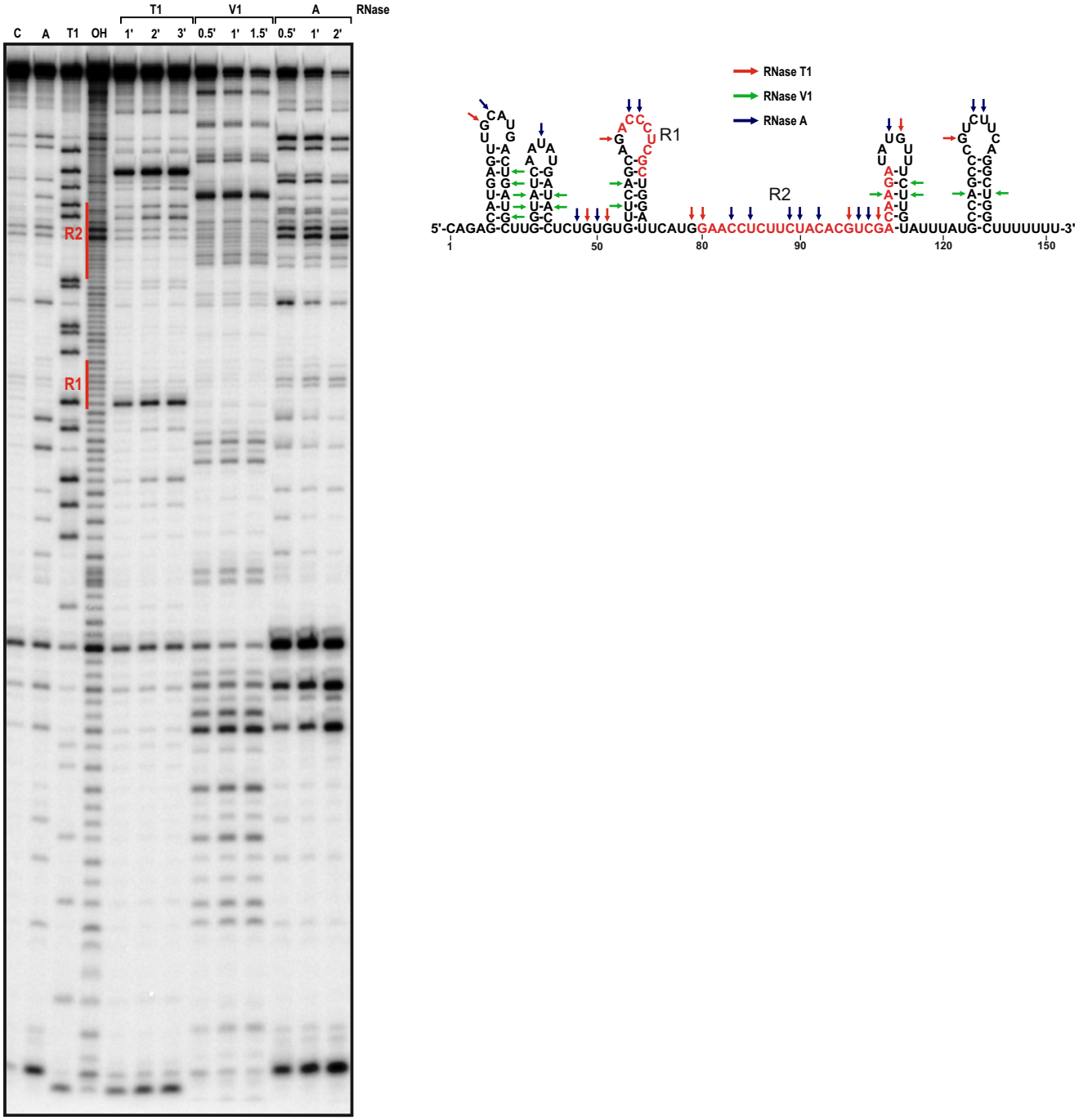

Figure S7

Papenfort *et al*., 2015

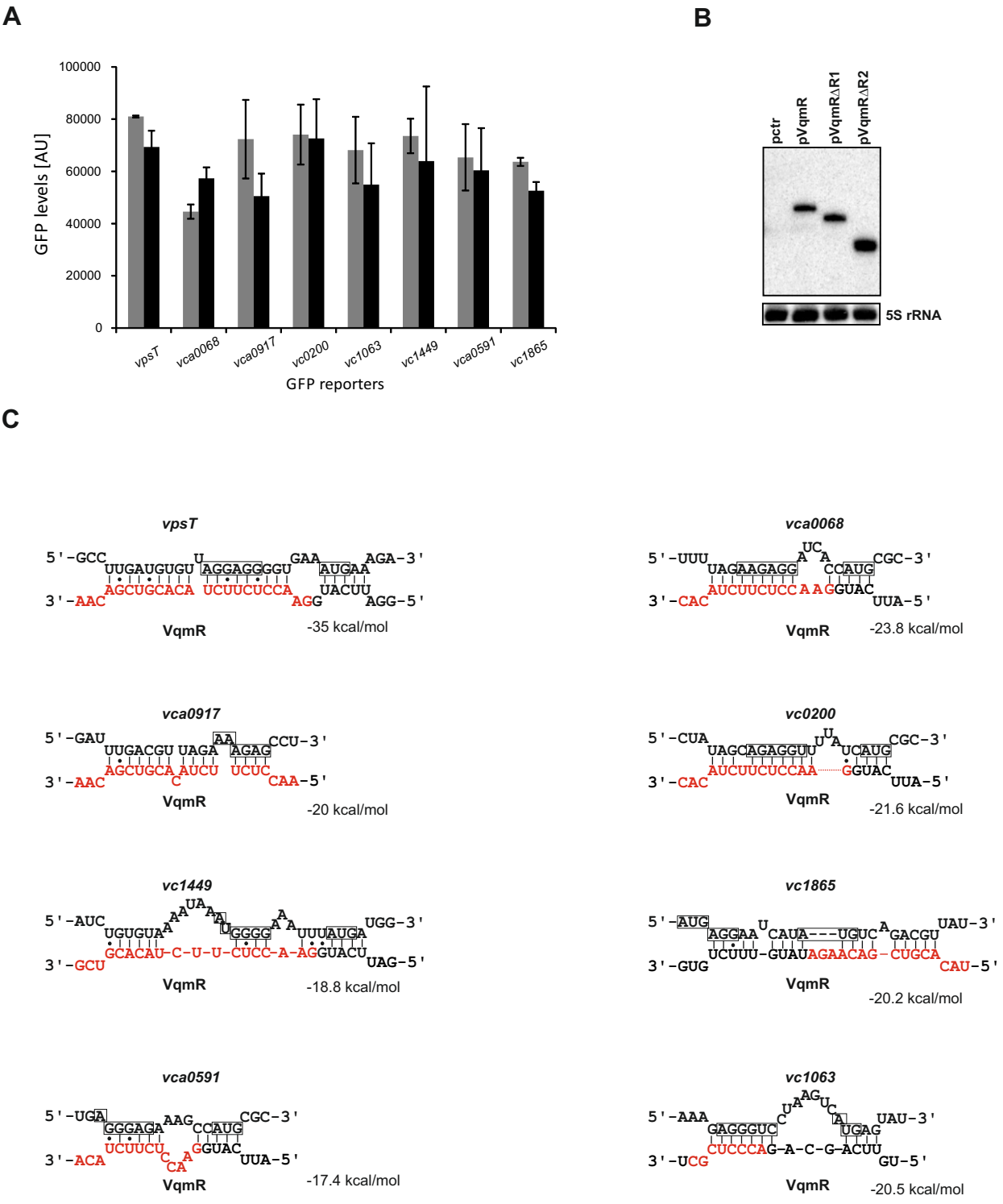

Figure S8

Papenfort *et al*., 2015