Prediction, microarray, and Northern blot analyses identify new intergenic small RNAs in *Aliivibrio salmonicida*

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Abstract

Bacterial small RNAs (sRNAs) are *trans*-encoded regulatory RNAs that typically bind mRNAs by short sequence complementarities and change the expression of the corresponding proteins. Some of the well characterized sRNAs serve critical steps in the regulation of important cellular processes, such as quorum sensing (Qrr), iron homeostasis (RyhB), oxidative stress (OxyS), and carbon metabolism (Spot42). However, many sRNAs remains to be identified, and the functional roles of sRNAs are known for only a small fraction. For example, of the hundreds of candidate sRNAs from members of the bacterial family *Vibrionaceae*, the function is known for only nine. We have in this study significantly contributed to the discovery and verification of new sRNAs in a representative of *Vibrioneceae*, i.e., the *Aliivibrio salmonicida*, which causes severe disease in farmed Atlantic salmon and other fishes. A computational search for intergenic non-coding (nc) RNAs in the 4.6 Mb genome identified a total of 252 potential ncRNAs (including 233 putative sRNAs). Depending on the set threshold value for fluorescence signal in our microarray approach, we identified 50-80 putative ncRNAs that are expressed under different growth conditions, twelve of which were verified by Northern blot analysis. In total we identified nine new sRNAs.

Introduction

Bacteria contain a diverse set of non-coding (nc) RNAs [1]. Some of these RNAs, such as RNaseP, tRNAs, rRNAs, and tmRNA, serve "housekeeping" functions, CRISPR RNAs protect the bacteria from viruses and plasmids [2], whereas other classes of ncRNAs serve as regulators in gene expression. *cis*-encoded RNA regulators are typically part of mRNAs and located in front of protein coding regions. They change the expression of the corresponding protein by binding to small metabolites (i.e., riboswitches) [3]. Other regulatory RNAs are transcribed in *trans* from separate promoters located in intergenic regions (IGRs), and, finally, some regulatory RNAs are transcribed from protein coding regions, but from the opposite strand (i.e., anti-sense RNAs).

The majority of *trans*-encoded regulatory RNAs in bacteria are small in size (hence the name small RNAs, or sRNAs) and act by targeting mRNAs by short imperfect sequence complementarities, thus, modulating expression of their targets [4]. The result can be change in cellular processes, such as iron homeostasis [5, 6], quorum sensing [7], sugar metabolism [8], oxidative stress [9], and virulence [10]. sRNAs were first discovered in *Escherichia coli* 30 years ago [11, 12], but their abundance and important functional roles have only recently been acknowledged. By the end of the 20th century, only 10 sRNA were known in *E. coli* [13], but this quickly changed when several research labs started genome-wide systematic searches [14-17]. After these initial studies in *E. coli*, a number of similar works have resulted in a growing list of sRNAs, or putative sRNAs, from other bacteria (mostly pathogens), e.g., *Bacillus subtilis* [18], *Vibrio cholerae* [19, 20], *Pseudomonas aeruginosa* [21], *Staphylococcus aureus* [22], and *Listeria monocytogenes* [23]. As of November 2010, the sRNAMap database lists 79 sRNAs in *E. coli* strain K12 MG1655 (87 in all *E. coli*) and a total of 397 in 28 bacterial species [24]. Several approaches, such as bioinformatic prediction, microarray, RNA-seq, Hfq co-immunoprecipitation, and RNomics have proven useful in genome-wide detection of bacterial sRNAs [25], and we expect that a significant number of sRNAs will be discovered in future studies.

The relatively large *Vibrionaceae* family of gamma-proteobacteria contains a number of serious pathogens of humans (e.g., *V. cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*) and animals (e.g., *Vibrio coralliilyticus*, *Vibrio anguillarum*, and

Aliivibrio salmonicida) (reviewed in [26]). This group of bacteria is therefore of broad interest, and several recent efforts have focused on genome-wide discovery of sRNAs in representatives of *Vibrionaceae*, mostly using *V. cholerae* as the model [19, 20]. Together, these studies identified hundreds of putative sRNAs and verified the presence of thirteen by Northern blot analysis. Experimental evidence of specific roles of sRNAs of vibrios and aliivibrios (bacteria from the two genera *Vibrio* and *Aliivibrio*, respectively) are known for Qrr1-5 and the redundant CsrB, CsrC, and CrsD, which all regulate quorum sensing [27, 28], RyhB, which is involved in iron homeostasis [29], MicX [30] and VrrA [31], which regulate expression of outer membrane proteins, IGR7, which has a role in carbon metabolism [20], and MRB RNA I, which regulates plasmid replication [32].

We have in this study performed a genome-wide computational search for ncRNAs in intergenic regions with special focus on sRNAs in the genome of the Gram-negative and cold-adapted *A. salmonicida*. The bacterium is the causative agent of cold-water vibriosis (or "Hitra disease") in farmed Atlantic salmon, sea farmed rainbow trout, and captive Atlantic cod [33]. Computational predictions were used for custom design of a microarray chip containing ncRNA predictions as well as nearly all open reading frames. Twenty-eight ncRNAs, including 26 previously unrecognized sRNAs, were tested by Northern blot analysis.

Results and discussions

A. salmonicida total RNA is rich in RNAs of 150-200 nt

To evaluate the potential for finding new sRNAs in *A. salmonicida*, we initially cultivated the bacterium and sampled cells at early, mid, and late exponential phase (i.e., OD_{600} 0.15, 0.75, and 1.0). Total RNA from these samples was separated on a 5 % polyacrylamide/8 M urea gel (Fig. 1). Major bands were identified as 23S, 16S, and 5S rRNAs and multiple tRNAs based on predicted sizes and previous publications [e.g., 22]. Interestingly, a number of faint but distinct bands in the size range of 150-200 nt are clearly visible. The size range overlaps well with the expected sizes of bacterial sRNAs [24]. To evaluate if at least a fraction of these bands could correspond to sRNAs, or other types of ncRNAs, we isolated RNAs of 150-300 nt in length from the gel and cloned the corresponding cDNA in a shot-gun approach. Sanger sequencing of 110 clones revealed that the majority of these corresponded to rRNA (60 %) or mRNA (25 %) fragments, and only five clones mapped to intergenic regions. In summary, our initial survey showed that *A. salmonicida* total RNA contains numerous small RNA species. The majority of these correspond, however, to rRNA or mRNA fragments and only a very limited number (~4 %) map to IGRs (i.e., potential sRNAs).

Bioinformatic prediction of 252 putative ncRNAs

The experimental data described above provided us with limited information about the potential for finding sRNAs, so in a parallel approach, we used bioinformatics to predict ncRNA genes in IGRs. The *A. salmonicida* genome consists of two chromosomes and four plasmids [34]. From these, we extracted a total of 3308 IGRs, constituting 23 % of the genome. We considered only IGRs ≥50 nt in length, which delimited the number of IGRs to 2488 (approx. 21 % of total genome). The majority of these (1828) map to chromosome I.

A search in Rfam [35] identified 25 homologs of known ncRNAs (listed in Table S1), distributed among the categories sRNAs, riboswitches, RNaseP, and tmRNA (excluding tRNAs and rRNAs). Next, putative sRNAs were predicted based on three main criteria: (1) co-localization of putative promoters and/or transcription factor binding

sites and Rho-independent terminators (50 – 500 nt apart), (2) high sequence conservation between relatively closely related species, and (3) conservation of synteny of flanking genes. Fig. 2 shows how the 252 predicted ncRNAs (196 in ChrI and 56 in ChrII) are distributed in size from 65 to 392 nt. The majority are between 101-150 nt and 151-200 nt (73 and 92, respectively), and 87 % percent are ≤ 250 nt, which is in agreement with the typical size of sRNAs (233 of 252 predicted ncRNAs are candidate sRNAs). Promoters were predicted upstream of 48 by BProm (http://www.softberry.com) and Patser [36], Fur-boxes were predicted upstream of 14, and 89 were found associated with Rho-independent terminators using TransTerm. Compared to other available bioinformatic sRNA prediction tools, 192 of the predicted sRNAs were recovered by either sRNAfinder [37] or QRNA [15]. Finally, 222 are also found in one or more members of the *Vibrionaceae* family.

Global validation of ncRNA predictions using microarray

Next, we wanted to validate our ncRNA predictions on a global scale and used a custom designed microarray (*Vibrio salmonicida* V1.0.1 AROS) from Operon (now Eurofins) based on 70-mer oligos. The microarray contains oligos complementary to all the 252 predicted ncRNAs (and nearly all the 4282 protein coding genes, which are not considered here). We assumed that only a fraction of the sRNAs would be expressed under normal lab conditions, and we therefore subjected *A. salmonicida* to low iron conditions and oxidative stress. Cells were grown to mid exponential phase (OD_{600} ~0.5), subjected to sub-lethal stress conditions by adding 50 µM 2,2'-dipyridyl or 100 µM fresh hydrogen peroxide, respectively, and samples were collected 15, 30, and 60 min after the addition of chemicals. Treated samples were compared to untreated control samples. To find sRNAs with potential roles in quorum sensing, we also analyzed gene expression in a LitR deletion mutant *(ΔlitR)* of *A. salmonicida* (the *ΔlitR* mutant will be described in detail elsewhere). LitR is the homolog of HapR, which is the master regulator of quorum sensing in *V. cholerae*, (reviewed in [38]). Here, samples were collected at low, mid, and high exponential growth phase (OD_{600} 0.15, 0.5, and 0.8, respectively) and compared to identical samples of wild-type.

Table 1 shows a summary of the microarray results (complete lists are available in

Table S2), and Fig. 2 provides a graphical overview of how expressed ncRNAs are distributed into different size categories. Regardless of origin of samples (i.e., treated or untreated samples, mutant, or wt), 134, 142, and 152 ncRNAs produced fluorescence signals >2 fold above background and 7, 4, and 15 were differentially expressed under low iron, H_2O_2 conditions, or in *ΔlitR*, respectively. However, these numbers are likely inflated due to unspecific signals and/or false positive predictions. A more conservative estimate would be to only consider spots with Cy5 or Cy3 fluorescence intensities above certain threshold values. We considered three different threshold values, i.e., >50, >75, and >100. These were chosen based on the following observations and assumptions: Of the approximately ~4200 genes that are spotted on the chip, about 3600 were retained after bioinformatic analysis. With threshold values set at >50, >75, or >100, approximately 1200, 800, or 700 genes are retained, respectively, which would suggest that at least 33 % (1200/3600), 22 % (800/3600), or 19 % (700/3600) of the genes are expressed under our growth conditions when using microarray technology. These numbers are very modest when compared to recent RNA-seq data from for example *Bacillus anthracis*, which suggests expression of ~94 % of the entire genome [39]. Even though it is difficult to directly compare data from these two very different technologies, the data indicate that our set threshold value of >100 is relatively conservative because it is reasonable to believe that more than 19 % of the genes should be expressed during growth.

Table 1 shows that the average number of spots with fluorescence values above 50, 75, and 100 are 83, 63, and 50, respectively. These spots represent putative sRNAs. Given that the total number of sRNAs in *E. coli* is 87, and that *A. salmonicida* has a similar number of sRNAs, then it is reasonable to believe that many of the identified spot could represent genuine sRNAs.

Nine new sRNAs detected by Northern blot analysis

Candidate sRNAs from Table 1 were next tested by Northern blot analysis. The experimental setup was identical to that used for microarray, except that cell samples were collected 7.5, 15, and 30 min after treatment (instead of 15, 30, and 60 min) or at ODs 0.15, 0.5, 0.8, and 1.2 (OD 1.2 was added) for the *ΔlitR* mutant. Probes were

designed for 26 putative sRNAs, some of which were differentially expressed in the microarray experiment. The Qrr sRNA, RNaseP, and 5S rRNA were included as controls.

Fig. 3 shows a summary of resulting Phosphoimage scans of ten putative sRNAs (VSsrna 7, 21, 108, 145, 152, 185, and 190 on Chr I and VSAsrna 8, 17, and 46 on Chr II) and the three controls. Table 2 shows more details on these validated RNAs. VSsrna185 is a homolog of the B2 sRNA, which was found in *V. cholerae* [19]. Predicted and actual RNA sizes are indicated. Thirteen probes did not produce a visible signal, and probes for VSsrna 10, 42, and 115 on Chr I produced multiple bands that could not be resolved and are not shown. As expected, Qrr was differentially expressed in the Δ*litR* mutant, from 2 to 5.5 fold upregulation (compared to wild-type), which is in agreement with the microarray results (3.4 to 5.6 fold upregulation). In *V. cholerae*, the gene expression levels of Qrr are fine tuned by two feedback loops that involves HapR (i.e., the LitR homolog) and LuxO, with HapR indirectly activating expression of Qrr (see [40]). It is unclear to us why the level of Qrr is significantly higher in the *A. salmonicida* LitR mutant, but one possible explanation is that the *litR* mRNA target is absent and Qrr will therefore not be degraded with its target. It should be noted that the number of *qrr* genes varies between members of *Vibrionaceae,* for example, one in *A. salmonicida* and *Vibrio fischeri*, four in *V. cholera*, and five in *V. vulnificus* and *Vibrio harveyi* [41], which suggest species-specific regulation of *qrr* expression.

Probes targeted to VSsrna7, VSsrna17, VSsrna108, VSsrna152, and VSAsrna8 each produced one distinct band in close agreement with the predicted size. Probes against VSsrna21, VSsrna145, and VSsrna190 produced, in contrast, bands corresponding to larger RNAs than predicted, which is not unexpected since prediction of promoters and Rho-independent terminators is relatively unreliable, even in the model organism *E. coli*. The VSAsrna46 probe produced three distinct bands with the strongest band corresponding to an RNA close to the predicted size.

Interestingly, the resulting data suggest that several sRNAs are differentially expressed. In the *ΔlitR* mutant, VSsrna7, VSsrna108, VSAsrna8, and VSAsrna46 are all up-regulated during late exponential phase. VSsrna21 is in contrast down regulated ~100 fold. The on/off-like expression pattern of VSsrna21 indicates that it might have a direct role in quorum sensing. It does not share significant sequence conservation with any

known sRNAs but appears to be conserved in other bacterial species (Table 2). Under low iron conditions, VSsrna7 is up-regulated 2.4 and 2.9 folds, 7.5 and 15 min after treatment, respectively, whereas VSsrna190 is down regulated after treatment compared to the wild-type. Under oxidative stress, VSsrna7 and VSsrna108 are up-regulated 2.1 and 2.5 folds, respectively, 15 min after stimulation. The two latter ncRNAs were not noted as differentially expressed using microarray, which demonstrates the importance of using other direct methods, like Northern blot analysis for verification of results.

In summary, of the 28 microarray spots that were chosen for further investigation using Northern blot analysis, we convincingly identified 11 sRNAs from *A. salmonicida*, including nine new sRNAs, the homolog of B2 from *V. cholera*, and Qrr. Six of the new sRNAs are in close agreement with predicted sizes and three are longer than predicted (Fig. 3). Eight (VSsrna 7, 108, 145, 152, and 190 and VSAsrna 8, 17, and 46) produced spots with high intensities (>500) in microarray analysis, whereas one (VSsrna21) produced lower fluorescence signal (i.e., 76). We have tested with Northern blot analysis the majority of putative sRNAs that produced high-intensity spots in microarray analysis, and we find it reasonable to believe that many of these, if not all, are genuine sRNAs. We expect, however, that many more sRNAs remains to be discovered in *A. salmonicida*, also among the predicted RNAs that were identified with microarray analysis, but that remains to be further tested.

Comparison to other ncRNA studies from the family *Vibrionaceae*

A few studies have during recent years reported the discovery of ncRNAs in related members of *Vibrionaceae* [19, 20, 42], and we wanted to compare our findings with these results. Twenty-five of our predicted ncRNAs are homologs of known ncRNAs found in Rfam and are distributed among the classes riboswitch (10), RNaseP (1), tmRNA (1), sRNA (6), SRP-RNA (1), anti-sense RNA (1), t44 (1), group II intron (1), and mRNA leaders (3) (see Table S1 for details). Compared to results from *V. cholera*, our predictions include 14 of 27 previously verified or putative IGR ncRNAs [19, 20, 42], including three of six ncRNAs (sRNApred B2, B4, and C1) that were predicted by sRNAPredict and confirmed by Northern analysis [19] and four of the seven IGR sRNAs that were found using direct cloning and parallel sequencing [20]. Of the seven IGR

RNAs from the latter study, our prediction did not include IGR1, IGR4, and IGR6. IGR1 might be present in *A. salmonicida* (blastn E-value = 0.065 and total score = 32), whereas IGR4 and IGR6 are only found in *V. cholerae.*

Using RNA-seq, Liu et al. [20] reported 500 IGR RNAs in *V. cholerae* that were considered as putative sRNAs. We removed from this list redundant/overlapping RNA sequences and compared the resulting 251 non-redundant IGR sequences (199 and 52 in chromosomes I and II, respectively) with our 252 predicted ncRNAs using blastn and the search criteria E-value ≤ 0.01 and total score ≥ 30. This search identified 21 significant hits (see Table S1 for details). Nine hits represent homologous of previously known ncRNAs in Rfam (six riboswitches and three sRNAs) and 12 represent novel sRNAs (four of which have been confirmed with Northern blot analysis).

In a recent study, identification of ncRNAs was performed on environmental vibrios [42] using a combination of *in silico* search (Rfam) followed by microarray-based expression profiling. Microarray analysis revealed 21 ncRNAs that were expressed in mid-exponential phase, and a subset of six ncRNAs were tested with reverse transcription PCR (RT-PCR). Our predictions identified 17 of these 21 ncRNAs.

Concluding remarks

We have used computational and experimental methods to identify intergenic ncRNAs (mostly sRNAs) in the marine bacterium *A. salmonicida*. This is the first genome-wide study of its kind for a fish pathogen. Of the 252 tested ncRNAs, our microarray analysis suggests that approximately ~50 (i.e., when using a conservative threshold value) are expressed under our laboratory growth conditions, and Northern blot analysis verified expression of nine new sRNAs. Our prediction list and expression profiling data also includes sRNA candidates recently found in *V. cholerae* with RNA-seq and other methods. One of our current goals is to study the presumably important roles of sRNAs in the development of cold-water vibriosis in Atlantic salmon (and other marine fish species) caused by *A. salmonicida*. By comparing our data with results from other representatives of *Vibrionaceae*, such as the infamous human pathogen *V. cholerae*, we are discovering sRNAs that are broadly distributed in *Vibrionaceae* as well as sRNAs that are limited to aliivibrios and *A. salmonicida* in particular. These analyses will

hopefully guide us to better understand the biological roles of sRNAs in this important group of pathogens.

Materials and Methods

Bacterial strains and growth conditions

A. salmonicida LFI1238 [34] and an isogenic *ΔlitR* mutant (Bjelland et al. unpublished) were cultured in LB medium containing 2.5 % NaCl at 12 °C and 200 rpm. Cells were subjected to H_2O_2 or 2,2`-dipyridyl after growth in 200 ml culture flasks to mid-exponential phase ($OD_{600}$ ~0.5). Cells were next split into three equally sized cultures. $H_2O_2$ was added to one flask to a final concentration of 100 μM, the iron chelator 2,2`-dipyridyl was added to one flask to a final concentration of 50 μM, and, finally, one flask was used as the non-treated control. Two 5 ml samples were collected 7.5, 15, and 30 min after treatment, cells were harvested by centrifugation (3,500 × g, 10 min), flash-frozen, and kept at -70 °C prior to RNA isolation. The construction and characterization of the *A. salmonicida ΔlitR* mutant will be described elsewhere (Bjelland et al., unpublished) but was used here to find sRNAs that have potential roles in quorum sensing. The *ΔlitR* mutant was grown in 60 ml cultures and samples were collected at OD_{600} 0.15, 0.5, and 0.8 for microarray analysis and OD_{600} 0.15, 0.5, 0.8 and 1.2 for Northern blotting. Wild type strain was used as control. Samples were subsequently treated as described above.

Northern blot analyses

Total RNA was isolated from bacterial cultures using the isol-RNA reagent (5 PRIME) and quantified with NanoDrop (Thermo Fisher Scientific). Approximately 10 μg of total RNA was separated on 5 % polyacrylamide/8 M urea denaturing gels and transferred to a positively charged Hybond-N+ nylon membrane (GE Healthcare) by a TE70 series SemiPhor Semi-Dry Transfer Unit (Hoefer, Inc.). Subsequent procedures were done essentially as previously described [6]. Briefly, RNAs were detected on membranes by using [α-^{32}P] dCTP-labeled double-stranded DNA probes, and signals were collected on phosphoimaging screens (Fujifilm) and scanned on a BAS-5000 phosphoimager (Fujifilm). The ImageGauge software v4.0 (Fujifilm) was used to measure the strength of signals, and the 5S ribosomal RNA was used to normalize the resulting values.

Microarray analyses

Microarray analysis was run as described in [43]. Briefly, DNA was removed from total RNA preparations prior to a cleanup step with RNeasy MinElute spin columns (Qiagen). The quality of RNA was tested, and cDNA was constructed from 15 μg purified RNA using the Aminoallyl cDNA Labeling Kit (Ambion) and CyDye™ Post-Labeling Reactive Dye Pack (GE Healthcare) for labelling. Labelled samples were hybridized to "*Vibrio salmonicida* V1.0.1 AROS" slides (Eurofins), and slides were subsequently washed. Experiments were run in triplets. Finally, slides were scanned, and resulting expression data were analysed using J-Express Pro v2.7 [44]. Microarray data has been uploaded to The NCBI Gene Expression Omnibus (GEO) database and is available through accession number GSE25558. Expression data from H_2O_2-treated cells was extracted from GSE20082 [43].

Computational prediction of sRNAs

The search for potential sRNA genes in *A. salmonicida* was limited to IGRs. We define an IGR as the region between two annotated genes on either strand that is delimited by its closest left and right flanking genes. Briefly, our sRNA genes were identified by searching for co-localization of genetic features that are associated with bacterial sRNA genes. These include (1) the presence of putative promoters and/or transcription factor binding sites (TFBSs) 50 – 500 nt upstream of Rho-independent terminators, (2) sequence conservation between phylogenetically related species, and (3) conservation of synteny of flanking genes.

BProm (http://www.softberry.com), a bacterial σ^{70} promoter recognition program, was used to predict -10 and -35 promoter sequences. Specific PSSMs for σ^{54} and σ^{38} were used as input to Patser [36] to scan the *A. salmonicida* IGRs for σ^{54} and σ^{38} promoter sequences. Prediction of potential Fur binding sites was done as previously described [6]. TransTerm [45] was used to predict Rho-independent terminators in both the chromosomes of *V. salmonicida*. TransTerm searches were conducted with the confidence threshold of 90 %. The genome viewer and annotation tool Artemis [46] and a python script were used to visualize the predicted sequence features described above. The ncRNA prediction programs sRNAfinder [37] and QRNA [15] were run for comparison and validation of our predictions.

Compilation of a *Vibrionaceae* IGR database and search for sRNA homologs

The IGRs from *A. salmonicida* were used to search for homologous IGRs in the six *Vibrionaceae* genomes of *Vibrio cholerae* O1 biovar eltor str. N16961, *V. fischeri* ES114, *Vibrio parahaemolyticus* RIMD 2210633, *Vibrio vulnificus* CMCP6, *Vibrio vulnificus* YJ016, and *Photobacterium profundum* SS9. Sequences were extracted from GenBank (ftp://ftp.ncbi.nlm.nih.gov/genbank/genomes/Bacteria/). An IGR database was constructed based on these six genomes and the gene annotation as provided at NCBI's Complete Microbial Genomes (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). Only IGRs ≥ 50 nt in length were used. The Formatdb option was used to format the IGR database. StandAlone BLAST from NCBI (ftp://ftp.ncbi.nih.gov/blast/) was used to search for homologs of putative sRNAs. Blastn search parameters were set to word size = 7 and E-value ≤ 0.1.

The search for homologs of known ncRNAs was performed using the cmsearch program, which is part of the Infernal software package [47], and the Rfam database of non-coding RNA families [35]. The lower cmsearch cut-off score was set to 25. Sequences corresponding to tRNAs and rRNAs were excluded.

Supporting Information

Table S1 Detailed information on predicted ncRNAs from intergenic regions of chromosomes I and II of *A. salmonicida*.

Table S2 Complete microarray datasets with fold change and fluorescence intensity values for each predicted ncRNA.

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Figure legends

Figure 1. *A. salmonicida* total RNA from early, mid, and late exponential growth phase separated on a 5 % denaturing polyacrylamide gel and stained with ethidium bromide. The positioning of 23S, 16S, and 5S rRNAs and tRNAs were inferred from the predicted sizes. A number of distinct bands can be seen in the size range of approx. 150-200 nt. Asterisk indicates a band of approx. 300 nt that corresponds to a 23S rRNA fragment that was identified by gel isolation, cDNA synthesis, and cloning.

Figure 2. Size distribution of predicted ncRNAs. Predicted ncRNAs are shown in dark grey bars, ncRNAs that produced microarray fluorescence signal >2 fold above background are shown in light grey bars, and, finally, ncRNAs that produced microarray fluorescence signal >100 are shown in open bars.

Figure 3. Northern blot analysis of selected ncRNAs. Radio-labeled double-stranded DNA probes that were specific for predicted sRNAs or one of the three control ncRNAs (Qrr, RNaseP, and 5S rRNA) were hybridized to the membrane with total RNA from *A. salmonicida* wild-type or *ΔlitR* mutant. Prior to Northern blot analysis, wild-type cells were grown to mid-exponential phase and treated with 50 μM 2,2'-dipyridyl or 100 μM hydrogen peroxide. Untreated (control) cells were used as control. Samples were collected 7.5, 15, and 30 min after treatment. Numbers to the right represent actual and predicted RNA sizes in nt. RNAs from chromosome I (Chr I) or II (Chr II) are indicated. The probe targeted against VSsrna46 produced multiple bands, and band sizes that did not correspond to the predicted size are shown in parenthesizes. 5S rRNA was used to normalize the strength of signals between lanes as measured with phosphoimaging. The VSsrna185 corresponds to the B2 sRNA that was recently identified in *V. cholerae* [19]

Tables

Table 1. Summary of ncRNA microarray analysis.

		Fold change		Threshold		
	Total[1]	**≥2 ×**	**≥1.5 ×**	**>50**	**>75**	**>100**
Low iron	134	7	22	85	68	52
Oxidative stress	142	4	26	91	62	50
ΔlitR	152	15	50	72	60	48

[1] Total number of ncRNAs with fluorescence signal 2× above background.

Table 2. Details of experimentally validated sRNAs.

ncRNA Id	Flanking genes[1]	Start[2]	End[2]	Length[3]	Strand[4]	Sequence conservation[5]	Promoter[6]	Terminator[7]	Type[8]
VSsrna7	VSAL_I0041/VSAL_I0042	44694	44491	204	< < >	+	-	-	Novel
VSsrna21	VSAL_I0222/VSAL_I0223	267228	267063	166	> < <	+++	SigmaS, Fur-box	+	Novel
VSsrna108	VSAL_I1796/VSAL_I1797	1919211	1919404	194	> > >	+	Fur-box	+	Novel
VSsrna114	VSAL_I1873/VSAL_I1874	2009822	2009717	106	> < >	++	Sigma54	+	Qrr
VSsrna145	VSAL_I2441/VSAL_I2442	2615332	2615451	120	> > >	+++	-	-	Novel
VSsrna152	VSAL_I2535/VSAL_I2536	2717523	2717659	137	> > >	++	Fur-box	+	Novel
VSsrna165	VSAL_I2652/VSAL_I2653	2872308	2871972	337	< < <	+++	-	+	RNaseP_bact_a (rnpB) B2 *Vibrio Cholerae*, Livny *et al*., 2005
VSsrna185	VSAL_I2907/VSAL_I2908	3152443	3152724	282	< > <	+++	Sigma70	-	
VSsrna190	VSAL_I2972/VSAL_I2973	3219049	3219240	192	< > <	+++	Sigma70	+	Novel
VSAsrna8	VSAL_II0231/VSAL_II0232	254998	255222	226	> > <	+++	SigmaS	+	Novel
VSAsrna17	VSAL_II0520/VSAL_II0521	580917	580759	160	< < <	+	-	-	Novel
VSAsrna46	VSAL_II0920/VSAL_II0921	1006466	1006637	173	> > <	+	-	+	Novel

[1] The genes numbers for the up- and downstream *A. salmonicida* ORFs

[2,3] Predcited sRNA coordinates and lenght (nt)

[4] Genes encoded on plus strand are denoted with > and genes encoded on the minus strand are denoted with <.

[5] BLASTN was used to search for sequence conservation in other bacteria. + :sequence conservation mainly in Aliivibrio; ++ :sequence conservation primarily in *Vibrionaceae* family; +++ :sequence was conserved in many bacterial species.

[6] Promoter prediction with Bprom software (Softberry, Mount Kisco, NY) and in-house PSSMs and using Patser software [36].

[7] Rho-independent terminator as predicted by TransTerm software [45].

[8] sRNAs were quereied against Rfam database [35] and other experimentally validated *Vibrionaceae* sRNAs. Homologs to known sRNAs are indicated.

Figures

Fig.1

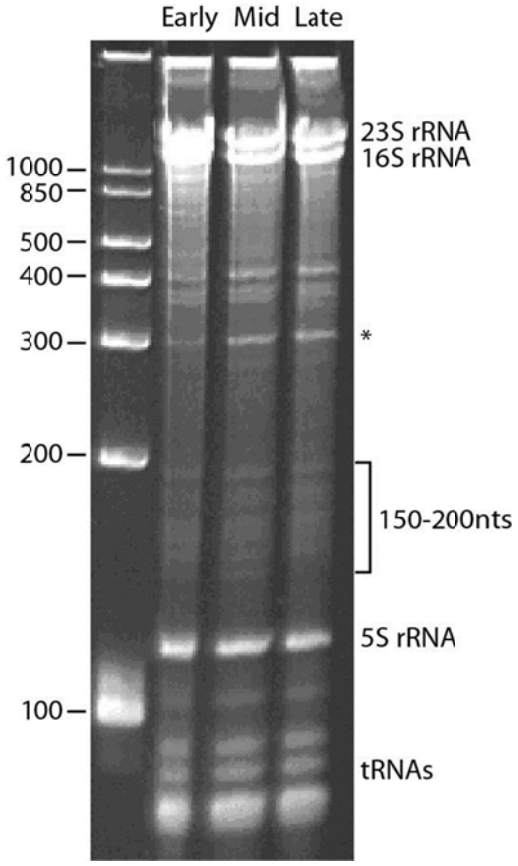


Fig. 2.

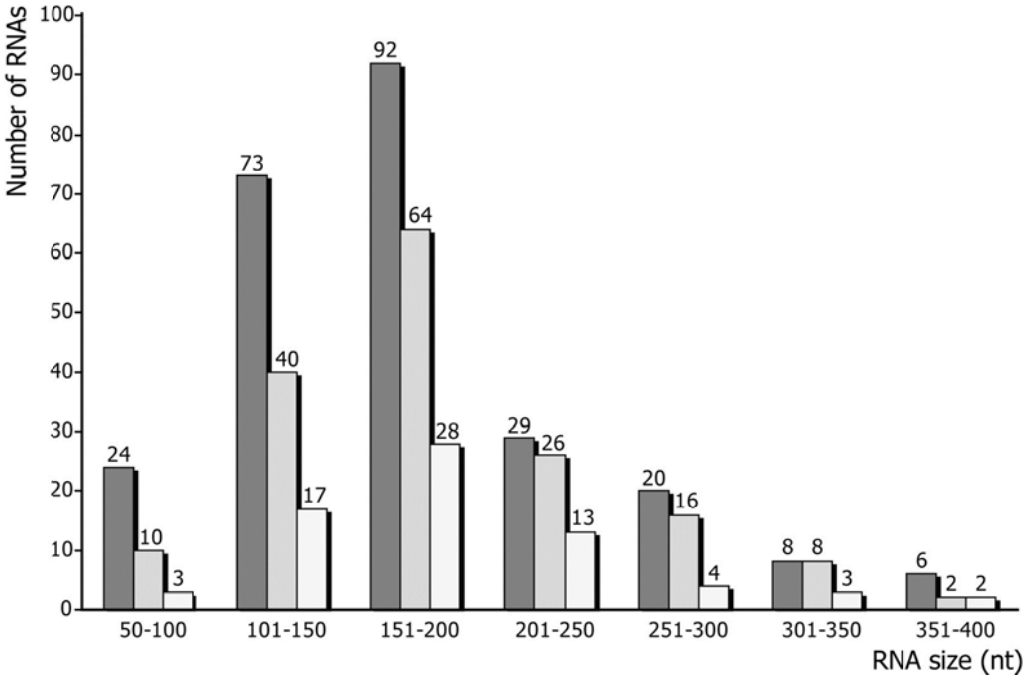
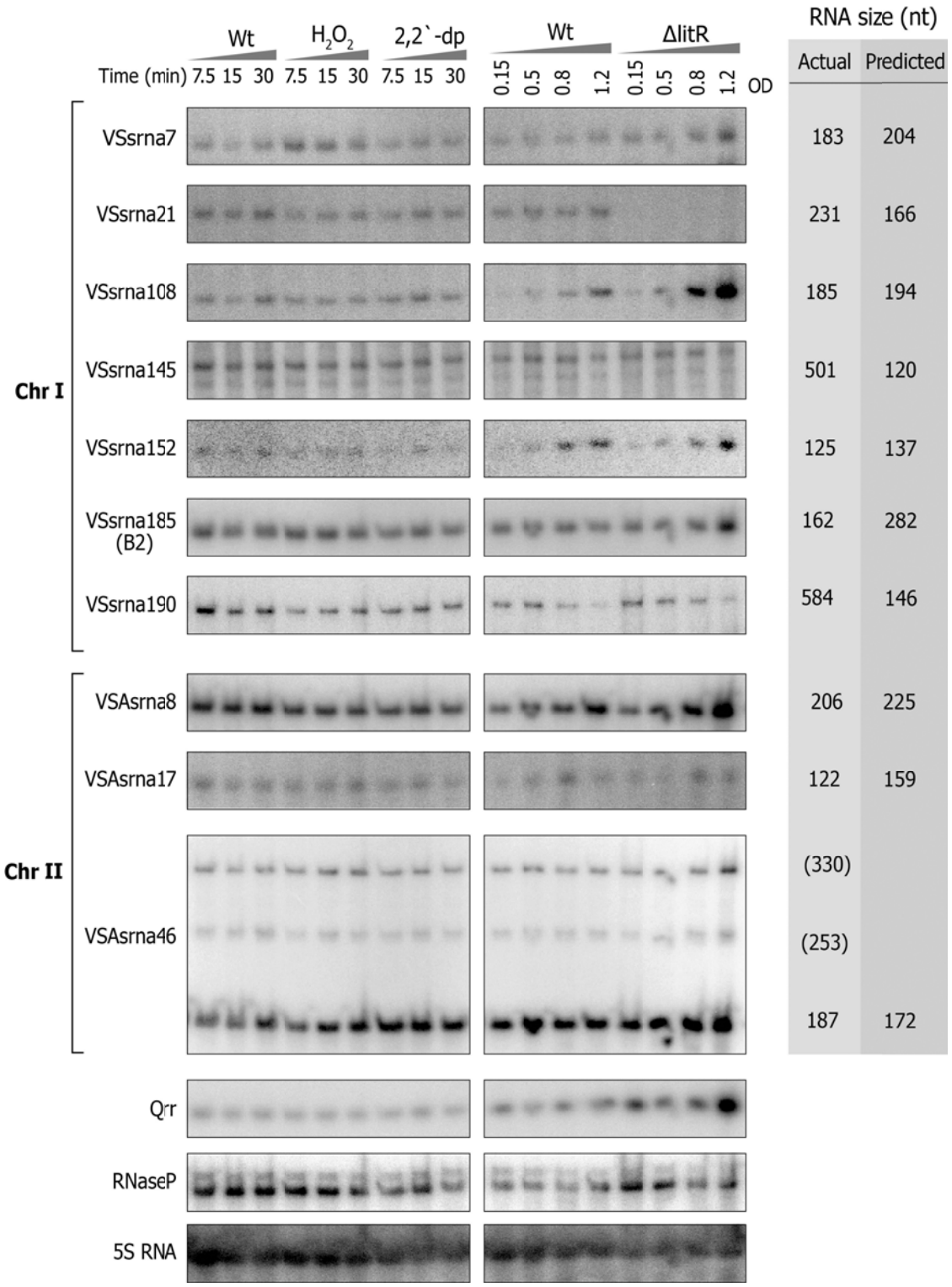

Number of RNAs
100
90
80
70
60
50
40
30
20
10
0
24
10
3
73
40
17
92
64
28
29
26
13
20
16
4
8
8
3
6
2
2
50-100
101-150
151-200
201-250
251-300
301-350
351-400
RNA size (nt)

Fig. 3


Wt
H2O2
2,2`-dp
Wt
ΔlitR
Time (min) 7.5 15 30 7.5 15 30 7.5 15 30
0.15 0.5 0.8 1.2 0.15 0.5 0.8 1.2 OD
RNA size (nt)
Actual Predicted
Chr I
VSsrna7 183 204
VSsrna21 231 166
VSsrna108 185 194
VSsrna145 501 120
VSsrna152 125 137
VSsrna185 (B2) 162 282
VSsrna190 584 146
Chr II
VSAsrna8 206 225
VSAsrna17 122 159
VSAsrna46 (330) (253) 187 172
Qrr
RNaseP
5S RNA

Supplement file 1 (Table S1) - Chr I

ncRNA Id	upstream gene	donwstream gene	Start	Stop	Length	Terminator[1]	Sigma70[2]	SigmaS[3]	Sigma54[3]	Fur-box[4]	Predicted by sRNAFinder[5]
VSsrna1	VSAL_I0017	VSAL_I0018	18808	18901	94	Y					
VSsrna2	VSAL_I0018	VSAL_I0019	19334	19617	284	Y					
VSsrna3	VSAL_I0019	VSAL_I0020	20957	21238	282						
VSsrna4	VSAL_I0020	VSAL_I0021	22297	22495	199	Y					
VSsrna5	VSAL_I0021	VSAL_I0022	23777	23514	264						
VSsrna6	VSAL_I0027	VSAL_I0028	29486	29220	267						
VSsrna7	VSAL_I0041	VSAL_I0042	44694	44491	204						Y
VSsrna8	VSAL_I0046	VSAL_I0047	50548	50724	177		Y				Y
VSsrna9	VSAL_I0048	VSAL_I0049	52409	52332	78						Y
VSsrna10	VSAL_I0050	VSAL_I0051	54553	54707	155						Y
VSsrna11	VSAL_I0061	VSAL_I0062	65235	65406	172	Y					
VSsrna12	VSAL_I0062	VSAL_I0063	66942	66820	123	Y					
VSsrna13	VSAL_I0147	VSAL_I0148	187060	187299	240						
VSsrna14	VSAL_I0148	VSAL_I0149	188560	188696	137						Y
VSsrna15	VSAL_I0150	VSAL_I0149	189725	189818	94						
VSsrna16	VSAL_I0189	VSAL_I0188	230612	230411	202	Y					Y
VSsrna17	VSAL_I0193	VSAL_I0194	234088	234222	135						Y
VSsrna18	VSAL_I0197	VSAL_I0198	238984	239140	157						Y
VSsrna19	VSAL_I0222	VSAL_I0223	266785	266480	306	Y		Y			Y
VSsrna20	VSAL_I0222	VSAL_I0223	266923	267092	170	Y		Y			Y
VSsrna21	VSAL_I0222	VSAL_I0223	267228	267063	166	Y		Y		Y	Y
VSsrna22	VSAL_I0226	VSAL_I0227	271594	271779	186	Y				Y	Y
VSsrna23	VSAL_I0227	VSAL_I0228	275173	275294	122	Y					Y
VSsrna24	VSAL_I0227	VSAL_I0228	275570	275635	66	Y					Y
VSsrna25	VSAL_I0240	VSAL_I0241	286472	286317	156						Y
VSsrna26	VSAL_I0286	VSAL_I0287	334965	335150	186	Y					
VSsrna27	VSAL_I0288	VSAL_I0289	336999	337106	108	Y					Y
VSsrna28	VSAL_I0348	VSAL_I0349	380531	380644	115						
VSsrna29	VSAL_I0377	VSAL_I0378	414656	414838	183	Y					Y
VSsrna30	VSAL_I0440	VSAL_I0441	489471	489661	191	Y		Y			
VSsrna31	VSAL_I0443	VSAL_I0445	497473	497299	175						Y
VSsrna32	VSAL_I0460	VSAL_I0461	517755	517871	117						
VSsrna33	VSAL_I0476	VSAL_I0477	533475	533606	132						
VSsrna34	VSAL_I0503	VSAL_I0504	562458	562532	75						Y

VSsrna35	VSAL_I0519	VSAL_I0520	574278	574079	200	Y					
VSsrna36	VSAL_I0523	VSAL_I0524	578124	578237	114	Y					
VSsrna37	VSAL_I0534	VSAL_I0535	590893	591015	123						
VSsrna38	VSAL_I0557	VSAL_I0558	611552	611673	122	Y		Y		Y	Y
VSsrna39	VSAL_I0566	VSAL_I0567	622676	622814	139						
VSsrna40	VSAL_I0568	VSAL_I0569	625387	625585	199						Y
VSsrna41	VSAL_I0588	VSAL_I0589	651057	650860	198						Y
VSsrna42	VSAL_I0595	VSAL_I0596	657826	657937	112	Y		Y		Y	
VSsrna43	VSAL_I0600	VSAL_I0601	664197	664316	120						
VSsrna44	VSAL_I0601	VSAL_I0602	664603	664785	183	Y					
VSsrna45	VSAL_I0605	VSAL_I0606	670104	670267	164						Y
VSsrna46	VSAL_I0616	VSAL_I0617	679366	679256	111	Y		Y			
VSsrna47	VSAL_I0617	VSAL_I0618	680736	680958	223						
VSsrna48	VSAL_I0618	VSAL_I0619	681505	681341	165						
VSsrna49	VSAL_I0619	VSAL_I0620	682284	682400	117						
VSsrna50	VSAL_I0630	VSAL_I0631	696814	696915	102						
VSsrna51	VSAL_I0643	VSAL_I0644	717610	717680	71	Y					
VSsrna52	VSAL_I0662	VSAL_I0663	735232	735441	210						Y
VSsrna53	VSAL_I0675	VSAL_I0676	754044	754169	126						
VSsrna54	VSAL_I0684	VSAL_I0685	770160	770274	115						Y
VSsrna55	VSAL_I0702	VSAL_I0703	789582	789818	237						Y
VSsrna56	VSAL_I0734	VSAL_I0735	819651	819379	273						Y
VSsrna57	VSAL_I0736	VSAL_I0737	821241	821451	211						Y
VSsrna58	VSAL_I0737	VSAL_I0738	823090	822919	172						Y
VSsrna59	VSAL_I0750	VSAL_I0751	841758	841901	144	Y				Y	Y
VSsrna60	VSAL_I0755	VSAL_I0756	844341	844475	135						
VSsrna61	VSAL_I0761	VSAL_I0762	850806	851060	255						Y
VSsrna62	VSAL_I0780	VSAL_I0781	866237	866373	137						
VSsrna63	VSAL_I0804	VSAL_I0805	894841	894681	161	Y					Y
VSsrna64	VSAL_I0814	VSAL_I0815	905083	904831	253	Y					Y
VSsrna65	VSAL_I0816	VSAL_I0817	907133	906837	297		Y				Y
VSsrna66	VSAL_I0819	VSAL_I0820	910412	910342	71						
VSsrna67	VSAL_I0820	VSAL_I0821	911773	912000	228	Y					Y
VSsrna68	VSAL_I0830	VSAL_I0831	925875	926053	179		Y				Y
VSsrna69	VSAL_I0942	VSAL_I0943	1040569	1040669	101	Y					Y
VSsrna70	VSAL_I0973	VSAL_I0974	1076277	1076470	194		Y				Y
VSsrna71	VSAL_I1050	VSAL_I1051	1142934	1143065	132						
VSsrna72	VSAL_I1051	VSAL_I1052	1144296	1144520	225		Y				

VSsrna73	VSAL_I1073	VSAL_I1074	1166803	1166668	136	Y					Y
VSsrna74	VSAL_I1109	VSAL_I1110	1207494	1207395	100						Y
VSsrna75	VSAL_I1132	VSAL_I1133	1236029	1236412	384	Y					Y
VSsrna76	VSAL_I1158	VSAL_I1159	1262213	1262453	241						Y
VSsrna77	VSAL_I1185	VSAL_I1186	1293804	1293881	78	Y		Y			
VSsrna78	VSAL_I1219	VSAL_I1220	1326789	1326920	132	Y	Y				Y
VSsrna79	VSAL_I1229	VSAL_I1230	1339927	1339843	85						
VSsrna80	VSAL_I1294	VSAL_I1295	1405550	1405690	141						
VSsrna81	VSAL_I1310	VSAL_I1311	1421783	1422029	247						Y
VSsrna82	VSAL_I1322	VSAL_I1323	1434287	1434143	145						Y
VSsrna83	VSAL_I1334	VSAL_I1335	1449996	1450187	192	Y	Y				Y
VSsrna84	VSAL_I1336	VSAL_I1337	1452534	1452380	155						Y
VSsrna85	VSAL_I1346	VSAL_I1347	1462526	1462657	132						
VSsrna86	VSAL_I1368	VSAL_I1369	1490089	1489981	109						Y
VSsrna87	VSAL_I1384	VSAL_I1385	1501603	1501792	190		Y				Y
VSsrna88	VSAL_I1396	VSAL_I1397	1513487	1513371	117	Y					
VSsrna89	VSAL_I1457	VSAL_I1458	1577195	1577369	175		Y				Y
VSsrna90	VSAL_I1534	VSAL_I1535	1635498	1635587	90						
VSsrna91	VSAL_I1548	VSAL_I1549	1658307	1658447	141						Y
VSsrna92	VSAL_I1559	VSAL_I1560	1670913	1671095	183						Y
VSsrna93	VSAL_I1591	VSAL_I1592	1701193	1700993	201		Y				
VSsrna94	VSAL_I1602	VSAL_I1603	1710625	1710485	141						
VSsrna95	VSAL_I1623	VSAL_I1624	1730164	1730295	132		Y				
VSsrna96	VSAL_I1624	VSAL_I1625	1730995	1731128	134						Y
VSsrna97	VSAL_I1636	VSAL_I1637	1741744	1741905	162						
VSsrna98	VSAL_I1683	VSAL_I1684	1778987	1779136	150	Y					Y
VSsrna99	VSAL_I1766	VSAL_I1767	1879107	1879436	330	Y		Y			
VSsrna100	VSAL_I1767	VSAL_I1768	1882110	1882291	182						Y
VSsrna101	VSAL_I1768	VSAL_I1769	1883274	1883549	276						Y
VSsrna102	VSAL_I1774	VSAL_I1775	1892629	1892482	148						Y
VSsrna103	VSAL_I1786	VSAL_I1787	1904815	1905006	192						Y
VSsrna104	VSAL_I1787	VSAL_I1788	1905714	1905876	163		Y				Y
VSsrna105	VSAL_I1793	VSAL_I1794	1915899	1915765	135	Y					
VSsrna106	VSAL_I1795	VSAL_I1796	1917423	1917535	113		Y				Y
VSsrna107	VSAL_I1795	VSAL_I1796	1917522	1917697	176		Y				Y

VSsrna108	VSAL_I1796	VSAL_I1797	1919211	1919404	194	Y				Y	
VSsrna109	VSAL_I1814	VSAL_I1815	1938139	1938020	120	Y					Y
VSsrna110	VSAL_I1820	VSAL_I1821	1947322	1947068	255	Y				Y	
VSsrna111	VSAL_I1822	VSAL_I1823	1948795	1949046	252	Y		Y			Y
VSsrna112	VSAL_I1840	VSAL_I1841	1974015	1974136	122						
VSsrna113	VSAL_I1854	VSAL_I1855	1988965	1989137	173						
VSsrna114	VSAL_I1873	VSAL_I1874	2009822	2009717	106	Y			Y		Y
VSsrna115	VSAL_I1876	VSAL_I1877	2012879	2013068	190						
VSsrna116	VSAL_I1907	VSAL_I1908	2041266	2041625	360	Y		Y			
VSsrna117	VSAL_I1955	VSAL_I1956	2096287	2096646	360	Y	Y				Y
VSsrna118	VSAL_I1960	VSAL_I1961	2101271	2101470	200	Y	Y				
VSsrna119	VSAL_I1982	VSAL_I1983	2124284	2124611	328		Y				Y
VSsrna120	VSAL_I1983	VSAL_I1984	2125801	2125958	158	Y					Y
VSsrna121	VSAL_I2027	VSAL_I2028	2173504	2173761	258						Y
VSsrna122	VSAL_I2029	VSAL_I2030	2176828	2176955	128	Y					Y
VSsrna123	VSAL_I2031	VSAL_I2032	2178480	2178679	200						Y
VSsrna124	VSAL_I2032	VSAL_I2033	2180338	2180512	175		Y				Y
VSsrna125	VSAL_I2040	VSAL_I2041	2187392	2187206	187						Y
VSsrna126	VSAL_I2059	VSAL_I2060	2207191	2207272	82	Y					
VSsrna127	VSAL_I2066	VSAL_I2067	2216662	2216732	71	Y					Y
VSsrna128	VSAL_I2117	VSAL_I2118	2268944	2269143	200						Y
VSsrna129	VSAL_I2118	VSAL_I2119	2270080	2270005	76		Y				Y
VSsrna130	VSAL_I2144	VSAL_I2145	2302406	2302798	393	Y	Y				Y
VSsrna131	VSAL_I2147	VSAL_I2148	2305637	2305449	189						Y
VSsrna132	VSAL_I2198	VSAL_I2199	2363652	2363815	164						Y
VSsrna133	VSAL_I2203	VSAL_I2204	2368323	2368003	321	Y				Y	
VSsrna134	VSAL_I2212	VSAL_I2213	2380485	2380249	237		Y				Y
VSsrna135	VSAL_I2250	VSAL_I2251	2420415	2420642	228						Y
VSsrna136	VSAL_I2254	VSAL_I2255	2425584	2425847	264						
VSsrna137	VSAL_I2256	VSAL_I2257	2430456	2430758	303						Y
VSsrna138	VSAL_I2268	VSAL_I2269	2443176	2443374	199		Y				Y
VSsrna139	VSAL_I2272	VSAL_I2273	2448409	2448545	137						Y
VSsrna140	VSAL_I2317	VSAL_I2318	2491062	2491235	174						Y
VSsrna141	VSAL_I2329	VSAL_I2330	2505378	2505569	192						
VSsrna142	VSAL_I2379	VSAL_I2380	2550405	2550527	123	Y	Y				

VSsrna143	VSAL_I2396	VSAL_I2397	2566644	2566802	159	Y	Y				Y
VSsrna144	VSAL_I2427	VSAL_I2428	2598257	2598059	199	Y					Y
VSsrna145	VSAL_I2441	VSAL_I2442	2615332	2615451	120						Y
VSsrna146	VSAL_I2441	VSAL_I2442	2615482	2615592	111	Y					
VSsrna147	VSAL_I2470	VSAL_I2471	2644974	2644801	174	Y					Y
VSsrna148	VSAL_I2471	VSAL_I2472	2646915	2647114	200	Y				Y	Y
VSsrna149	VSAL_I2479	VSAL_I2480	2653856	2654219	364	Y					Y
VSsrna150	VSAL_I2492	VSAL_I2493	2671152	2671427	276						
VSsrna151	VSAL_I2492	VSAL_I2493	2671535	2671718	184						Y
VSsrna152	VSAL_I2535	VSAL_I2536	2717523	2717659	137	Y				Y	Y
VSsrna153	VSAL_I2539	VSAL_I2540	2721353	2721115	239						
VSsrna154	VSAL_I2540	VSAL_I2541	2721726	2721907	182	Y					Y
VSsrna155	VSAL_I2541	VSAL_I2542	2722635	2722490	146						
VSsrna156	VSAL_I2542	VSAL_I2543	2723291	2723479	189						
VSsrna157	VSAL_I2543	VSAL_I2544	2724501	2724360	142						
VSsrna158	VSAL_I2545	VSAL_I2546	2726539	2726676	138	Y					Y
VSsrna159	VSAL_I2556	VSAL_I2557	2739151	2738949	203		Y				
VSsrna160	VSAL_I2576	VSAL_I2577	2776105	2776245	141						Y
VSsrna161	VSAL_I2588	VSAL_I2589	2792526	2792703	178						Y
VSsrna162	VSAL_I2590	VSAL_I2591	2794296	2794452	157		Y				Y
VSsrna163	VSAL_I2617	VSAL_I2618	2832071	2832259	189		Y				Y
VSsrna164	VSAL_I2628	VSAL_I2629	2844887	2845123	237						Y
VSsrna165	VSAL_I2652	VSAL_I2653	2872308	2871972	337	Y					Y
VSsrna166	VSAL_I2662	VSAL_I2663	2881045	2880856	190						Y
VSsrna167	VSAL_I2682	VSAL_I2683	2906058	2905885	174	Y					Y
VSsrna168	VSAL_I2686	VSAL_I2687	2908977	2909072	96						Y
VSsrna169	VSAL_I2700	VSAL_I2701	2924582	2924764	183						Y
VSsrna170	VSAL_I2701	VSAL_I2702	2926410	2926219	192						Y
VSsrna171	VSAL_I2706	VSAL_I2707	2931685	2931778	94						Y
VSsrna172	VSAL_I2709	VSAL_I2710	2934183	2934064	120						Y
VSsrna173	VSAL_I2710	VSAL_I2711	2936143	2936276	134	Y					Y
VSsrna174	VSAL_I2711	VSAL_I2712	2936542	2936633	92						Y
VSsrna175	VSAL_I2727	VSAL_I2728	2954037	2953919	119						Y
VSsrna176	VSAL_I2740	VSAL_I2741	2966348	2966485	138						
VSsrna177	VSAL_I2740	VSAL_I2741	2966346	2966525	180						

VSsrna178	VSAL_I2748	VSAL_I2749	2977130	2977253	124						Y
VSsrna179	VSAL_I2771	VSAL_I2772	3001763	3001861	99						Y
VSsrna180	VSAL_I2772	VSAL_I2773	3003174	3003351	178						Y
VSsrna181	VSAL_I2829	VSAL_I2830	3067407	3067538	132						Y
VSsrna182	VSAL_I2866	VSAL_I2867	3108716	3108913	198	Y					Y
VSsrna183	VSAL_I2886	VSAL_I2887	3132517	3132287	231						
VSsrna184	VSAL_I2893	VSAL_I2894	3139678	3139830	153						
VSsrna185	VSAL_I2907	VSAL_I2908	3152443	3152724	282						Y
VSsrna186	VSAL_I2908	VSAL_I2909	3153279	3153407	129	Y					
VSsrna187	VSAL_I2927	VSAL_I2928	3171139	3171348	210						Y
VSsrna188	VSAL_I2939	VSAL_I2940	3186247	3186465	219						Y
VSsrna189	VSAL_I2940	VSAL_I2941	3188215	3188355	141						Y
VSsrna190	VSAL_I2972	VSAL_I2973	3219049	3219240	192	Y					Y
VSsrna191	VSAL_I2982	VSAL_I2983	3230503	3230616	114						Y
VSsrna192	VSAL_I2989	VSAL_I2990	3239041	3239236	196						Y
VSsrna193	VSAL_I3002	VSAL_I3003	3252392	3252631	240	Y					Y
VSsrna194	VSAL_I3002	VSAL_I3003	3253286	3253522	237	Y					Y
VSsrna195	VSAL_I3049	VSAL_I3050	3304388	3304515	128	Y					Y
VSsrna196	VSAL_I3049	VSAL_I3050	3304657	3304574	84	Y					Y

[1]Rho-independent terminator as predicted by TransTerm

[2]Predicted with Bprom software (http://linux1.softberry.com/berry.phtml)

[3]Predicted with in-house position specific scoring matrix using Patser software tool

[4]Predicted with vibrio-specific Fur-box matrix (Ahmad et. al., 2008) using Patser software tool

[5]sRNAfinder (Tjaden, 2008)

[6]QRNA (Rivas E, 2001)

[7]Y (high) = conserved through out *Vibrionaceae* family; Y (medium) = consevred in all *alivibrio* and few *vibrio sp* .; Y (little) = conserved only among *aliivibrio sp* .

NOTE: Y denotes a corresponding prediction. An empty cell denotes NO corresponding prediction.

Cont. supplement file 1

ncRNA Id	Predicted by QRNA[6]	Sequence conservation in *Vibrionaceae*[7]	Potential Homolog to	RFAM ID	RFAM Accession	Type	Description
VSsrna1	Y	Y (high)					
VSsrna2	Y	Y (high)					
VSsrna3		Y (high)					
VSsrna4	Y	Y (high)					
VSsrna5	Y	Y (high)					
VSsrna6		Y (high)					
VSsrna7		Y (little)					
VSsrna8		Y (little)					
VSsrna9		Y (medium)					
VSsrna10		Y (medium)					
VSsrna11		Y (medium)					
VSsrna12		Y (medium)					
VSsrna13		Y (high)					
VSsrna14	Y	Y (high)					
VSsrna15		Y (high)					
VSsrna16		Y (high)					
VSsrna17							
VSsrna18		Y (high)					
VSsrna19							
VSsrna20							
VSsrna21		Y (high)					
VSsrna22		Y (high)	RyhB	RyhB	RF00057	sRNA	RyhB RNA
VSsrna23		Y (high)	Spot 42	Spot 42	RF00021	sRNA	Spot 42 RNA
VSsrna24		Y (little)					
VSsrna25		Y (medium)					
VSsrna26							
VSsrna27		Y (little)					
VSsrna28		Y (little)	TPP Riboswitch (ORF2 *V. Cholerae* Liu et al., 2009)	TPP	RF00059	Riboswitch	TPP riboswitch (THI element)
VSsrna29		Y (medium)	C1 from *Vibrio Cholerae*, Livny *et a*l., 2005				
VSsrna30		Y (little)					
VSsrna31		Y (little)	Lysine riboswitch (VC0391/VC0392 IGR, Liu et al., 2009)	Lysine	RF00168	Riboswitch	Lysine riboswitch
VSsrna32		Y (medium)					
VSsrna33	Y	Y (medium)					
VSsrna34		Y					

VSsrna35		Y (little)					
VSsrna36		Y (medium)					
VSsrna37		Y (medium)					
VSsrna38		Y (little)					
VSsrna39		Y (little)					
VSsrna40		Y (little)					
VSsrna41		Y (little)					
VSsrna42							
VSsrna43		Y (medium)	Ribosomal S15 leader	S15	RF00114	Regulation by transcription termination	Ribosomal S15 leader
VSsrna44			sraG/psrO (VC0646/VC0647 IGR, Liu et al., 2009)				
VSsrna45		Y (little)					
VSsrna46		Y (medium)					
VSsrna47	Y	Y (high)					
VSsrna48	Y	Y (high)					
VSsrna49	Y	Y (high)					
VSsrna50		Y (medium)					
VSsrna51			sraD/micA				
VSsrna52		Y (little)					
VSsrna53		Y (high)					
VSsrna54		Y (little)					
VSsrna55		Y (little)					
VSsrna56	Y	Y (high)					
VSsrna57		Y (high)					
VSsrna58		Y (little)					
VSsrna59		Y (little)					
VSsrna60		Y (medium)					
VSsrna61		Y (high)					
VSsrna62		Y (medium)					
VSsrna63		Y (little)					
VSsrna64		Y (little)					
VSsrna65		Y (little)					
VSsrna66		Y (medium)	antisense RNA regulating IS element (RNA-out)/AS7	RNA-OUT	RF00240	Antisense	RNA-OUT
VSsrna67			sraA				
VSsrna68		Y (little)					
VSsrna69			isrA				
VSsrna70		Y (little)					
VSsrna71	Y	Y (little)					
VSsrna72	Y	Y (little)					

VSsrna73		Y (little)					
VSsrna74		Y (medium)	bacterial signal recognition particle (SRP bact)	SRP_bact	RF00169	Ribonucleoprotein	Bacterial signal recognition particle RNA
VSsrna75	Y	Y (high)	His Leader (also B4 from Vibrio Cholerae, Livny et al., 2005)	His_leader	RF00514	Regulation by transcription termination	Histidine operon leader
VSsrna76		Y (little)					
VSsrna77		Y (little)					
VSsrna78		Y (little)					
VSsrna79							
VSsrna80		Y (medium)					
VSsrna81		Y (medium)					
VSsrna82		Y (little)					
VSsrna83		Y (little)					
VSsrna84		Y (little)					
VSsrna85	Y	Y (medium)					
VSsrna86		Y (little)					
VSsrna87		Y (little)					
VSsrna88		Y (little)					
VSsrna89		Y (little)					
VSsrna90		Y (medium)					
VSsrna91		Y (little)					
VSsrna92		Y (little)					
VSsrna93		Y (medium)					
VSsrna94		Y (medium)					
VSsrna95	Y	Y (high)					
VSsrna96		Y (little)					
VSsrna97		Y (little)					
VSsrna98		Y (little)					
VSsrna99							
VSsrna100		Y (little)					
VSsrna101		Y (little)					
VSsrna102		Y (little)					
VSsrna103		Y (medium)					
VSsrna104		Y (medium)					
VSsrna105		Y (little)					
VSsrna106		Y (medium)	Glycine riboswitch	Glycine	RF00504	Riboswitch	Glycine riboswitch
VSsrna107		Y (medium)	Glycine riboswitch (VC1422/VC1423 IGR, Liu et al., 2009)	Glycine	RF00505	Riboswitch	Glycine riboswitch

VSsrna108		Y (little)					
VSsrna109		Y (little)					
VSsrna110		Y (high)	*V. Cholerae* IGR 5 Liu *et al*., 2009				
VSsrna111		Y (little)					
VSsrna112							
VSsrna113							
VSsrna114		Y (high)	Qrr	Qrr	RF00378	sRNA	Qrr RNA
VSsrna115		Y (medium)	MOCO RNA motif	MOCO_RNA_motif	RF01055	Riboswitch	Moco (molybdenum cofactor) riboswitch
VSsrna116		Y (little)					
VSsrna117		Y (medium)					
VSsrna118		Y (medium)					
VSsrna119		Y (little)	GEMM RNA motif	GEMM_RNA_motif	RF01051	Riboswitch	GEMM cis-regulatory element
VSsrna120		Y (little)					
VSsrna121		Y (medium)	VC2640/VC264 IGR, Liu et al., 2009				
VSsrna122		Y (little)					
VSsrna123		Y (little)					
VSsrna124		Y (little)					
VSsrna125		Y (medium)					
VSsrna126							
VSsrna127		Y (medium)	Group II catalytic intron	Intron_gpII	RF00029	Unknown	Group II catalytic intron
VSsrna128							
VSsrna129		Y (medium)					
VSsrna130		Y (medium)	*V. Cholerae* IGR 2 Liu *et al*., 2009 Lysine riboswitch				
VSsrna131		Y (little)					
VSsrna132		Y (little)					
VSsrna133		Y (medium)					
VSsrna134		Y (medium)					
VSsrna135		Y (little)					
VSsrna136							
VSsrna137		Y (little)					
VSsrna138		Y (little)					
VSsrna139		Y (little)					
VSsrna140		Y (little)	VC2260/VC2261 IGR, Liu et al., 2009				
VSsrna141		Y (high)					
VSsrna142		Y (high)	*V. Cholerae* IGR 7 Liu *et al*., 2009				

VSsrna143							
VSsrna144		Y (little)	t44 (VC2260/VC2261 IGR, Liu et al., 2009)	t44	RF00127	sRNA	t44 RNA
VSsrna145		Y (high)					
VSsrna146							
VSsrna147		Y (little)	tpke11				
VSsrna148		Y (little)					
VSsrna149		Y (little)	tmRNA/ssrA	tmRNA/ssrA	RF00023	Modulators of protein activity	transfer-messenger RNA
VSsrna150							
VSsrna151							
VSsrna152		Y (medium)	VC2472/VC2473 IGR, Liu et al., 2009				
VSsrna153		Y (high)					
VSsrna154	Y	Y (high)	SsrS/6s	SsrS/6s	RF00013	Modulators of protein activity	6S / SsrS RNA
VSsrna155		Y (high)					
VSsrna156	Y	Y (high)					
VSsrna157		Y (high)					
VSsrna158		Y (medium)					
VSsrna159		Y (medium)	threonine operon leader also has ryfA match	Thr_leader	RF00506	Regulation by transcription termination	Threonine operon leader
VSsrna160		Y (high)					
VSsrna161		Y (little)					
VSsrna162		Y (little)					
VSsrna163		Y (medium)					
VSsrna164		Y (little)					
VSsrna165		Y (high)	RNaseP_bact_a (rnpB)	RNaseP_bact_a	RF00010	Modulators of protein activity	Bacterial RNase P class A
VSsrna166		Y (little)					
VSsrna167		Y (medium)	*V. Cholerae* IGR 3 Liu *et al*., 2009				
VSsrna168							
VSsrna169		Y (little)					
VSsrna170		Y (little)					
VSsrna171							
VSsrna172		Y (medium)					
VSsrna173		Y (little)	VC2640/VC264 IGR, Liu et al., 2009				
VSsrna174		Y (little)					
VSsrna175		Y (little)					
VSsrna176	Y	Y (medium)					
VSsrna177		Y (medium)					

VSsrna178		Y (little)					
VSsrna179		Y (medium)					
VSsrna180		Y (little)					
VSsrna181		Y (medium)					
VSsrna182		Y (little)	Pseudomonas aeruginosa sRNA P26 (VC0327/VC0328 IGR, Liu et al., 2009)	P26	RF00630	sRNA	Pseudomonas sRNA P26
VSsrna183		Y (little)	Cobalamin Riboswitch (VC0154/VC0156 IGR, Liu et al., 2009)	Cobalamin	RF00174	Riboswitch	Cobalamin riboswitch
VSsrna184		Y (medium)					
VSsrna185		Y (high)	B2 from *Vibrio Cholerae*, Livny *et a*l., 2005				
VSsrna186		Y (medium)					
VSsrna187							
VSsrna188		Y (medium)					
VSsrna189		Y (medium)					
VSsrna190		Y (high)					
VSsrna191		Y (medium)	TPP Riboswitch (VC0060/VC0061 IGR, Liu et al., 2009)	TPP	RF00059	Riboswitch	TPP riboswitch (THI element)
VSsrna192							
VSsrna193		Y (medium)					
VSsrna194		Y (medium)	CsrB	CsrB	RF00018	sRNA	CsrB/RsmB RNA family
VSsrna195							
VSsrna196							

Supplement file 1 (Table S1) - Chr II

ncRNA Id	upstream gene	donwstream gene	Start	Stop	Length	Terminator[1]	Sigma70[2]	SigmaS[3]	Sigma54[3]	Fur-box[4]	Predicted by sRNAFinder[5]
VSAsrna1		VSAL_II0001	792	1031	241						Y
VSAsrna2	VSAL_II0014	VSAL_II0015	16638	16462	178						Y
VSAsrna3	VSAL_II0021	VSAL_II0022	32043	31834	211	Y			Y		
VSAsrna4	VSAL_II0073	VSAL_II0074	99325	99126	201						Y
VSAsrna5	VSAL_II0152	VSAL_II0153	179013	179300	289	Y			Y	Y	
VSAsrna6	VSAL_II0152	VSAL_II0153	179568	179241	329	Y			Y	Y	
VSAsrna7	VSAL_II0230	VSAL_II0231	253753	253942	191						Y
VSAsrna8	VSAL_II0231	VSAL_II0232	254998	255222	226	Y		Y			
VSAsrna9	VSAL_II0279	VSAL_II0280	311816	311629	189						Y
VSAsrna10	VSAL_II0342	VSAL_II0343	385403	385543	142						
VSAsrna11	VSAL_II0450	VSAL_II0451	512493	512699	208	Y			Y	Y	Y
VSAsrna12	VSAL_II0485	VSAL_II0486	544937	545034	99	Y		Y	Y		Y
VSAsrna13	VSAL_II0496	VSAL_II0497	555717	555403	316	Y				Y	Y
VSAsrna14	VSAL_II0500	VSAL_II0501	559785	559501	286						Y
VSAsrna15	VSAL_II0501	VSAL_II0502	561018	560924	96						Y
VSAsrna16	VSAL_II0502	VSAL_II0503	561915	561778	139						Y
VSAsrna17	VSAL_II0520	VSAL_II0521	580917	580759	160						
VSAsrna18	VSAL_II0521	VSAL_II0522	581911	582103	194						Y
VSAsrna19	VSAL_II0534	VSAL_II0535	594506	594705	201	Y					Y
VSAsrna20	VSAL_II0535	VSAL_II0536	596624	596822	200						Y
VSAsrna21	VSAL_II0535	VSAL_II0536	597328	597459	133						Y
VSAsrna22	VSAL_II0536	VSAL_II0537	598811	598974	165						Y
VSAsrna23	VSAL_II0536	VSAL_II0537	598811	598974	165						Y
VSAsrna24	VSAL_II0537	VSAL_II0538	599729	599860	133						Y
VSAsrna25	VSAL_II0543	VSAL_II0544	602798	602975	179						Y
VSAsrna26	VSAL_II0546	VSAL_II0547	604791	604992	203						Y
VSAsrna27	VSAL_II0547	VSAL_II0548	605934	606130	198						Y
VSAsrna28	VSAL_II0559	VSAL_II0560	614453	614265	190						Y
VSAsrna29	VSAL_II0559	VSAL_II0560	614869	614675	196						Y
VSAsrna30	VSAL_II0594	VSAL_II0595	651855	651681	176	Y					Y
VSAsrna31	VSAL_II0598	VSAL_II0599	656161	656316	157						Y
VSAsrna32	VSAL_II0635	VSAL_II0636	685714	685965	253						Y
VSAsrna33	VSAL_II0675	VSAL_II0676	742076	742214	140						

VSAsrna34	VSAL_II0694	VSAL_II0695	761449	761336	115						Y
VSAsrna35	VSAL_II0707	VSAL_II0708	775735	775645	92						Y
VSAsrna36	VSAL_II0737	VSAL_II0738	813919	813722	199						
VSAsrna37	VSAL_II0752	VSAL_II0753	826558	826415	145	Y					
VSAsrna38	VSAL_II0752	VSAL_II0753	826558	826415	145						Y
VSAsrna39	VSAL_II0783	VSAL_II0784	860842	860997	157	Y					
VSAsrna40	VSAL_II0785	VSAL_II0786	862395	862042	355						
VSAsrna41	VSAL_II0789	VSAL_II0790	867270	867361	93						
VSAsrna42	VSAL_II0815	VSAL_II0816	893588	893778	192						Y
VSAsrna43	VSAL_II0820	VSAL_II0821	901000	901248	250						Y
VSAsrna44	VSAL_II0867	VSAL_II0868	953719	953498	223	Y					
VSAsrna45	VSAL_II0915	VSAL_II0916	1003028	1002884	146	Y					Y
VSAsrna46	VSAL_II0920	VSAL_II0921	1006466	1006637	173	Y					Y
VSAsrna47	VSAL_II0942	VSAL_II0943	1030039	1030284	247	Y			Y		
VSAsrna48	VSAL_II0943	VSAL_II0944	1033092	1032931	163						
VSAsrna49	VSAL_II0954	VSAL_II0955	1044873	1044989	118						Y
VSAsrna50	VSAL_II0956	VSAL_II0957	1047174	1047028	148	Y		Y			Y
VSAsrna51	VSAL_II0996	VSAL_II0997	1091034	1091145	113						Y
VSAsrna52	VSAL_II1023	VSAL_II1024	1121687	1121505	184	Y					Y
VSAsrna53	VSAL_II1052	VSAL_II1053	1148987	1148802	187						
VSAsrna54	VSAL_II1080	VSAL_II1081	1177269	1177119	152						Y
VSAsrna55	VSAL_II1094	VSAL_II1095	1189728	1189982	256						Y
VSAsrna56	VSAL_II1094	VSAL_II1095	1189920	1189660	262	Y					Y

[1]Rho-independent terminator as predicted by TransTerm

[2]Predicted with Bprom software (http://linux1.softberry.com/berry.phtml)

[3]Predicted with in-house position specific scoring matrix using Patser software tool

[4]Predicted with vibrio-specific Fur-box matrix (Ahmad et. al., 2008) using Patser software tool

[5]sRNAfinder (Tjaden, 2008)

[6]QRNA (Rivas E, 2001)

[7]Y (high) = conserved through out *Vibrionaceae* family; Y (medium) = consevred in all *alivibrio* and few *vibrio sp* .; Y (little) = conserved only among *aliivibrio sp* .

NOTE: Y denotes a corresponding prediction. An empty cell denotes NO corresponding prediction.

Cont. supplement file 1

ncRNA Id	Predicted by QRNA[6]	Sequence conservation in *Vibrionaceae*[7]	Potential Homolog to	RFAM ID	RFAM Accession	Type	Description
VSAsrna1		Y (little)					
VSAsrna2		Y (little)					
VSAsrna3		Y (little)					
VSAsrna4		Y (little)					
VSAsrna5		Y (little)					
VSAsrna6		Y (little)					
VSAsrna7							
VSAsrna8		Y (high)					
VSAsrna9		Y (little)					
VSAsrna10		Y (medium)					
VSAsrna11		Y (little)					
VSAsrna12		Y (high)	VC1295/VC1296 IGR, Liu et al., 2009				
VSAsrna13							
VSAsrna14	Y	Y (high)	VCA0196/VCA0197 IGR, Liu et al., 2009				
VSAsrna15	Y	Y (high)					
VSAsrna16		Y (high)					
VSAsrna17	Y	Y (little)					
VSAsrna18		Y (little)	TPP Riboswitch	TPP	RF00059	Riboswitch	TPP riboswitch (THI element)
VSAsrna19		Y (little)					
VSAsrna20		Y (little)					
VSAsrna21		Y (little)					
VSAsrna22		Y (little)					
VSAsrna23		Y (little)					
VSAsrna24		Y (little)					
VSAsrna25		Y (little)					
VSAsrna26		Y (little)					
VSAsrna27		Y (little)					
VSAsrna28		Y (little)					
VSAsrna29		Y (little)					
VSAsrna30		Y (little)					
VSAsrna31		Y (little)					
VSAsrna32		Y (little)					
VSAsrna33		Y high					

VSAsrna34		Y (little)					
VSAsrna35		Y (little)					
VSAsrna36		Y (medium)					
VSAsrna37		Y (high)					
VSAsrna38		Y (little)					
VSAsrna39	Y	Y (high)					
VSAsrna40	Y	Y (high)					
VSAsrna41		Y (high)					
VSAsrna42		Y (little)					
VSAsrna43		Y (little)					
VSAsrna44		Y (medium)					
VSAsrna45		Y (little)					
VSAsrna46		Y (medium)					
VSAsrna47	Y	Y (high)	VC1322/VC1321 IGR Liu et al., 2009				
VSAsrna48	Y	Y (high)					
VSAsrna49		Y (medium)					
VSAsrna50			VC2448/VC2449 IGR Liu et al., 2009				
VSAsrna51		Y (medium)					
VSAsrna52		Y (little)					
VSAsrna53	Y	Y (high)					
VSAsrna54		Y (little)	yybP-ykoY element (VC0021/VC0022 IGR Liu et al., 2009)	yybP-ykoY	RF00080	Riboswitch	yybP-ykoY leader
VSAsrna55		Y (little)					
VSAsrna56							

Supplement file 2 (Table S2) - 2,2´-Dipyridyl

ncRNA ID	Fold change			Intensity data					
				LOW		MID		HIGH	
	LOW	MID	HIGH	Red	Green	Red	Green	Red	Green
VSAsrna11	1.10	0.83	0.92	147.00	133.04	30.00	36.51	97.67	106.60
VSAsrna12	0.90	1.04	1.01	59.33	66.30	19.00	18.29	50.00	49.71
VSAsrna13	0.00	0.00	0.97	32.77	34.00	0.00	0.00	10.50	10.76
VSAsrna14	0.99	0.00	1.16	31.00	31.47	13.64	15.25	18.67	16.09
VSAsrna17	0.92	0.82	0.91	1261.00	1355.69	235.33	288.28	447.00	490.94
VSAsrna20	1.12	1.12	1.03	96.67	83.67	25.67	23.01	85.67	83.31
VSAsrna21	1.00	0.91	0.93	43.00	43.19	14.08	14.23	30.00	32.54
VSAsrna22	1.15	1.28	1.19	44.00	38.13	15.16	12.90	35.00	29.51
VSAsrna24	1.15	1.05	0.99	51.00	44.25	14.06	11.73	26.00	26.64
VSAsrna25	1.52	0.00	0.88	90.00	59.57	19.47	19.38	39.67	45.02
VSAsrna26	1.22	1.18	1.07	278.00	227.88	43.00	36.51	140.97	130.82
VSAsrna27	0.68	0.91	1.11	33.00	48.20	9.00	9.92	15.33	13.76
VSAsrna29	1.05	1.01	0.97	48.64	41.10	10.89	13.69	13.00	13.38
VSAsrna3	1.13	0.94	1.07	67.00	56.86	13.50	14.38	44.33	41.45
VSAsrna31	1.13	1.11	1.15	520.33	466.07	114.00	102.76	258.33	226.06
VSAsrna32	0.87	0.90	1.03	40.50	46.74	14.83	17.00	16.67	16.23
VSAsrna43	1.12	1.50	1.16	89.00	78.65	28.50	19.16	62.00	54.19
VSAsrna44	1.24	0.65	0.91	76.00	61.06	30.00	46.33	45.33	49.40
VSAsrna45	1.18	1.12	1.20	31.77	30.48	0.00	0.00	14.00	11.78
VSAsrna46	1.23	1.05	1.18	4850.67	3824.14	1004.67	1007.41	2574.67	2172.76
VSAsrna47	0.79	0.77	0.93	52.80	42.28	0.00	0.00	12.67	13.93
VSAsrna48	1.14	1.18	1.10	60.50	54.16	13.53	14.01	30.67	28.04
VSAsrna5	1.00	0.71	1.29	141.67	133.27	26.00	36.65	65.33	50.75
VSAsrna52	1.31	1.14	1.31	214.00	156.46	51.33	46.04	146.33	111.33
VSAsrna53	1.23	1.09	1.17	37.44	36.63	12.56	17.42	12.50	10.74
VSAsrna54	0.96	0.96	0.98	112.33	118.33	29.67	30.60	84.33	86.81
VSAsrna56	1.00	1.17	1.00	75.33	75.38	27.00	23.25	84.67	84.52
VSAsrna6	1.02	0.85	0.91	83.67	85.23	18.00	21.29	44.00	48.37
VSAsrna7	1.15	1.41	1.19	90.67	75.42	30.00	21.83	76.67	64.82
VSAsrna8	1.39	1.21	1.31	3140.67	2142.62	726.67	613.99	1591.67	1216.09
VSsrna10	4.57	4.08	1.13	2215.00	482.55	312.33	79.33	183.00	161.59
VSsrna101	0.00	1.33	1.21	0.00	0.00	0.00	0.00	13.00	10.81
VSsrna105	1.22	1.27	1.11	39.23	36.52	0.00	0.00	16.33	14.73
VSsrna106 gcvT riboswitch element	1.15	1.08	1.01	92.67	78.00	21.33	20.45	71.67	71.14
VSsrna107 gcvT riboswitch element	1.07	1.24	1.05	88.00	78.35	26.00	20.76	43.33	41.44
VSsrna108	1.12	0.85	0.91	97.33	81.16	21.00	25.78	52.67	57.99
VSsrna11	1.36	1.20	1.15	72.00	50.37	15.00	12.54	29.00	25.37

VSsrna111	1.43	1.24	1.47	66.67	42.88	14.00	11.29	26.50	17.92
VSsrna112	1.40	1.21	0.79	38.00	27.13	14.58	18.40	12.00	15.20
VSsrna113	1.05	1.28	0.96	105.67	99.19	33.33	27.03	86.33	90.08
VSsrna114 small RNA Qrr	0.99	0.93	1.22	750.00	791.69	140.67	157.43	309.67	255.34
VSsrna115	1.26	1.34	1.23	599.00	458.65	150.00	115.03	424.00	345.35
VSsrna12	1.38	0.82	0.89	79.33	56.69	15.00	18.71	28.67	32.23
VSsrna121	1.00	1.08	1.07	32.57	37.95	0.00	0.00	14.00	13.06
VSsrna123	1.22	0.96	1.04	91.67	72.04	23.67	24.69	58.67	56.40
VSsrna126	0.92	0.86	1.02	96.00	108.94	26.00	30.25	49.33	48.67
VSsrna133	0.79	0.78	0.96	40.00	50.59	12.07	13.72	27.00	29.15
VSsrna135	1.28	1.34	1.01	74.33	59.70	19.50	14.49	54.00	53.74
VSsrna140	1.02	0.00	0.90	56.00	56.98	13.55	17.75	17.67	19.77
VSsrna144	1.09	1.03	0.98	5121.67	4744.03	1089.67	1062.58	2973.67	3067.32
VSsrna145	1.01	1.14	0.88	681.33	670.89	130.00	115.20	321.67	369.21
VSsrna147	0.97	0.75	1.05	140.00	134.27	28.00	37.87	49.33	46.50
VSsrna148	0.85	0.73	0.93	0.00	0.00	19.00	26.14	0.00	0.00
VSsrna149 tmRNA	1.02	0.75	1.28	36182.67	35616.41	7633.00	10029.03	15929.33	12475.85
VSsrna150	0.74	0.86	1.01	52.00	70.11	13.30	19.08	15.50	15.41
VSsrna151	1.34	1.58	0.92	298.33	213.01	95.00	60.01	225.00	243.16
VSsrna152	1.20	1.09	1.18	159.50	132.62	23.00	23.38	35.00	30.08
VSsrna153	0.98	0.97	1.14	67.00	68.32	15.27	15.16	35.00	30.74
VSsrna154 small RNA 6S / SsrS RNA	0.91	0.67	1.10	818.00	868.04	140.33	215.68	267.67	242.84
VSsrna159 threonine operon leader	1.31	1.31	1.27	38.40	38.35	0.00	0.00	16.00	12.59
VSsrna16	1.02	1.03	0.97	171.00	158.90	41.33	39.85	124.67	128.75
VSsrna160	0.58	0.52	0.56	46.41	48.28	15.05	16.10	18.00	32.08
VSsrna165	0.89	0.66	0.99	31792.67	35551.34	6074.67	8994.17	14868.33	15147.63
VSsrna167	1.29	1.10	1.28	71.67	50.47	19.00	17.35	43.00	33.78
VSsrna169	1.06	1.13	1.07	485.33	441.38	117.00	103.54	328.67	307.83
VSsrna17	1.10	1.15	0.93	102.00	89.45	23.00	20.43	51.33	54.88
VSsrna170	0.92	0.00	0.89	71.50	79.33	16.38	19.16	40.33	48.62
VSsrna172	1.29	1.21	1.13	1882.67	1404.34	392.33	326.67	884.67	782.37
VSsrna173	0.52	0.60	0.87	25.00	48.40	15.04	20.78	17.50	20.04
VSsrna174	0.68	0.75	1.26	27.00	39.48	0.00	0.00	15.00	11.91
VSsrna180	0.78	0.79	0.95	103.00	131.42	23.93	28.86	70.12	77.34
VSsrna182	1.10	1.04	0.95	47.00	42.88	14.90	16.41	23.00	24.11
VSsrna183 cobalamin riboswitch	1.01	1.19	0.92	224.67	217.18	43.00	36.23	106.33	115.14
VSsrna184	1.14	1.12	1.06	24.50	22.10	13.00	11.57	23.67	22.93
VSsrna185	2.21	1.62	1.72	6811.00	2908.85	788.67	487.04	2035.33	1172.44
VSsrna187	1.33	1.62	1.26	802.67	604.56	252.67	157.96	863.33	686.42
VSsrna189	1.23	1.11	1.17	43.50	35.55	0.00	0.00	18.50	16.04
VSsrna190	1.34	1.32	1.13	2052.33	1485.02	484.33	368.52	974.00	858.25

VSsrna191 TPP riboswitch	1.01	1.01	0.94	44.00	43.62	0.00	0.00	18.50	19.94
VSsrna192	1.01	1.05	1.10	50.15	34.74	0.00	0.00	14.67	13.34
VSsrna193 CsrA-regulating small RNA CsrB3	1.02	0.98	1.82	32779.33	32253.62	15899.33	16204.99	18539.67	10211.61
VSsrna194 CsrA-regulating small RNA CsrB2	1.08	0.92	1.09	28035.67	24872.77	5079.00	5535.07	13356.00	12256.43
VSsrna195	1.00	0.89	0.93	149.33	152.98	35.50	40.91	72.00	78.52
VSsrna196	0.89	0.87	0.88	156.33	168.29	37.33	43.10	79.00	85.58
VSsrna20	1.32	1.38	1.07	45.00	34.09	15.70	18.61	16.33	15.45
VSsrna21	1.15	1.05	1.14	58.50	51.00	12.00	11.47	30.00	26.40
VSsrna22	3.94	4.74	16.30	262.00	67.23	65.00	13.72	274.33	17.12
VSsrna23 small RNA Spot 42	1.20	0.97	1.03	150.67	121.45	30.67	32.97	60.33	59.13
VSsrna24	1.50	1.32	1.22	854.67	546.23	168.00	139.05	333.67	278.38
VSsrna25	0.00	0.00	0.84	0.00	0.00	0.00	0.00	11.00	13.14
VSsrna26	0.98	1.01	0.86	30.78	34.06	12.40	17.10	8.00	9.29
VSsrna28	1.11	1.00	1.00	187.67	167.48	48.67	48.91	165.00	165.85
VSsrna3	3.75	1.49	1.17	156.50	42.02	18.63	21.09	24.00	20.93
VSsrna30	1.21	1.33	1.28	40.00	32.98	15.00	17.55	20.67	16.19
VSsrna31 lysine riboswitch	1.10	1.14	1.21	460.67	407.34	121.33	107.02	393.33	322.87
VSsrna32	1.12	1.21	1.21	50.50	44.89	14.71	13.52	32.00	26.52
VSsrna33	1.28	1.27	1.07	657.67	513.00	107.67	80.73	336.50	314.89
VSsrna35	1.46	0.00	1.06	48.00	32.89	14.07	16.16	15.00	13.80
VSsrna37	1.05	0.00	1.15	34.04	33.58	0.00	0.00	13.33	11.53
VSsrna38	1.14	1.16	0.90	45.00	40.90	14.06	14.63	14.00	15.45
VSsrna40	0.75	0.74	0.90	25.00	33.54	0.00	0.00	10.00	11.08
VSsrna42	0.95	0.96	1.06	10056.33	10706.22	2420.33	2590.98	4892.00	4727.17
VSsrna43 ribosomal S15 leader	1.36	1.45	1.10	6370.33	4465.04	1347.67	943.00	2279.00	2098.93
VSsrna44 VSsrna44 638438:638620	1.29	1.22	1.14	283.67	217.48	75.67	63.80	132.00	116.51
VSsrna47	0.97	0.95	1.12	45.00	46.48	12.65	13.65	29.33	26.32
VSsrna49	1.00	1.00	0.98	347.33	339.26	79.67	79.31	236.33	243.08
VSsrna52	1.31	1.05	1.18	1680.00	1244.23	455.67	445.96	1324.67	1134.42
VSsrna53	1.27	1.16	1.30	88.00	71.52	18.00	16.10	52.00	40.51
VSsrna55 CsrA-regulating small RNA CsrB1	1.10	0.99	1.16	34186.67	29990.32	5772.33	5836.97	12331.33	10526.88
VSsrna57	1.04	1.08	1.11	36.00	34.64	11.62	11.31	22.33	20.17
VSsrna60	1.05	1.32	0.93	92.00	85.67	29.33	22.36	95.67	102.90
VSsrna62	1.16	0.90	1.07	118.33	97.94	23.00	25.67	74.33	70.06
VSsrna63	1.00	1.05	1.04	34.50	34.79	11.89	12.24	21.33	20.58
VSsrna64	0.85	0.47	0.86	40.50	48.88	17.01	20.74	16.50	19.25
VSsrna67	1.49	1.92	1.33	1168.00	744.89	327.00	171.93	753.00	572.05
VSsrna68	1.33	1.48	1.09	36.00	27.13	16.99	22.14	14.50	13.34

VSsrna7	1.17	1.13	1.30	94.33	76.91	24.67	22.89	47.67	36.94
VSsrna70	1.55	1.03	0.89	59.50	39.05	16.62	22.97	10.00	11.21
VSsrna71	0.83	0.82	0.00	22.00	27.56	0.00	0.00	0.00	0.00
VSsrna72	1.36	1.18	2.12	102.00	75.33	18.00	15.32	65.33	31.36
VSsrna73	0.66	0.00	0.08	0.00	0.00	42.95	55.11	22.00	285.40
VSsrna75	1.65	1.67	1.40	316.67	181.52	83.00	50.67	206.67	148.94
VSsrna76	1.17	1.18	1.23	29.00	24.82	0.00	0.00	18.00	14.58
VSsrna77	0.00	0.90	0.90	0.00	0.00	0.00	0.00	15.33	16.99
VSsrna78	1.07	0.96	1.04	106.00	97.01	25.00	26.30	95.33	91.18
VSsrna81	0.00	0.00	1.39	62.94	41.23	13.41	13.17	27.33	19.95
VSsrna83	0.88	0.73	0.90	247.67	307.90	72.00	99.26	156.67	174.16
VSsrna85	1.33	1.50	1.09	62.00	46.64	14.33	11.72	22.00	20.20
VSsrna87	1.10	1.14	1.20	40.00	36.43	9.00	7.87	16.50	13.80
VSsrna89	1.00	1.12	1.02	152.33	149.91	39.67	36.39	104.33	102.71
VSsrna9	0.87	0.73	0.99	86.33	98.64	20.33	27.53	51.33	52.26
VSsrna93	1.36	1.41	1.11	296.33	219.05	58.33	42.17	191.67	170.72
VSsrna97	1.25	1.42	1.08	330.00	262.17	106.33	75.39	290.00	269.25
VSsrna99	0.96	1.01	0.93	35.00	36.44	12.22	13.88	18.00	19.20

Supplement file 2 (Table S2) - Hydrogen peroxide

ncRNA ID	Fold change			Intensity data					
				LOW		MID		HIGH	
	LOW	MID	HIGH	Red	Green	Red	Green	Red	Green
VSAsrna11	0.73	1.25	0.99	36.67	52.72	256.33	207.85	62.33	63.00
VSAsrna12	1.01	1.19	1.26	14.33	14.24	37.33	31.65	15.67	12.42
VSAsrna13	1.05	1.12	1.05	0.00	0.00	14.00	12.50	121.28	120.51
VSAsrna14	0.00	1.01	0.95	0.00	0.00	13.00	12.90	34.45	35.80
VSAsrna17	0.82	0.97	1.55	168.00	203.83	598.67	620.29	167.00	107.99
VSAsrna18	1.10	1.13	0.91	0.00	0.00	16.67	14.71	77.81	77.18
VSAsrna19	0.83	0.70	0.63	0.00	0.00	9.00	12.83	59.89	60.98
VSAsrna20	0.92	0.95	1.02	26.67	28.95	64.00	67.51	20.00	19.61
VSAsrna21	0.85	0.80	0.83	11.85	12.52	22.00	27.53	9.81	9.66
VSAsrna22	1.24	0.90	0.92	13.50	10.91	25.33	28.68	10.01	10.13
VSAsrna24	1.12	0.82	1.01	12.00	10.68	25.67	31.40	11.00	10.93
VSAsrna25	0.86	0.88	0.86	16.00	18.78	41.67	47.47	12.29	13.61
VSAsrna26	0.94	1.02	1.08	49.00	51.95	123.33	121.31	40.67	37.45
VSAsrna27	0.96	0.96	0.71	0.00	0.00	12.00	12.53	57.60	58.49
VSAsrna29	0.97	0.93	0.94	13.07	13.80	16.00	17.30	30.74	33.48
VSAsrna3	1.13	1.34	1.23	16.50	14.63	49.33	37.05	15.67	12.81
VSAsrna31	1.03	1.33	1.14	45.82	40.16	151.33	98.24	36.14	25.46
VSAsrna32	0.96	1.15	1.24	0.00	0.00	20.00	17.45	11.03	9.93
VSAsrna36	1.03	1.03	1.13	0.00	0.00	11.00	10.71	64.68	65.91
VSAsrna43	0.90	1.01	1.57	17.33	19.37	54.33	54.30	28.33	18.32
VSAsrna44	1.00	0.98	1.42	14.00	14.14	52.33	55.91	24.00	17.00
VSAsrna45	1.18	1.13	1.36	0.00	0.00	10.50	9.70	58.74	60.48
VSAsrna46	1.10	1.13	1.44	1023.67	939.31	3334.67	3023.99	936.00	651.40
VSAsrna48	1.03	1.01	0.90	13.67	13.53	41.33	42.13	12.00	13.29
VSAsrna5	0.86	1.08	1.13	21.00	24.67	42.00	38.73	17.00	15.11
VSAsrna52	1.34	1.05	1.45	45.00	35.43	104.67	111.72	50.33	34.73
VSAsrna54	0.99	1.11	1.17	31.00	32.14	92.00	83.24	22.33	19.26
VSAsrna56	1.13	1.08	1.36	30.33	26.79	80.00	73.57	22.00	16.33
VSAsrna6	0.96	1.09	1.17	23.67	24.64	49.00	45.01	18.50	15.70
VSAsrna7	1.03	0.96	1.49	35.33	34.57	89.33	98.96	37.00	24.63
VSAsrna8	1.48	1.19	1.40	841.67	576.93	1563.00	1372.04	514.33	367.42

VSsrna1	2.38	2.38	2.17	16.06	14.02	40.00	16.83	14.96	11.57
VSsrna10	1.09	1.26	1.36	89.00	83.47	235.67	190.79	85.67	63.24
VSsrna101	0.94	1.10	1.27	0.00	0.00	12.50	11.36	79.73	80.09
VSsrna105	0.00	0.95	0.91	0.00	0.00	10.00	10.56	45.77	48.42
VSsrna106	1.21	1.39	1.28	26.33	21.98	69.00	51.07	19.00	14.79
VSsrna107	1.22	1.42	1.13	19.00	15.55	70.00	49.53	14.67	13.03
VSsrna108	1.20	1.08	1.26	17.33	15.69	61.67	60.68	20.00	16.04
VSsrna11	1.43	1.24	1.16	15.00	10.47	27.67	22.75	10.61	10.06
VSsrna111	1.27	0.98	0.96	15.50	12.77	34.67	37.78	13.33	14.03
VSsrna112	0.82	0.81	0.82	0.00	0.00	19.33	24.88	9.75	9.23
VSsrna113	0.90	0.88	0.71	24.33	27.27	57.67	67.11	13.00	18.39
VSsrna114	0.98	1.58	1.95	133.33	143.86	231.00	190.34	70.00	38.79
VSsrna115	1.36	1.29	1.51	148.33	108.73	442.67	346.22	141.00	93.62
VSsrna119	1.16	1.21	1.29	0.00	0.00	15.00	13.01	179.30	177.06
VSsrna12	0.99	1.12	0.96	14.50	14.64	33.00	30.15	11.00	11.56
VSsrna121	1.00	1.15	1.14	10.98	11.85	12.67	11.27	83.86	84.11
VSsrna122	1.10	1.11	1.10	75.25	70.38	207.00	187.11	53.47	45.64
VSsrna123	1.05	0.99	1.01	33.67	33.01	64.67	66.96	20.33	20.12
VSsrna126	1.07	1.15	1.32	18.67	17.51	53.00	46.25	19.67	14.85
VSsrna133	1.11	1.08	1.06	19.83	18.88	48.00	44.35	15.47	13.29
VSsrna135	0.82	0.94	1.08	16.00	19.67	49.67	53.87	17.33	16.58
VSsrna137	1.06	1.01	0.96	0.00	0.00	13.00	12.88	34.72	36.06
VSsrna140	0.00	0.99	1.50	10.58	12.04	23.33	23.95	11.00	7.31
VSsrna144	0.84	0.99	0.85	1220.67	1451.43	4062.33	4119.60	819.67	965.28
VSsrna145	1.38	1.24	1.28	204.00	153.60	643.67	527.62	151.00	117.76
VSsrna147	1.03	1.58	2.15	35.67	35.56	70.33	45.43	25.67	11.94
VSsrna149	1.15	1.50	0.72	10265.33	9105.14	20977.00	14008.46	4564.67	6303.36
VSsrna150	1.15	1.15	1.14	0.00	0.00	15.00	13.00	179.36	177.12
VSsrna151	0.73	0.84	1.04	45.33	62.95	140.00	175.70	38.67	37.05
VSsrna152	1.21	1.30	1.80	15.00	12.59	54.67	48.83	22.00	12.44
VSsrna153	1.02	1.07	0.98	9.67	9.49	26.00	24.35	9.00	9.15
VSsrna154	1.04	1.50	1.76	178.33	175.98	443.50	313.42	185.50	110.76
VSsrna156	0.89	0.83	0.70	0.00	0.00	12.67	15.95	61.38	61.98
VSsrna159	1.09	1.12	1.09	0.00	0.00	16.67	15.52	61.85	61.71
VSsrna16	0.94	0.99	1.04	38.00	40.77	107.00	109.93	27.33	26.46
VSsrna165	0.92	1.09	0.72	6980.33	7655.07	16832.33	15598.24	3538.67	4995.74

VSsrna167	1.23	1.23	1.34	17.33	14.15	39.33	33.66	12.00	8.98
VSsrna169	1.26	1.05	1.27	168.33	134.76	368.67	356.55	80.33	63.94
VSsrna17	1.00	1.04	1.07	28.67	28.77	83.33	82.11	23.00	21.40
VSsrna170	0.96	1.01	0.93	0.00	0.00	15.50	14.95	79.87	79.87
VSsrna172	1.09	1.04	1.15	373.67	344.66	835.33	823.63	334.00	289.83
VSsrna174	0.80	0.77	0.67	11.05	10.93	14.33	19.27	49.37	49.77
VSsrna18	1.04	1.03	1.10	11.95	12.92	13.33	13.07	41.23	42.41
VSsrna180	0.97	0.96	1.02	14.48	14.31	32.00	33.28	11.47	11.33
VSsrna182	1.28	1.27	1.48	27.84	25.25	77.67	61.77	30.00	20.16
VSsrna183	0.97	0.95	0.98	71.67	73.94	154.00	161.88	38.33	39.08
VSsrna184	0.00	1.14	0.89	11.01	11.38	26.33	23.98	9.00	10.16
VSsrna185	2.14	1.14	1.08	2513.33	1258.44	5113.00	5065.04	1318.33	1225.72
VSsrna187	0.92	0.74	0.66	298.00	325.11	723.00	987.56	206.67	308.68
VSsrna189	0.83	0.78	0.80	0.00	0.00	13.50	17.17	58.57	59.19
VSsrna190	1.41	1.16	1.71	728.00	514.26	1583.33	1419.83	588.00	345.55
VSsrna191	1.13	1.01	0.96	0.00	0.00	14.00	13.93	79.61	79.81
VSsrna192	1.04	1.09	1.10	0.00	0.00	17.00	15.71	56.67	56.54
VSsrna193	1.15	2.05	1.06	23327.33	20399.21	27479.00	13449.18	18132.67	17336.48
VSsrna194	0.95	1.26	1.22	6410.00	6710.60	17740.33	14498.70	6413.00	5248.29
VSsrna195	0.96	1.04	1.20	32.00	33.35	87.33	87.32	25.33	21.63
VSsrna196	1.01	1.06	1.26	38.00	39.06	125.00	118.98	37.00	29.54
VSsrna20	1.01	1.11	1.28	13.97	13.69	28.00	26.51	12.00	9.28
VSsrna21	0.71	0.97	1.06	10.00	14.09	36.67	39.87	14.67	13.75
VSsrna22	1.89	1.46	1.19	28.33	15.46	60.67	42.93	16.00	13.66
VSsrna23	1.11	1.29	1.31	47.33	46.06	151.33	137.08	46.00	35.15
VSsrna24	0.78	0.98	1.21	104.33	139.89	637.33	820.58	290.00	243.18
VSsrna25	1.09	1.11	1.11	0.00	0.00	12.00	10.98	75.97	76.54
VSsrna28	1.06	1.09	1.11	46.00	43.35	121.67	112.81	29.67	26.92
VSsrna3	0.00	1.72	1.29	0.00	0.00	24.00	13.97	11.00	8.54
VSsrna30	1.08	1.14	1.27	9.00	8.32	23.00	20.77	9.25	8.70
VSsrna31	1.43	1.39	1.39	132.33	93.23	337.67	244.39	95.33	68.60
VSsrna32	1.16	1.20	1.30	10.00	8.57	20.67	17.28	9.48	9.00
VSsrna33	0.94	0.90	0.85	160.33	171.09	332.67	375.38	76.00	89.15
VSsrna35	0.00	0.89	0.81	0.00	0.00	14.33	16.06	63.88	64.41
VSsrna37	1.04	1.06	1.11	11.31	12.26	12.50	12.07	66.80	67.44
VSsrna38	0.83	1.02	0.98	11.77	11.37	25.00	24.14	9.09	9.58

VSsrna40	0.82	1.33	1.76	0.00	0.00	16.00	11.96	185.71	183.21
VSsrna41	0.00	1.57	1.84	11.99	12.93	13.50	8.63	92.65	92.60
VSsrna42	1.20	1.07	1.52	3208.67	2824.99	5813.00	5505.74	2303.33	1521.80
VSsrna43	1.09	1.02	1.48	1235.00	1167.76	3968.33	4060.96	939.33	633.38
VSsrna44	0.91	1.17	1.29	57.33	64.15	242.33	237.29	87.00	68.60
VSsrna46	1.04	0.98	0.99	11.46	12.54	11.33	11.60	66.07	66.96
VSsrna47	0.94	1.03	1.06	11.50	12.33	26.00	25.23	9.18	9.67
VSsrna49	0.86	0.88	0.92	41.00	48.05	112.33	127.84	22.67	24.72
VSsrna52	0.99	1.08	1.43	368.67	380.17	1289.67	1290.01	515.33	361.77
VSsrna53	1.03	0.93	1.36	12.00	11.69	31.00	34.97	15.00	11.18
VSsrna55	0.93	1.28	1.37	6498.67	7085.99	12146.67	9515.54	4975.67	3720.58
VSsrna56	0.94	0.86	0.97	15.91	16.84	9.00	10.46	41.78	44.71
VSsrna57	1.05	1.03	1.00	10.79	11.82	20.00	19.45	10.09	9.80
VSsrna60	0.88	0.88	0.93	27.00	30.61	55.33	62.50	17.67	18.98
VSsrna61	0.97	0.93	0.88	11.67	12.62	12.00	12.96	50.94	51.98
VSsrna62	1.21	1.12	1.08	27.33	22.77	36.33	32.69	15.00	13.75
VSsrna63	1.03	1.07	1.12	10.00	9.81	27.00	24.84	9.43	9.49
VSsrna64	0.98	1.11	1.16	0.00	0.00	17.00	15.28	63.95	63.56
VSsrna65	1.14	1.66	1.91	14.85	12.79	26.67	16.54	13.43	9.92
VSsrna67	1.02	1.37	1.67	365.33	361.92	839.67	632.59	252.67	152.34
VSsrna68	1.16	1.26	1.14	9.83	9.76	16.33	13.26	126.58	125.34
VSsrna7	1.19	1.24	1.77	20.33	17.37	61.33	53.71	23.33	13.37
VSsrna70	1.32	1.20	1.08	20.33	15.77	58.00	51.21	13.50	12.43
VSsrna71	0.98	1.23	1.23	9.00	9.19	15.00	12.80	280.62	275.38
VSsrna72	0.98	1.01	0.87	17.00	17.45	36.33	36.47	10.50	12.06
VSsrna75	1.15	1.21	1.43	66.00	60.16	200.67	187.34	84.00	59.26
VSsrna76	1.14	1.37	1.41	11.28	11.66	19.67	14.97	47.10	45.49
VSsrna77	1.01	1.05	1.37	15.30	14.92	32.00	32.68	15.50	11.31
VSsrna78	1.34	1.44	1.78	28.67	22.64	93.00	67.18	41.67	23.33
VSsrna8	1.04	1.08	1.09	13.38	14.29	10.50	9.73	58.52	60.27
VSsrna81	1.03	1.07	1.11	11.15	12.75	17.33	17.53	36.32	36.89
VSsrna83	0.70	1.13	1.30	70.33	101.27	239.67	239.56	94.00	73.88
VSsrna87	0.98	1.10	1.25	0.00	0.00	14.67	13.43	123.84	122.93
VSsrna89	1.02	0.97	1.06	49.33	48.35	103.67	107.45	29.67	27.74
VSsrna9	0.84	0.83	0.87	18.00	21.51	34.67	42.95	11.09	13.43
VSsrna91	0.90	0.75	0.83	0.00	0.00	9.00	12.05	57.75	59.15

VSsrna93	0.58	0.79	0.92	34.33	59.05	93.00	119.66	35.33	38.06
VSsrna97	0.94	0.99	1.02	62.33	66.92	158.67	168.62	47.33	46.42
VSsrna99	1.14	1.19	1.30	0.00	0.00	21.00	18.65	11.00	8.47

Supplement file 2 (Table S2) - *litR* mutant

ncRNA ID	Fold change			Intensity data					
				LOW		MID		HIGH	
	LOW	MID	HIGH	Red	Green	Red	Green	Red	Green
VSAsrna1	1.97	1.49	0.92	14.21	14.16	29.93	24.98	10.00	10.90
VSAsrna11	1.78	1.72	1.96	157.33	88.29	244.67	139.31	460.33	264.58
VSAsrna12	1.28	1.30	1.20	18.67	14.46	23.67	18.07	54.33	46.28
VSAsrna13	1.18	1.04	1.38	9.00	7.61	10.00	9.58	26.00	19.75
VSAsrna14	1.24	1.22	1.18	17.00	13.75	13.67	11.58	16.00	13.50
VSAsrna17	1.31	1.08	1.18	862.33	654.07	512.67	450.11	764.00	666.49
VSAsrna18	1.43	1.24	1.17	22.00	15.61	15.00	12.12	31.00	27.14
VSAsrna20	0.89	0.75	0.96	36.33	41.97	36.33	49.93	163.33	171.47
VSAsrna21	0.94	0.99	1.02	14.31	16.01	14.34	13.40	21.00	20.61
VSAsrna22	0.90	0.87	1.31	14.50	15.98	22.00	25.36	69.00	56.30
VSAsrna24	0.68	0.81	1.04	10.00	14.62	14.00	17.28	25.33	26.48
VSAsrna25	1.08	0.74	1.10	16.00	15.42	22.00	29.66	61.33	56.52
VSAsrna26	0.99	1.15	0.97	39.67	40.94	43.00	40.88	109.50	113.32
VSAsrna27	1.17	1.11	1.06	0.00	0.00	0.00	0.00	15.67	15.41
VSAsrna29	0.97	0.92	0.96	14.91	17.45	22.56	20.81	13.00	13.50
VSAsrna3	1.06	0.81	1.31	12.00	11.27	12.00	14.76	21.67	17.45
VSAsrna31	1.65	1.87	1.35	68.33	43.48	74.00	39.20	126.00	91.34
VSAsrna32	0.00	1.32	1.21	14.21	11.43	15.00	11.33	25.50	21.87
VSAsrna35	1.16	1.14	1.12	0.00	0.00	0.00	0.00	27.00	25.41
VSAsrna36	1.00	0.99	0.95	0.00	0.00	29.76	24.60	12.00	12.61
VSAsrna43	0.97	1.92	1.33	15.00	15.49	23.67	12.39	32.00	26.00
VSAsrna44	1.48	1.24	1.90	25.33	17.84	33.00	29.52	129.00	75.17
VSAsrna45	1.20	1.12	1.06	0.00	0.00	0.00	0.00	17.00	16.05
VSAsrna46	2.12	1.22	1.49	2368.33	1101.92	2177.00	1728.29	5094.00	3582.79
VSAsrna47	0.91	1.28	1.04	31.41	23.46	19.66	16.62	15.50	14.95
VSAsrna48	0.97	0.69	0.70	15.00	16.41	18.00	26.03	21.33	28.94
VSAsrna5	1.02	0.90	1.25	20.67	20.48	25.00	27.24	91.00	75.99
VSAsrna52	1.46	0.86	1.85	143.67	99.03	122.00	147.22	405.33	237.51
VSAsrna53	1.46	1.36	1.46	14.39	12.95	27.73	22.44	12.00	8.21
VSAsrna54	1.00	1.20	1.10	42.33	41.78	47.50	42.93	39.00	34.95
VSAsrna56	0.96	0.94	1.12	52.67	55.10	41.33	42.86	97.00	91.34
VSAsrna6	1.14	1.06	1.15	26.33	23.09	30.00	28.05	69.33	61.97
VSAsrna7	3.73	2.90	1.94	114.33	40.38	115.67	42.11	127.67	79.16
VSAsrna8	2.31	1.63	1.55	679.67	287.74	916.00	570.61	6044.00	4984.45
VSsrna1	2.27	2.61	1.51	41.33	19.09	25.00	11.33	28.50	18.93
VSsrna10	1.31	1.43	1.77	76.33	59.36	98.33	69.40	288.67	163.77
VSsrna103	0.92	0.98	0.83	0.00	0.00	0.00	0.00	12.00	14.40
VSsrna106	1.35	1.29	1.32	37.67	29.76	45.33	35.05	60.33	44.70

VSsrna107	1.51	1.24	1.30	38.67	27.86	46.67	37.90	73.00	56.06
VSsrna108	1.40	0.81	1.50	56.67	40.42	80.33	95.85	754.00	598.50
VSsrna11	1.08	1.30	1.32	13.00	11.99	13.00	9.80	19.33	14.84
VSsrna111	1.19	1.10	1.65	62.67	54.20	82.67	76.31	200.67	145.37
VSsrna112	0.93	0.97	1.04	10.00	10.71	16.12	14.67	14.00	13.49
VSsrna113	1.62	1.67	0.73	24.00	23.28	26.33	16.81	81.67	109.74
VSsrna114 Small RNA qrr	5.55	3.38	4.17	510.00	93.13	335.33	102.52	811.33	199.97
VSsrna115	1.80	1.50	1.81	178.67	100.91	308.33	201.56	854.00	511.75
VSsrna118	1.55	1.66	1.15	0.00	0.00	0.00	0.00	13.50	11.68
VSsrna119	1.03	1.08	1.06	0.00	0.00	0.00	0.00	15.00	13.75
VSsrna12	1.48	1.03	1.41	18.00	12.19	16.00	15.96	26.00	18.10
VSsrna121	1.22	0.94	1.09	16.00	13.12	15.00	15.84	30.00	27.71
VSsrna123	1.17	0.82	1.05	14.00	11.97	13.00	15.80	32.00	33.25
VSsrna126	0.91	1.01	1.02	13.00	14.27	15.00	14.81	33.33	34.07
VSsrna131	0.85	0.86	0.67	0.00	0.00	0.00	0.00	8.00	11.87
VSsrna133	1.39	1.18	1.02	21.00	15.15	12.00	10.15	82.33	80.25
VSsrna135	1.04	0.93	1.42	11.00	10.61	14.00	15.10	26.67	19.34
VSsrna140	1.31	1.11	1.31	21.50	16.85	15.00	13.67	34.33	28.46
VSsrna143	0.00	0.00	1.11	0.00	0.00	0.00	0.00	15.00	13.49
VSsrna144	0.84	0.98	0.77	1207.00	1432.14	1244.33	1309.44	1179.33	1537.88
VSsrna145	1.19	1.05	1.36	301.00	253.74	216.33	212.66	367.00	271.11
VSsrna147	1.66	1.14	1.39	78.33	46.25	84.67	70.84	55.00	39.52
VSsrna148	1.04	1.05	1.06	14.38	13.41	27.59	23.43	10.00	9.55
VSsrna149	0.92	0.98	1.07	8585.33	9231.57	12272.33	12057.43	15889.00	15221.63
VSsrna150	1.24	1.11	1.01	13.03	14.57	14.71	14.59	18.00	17.73
VSsrna151	1.39	1.24	0.80	79.33	74.98	38.33	35.99	170.33	201.02
VSsrna152	2.07	1.50	1.55	44.00	21.68	40.00	29.59	182.50	119.96
VSsrna153	1.09	1.15	1.23	15.77	16.71	17.07	16.25	25.00	20.81
VSsrna154	1.69	0.92	1.41	235.00	137.95	282.00	307.82	605.00	448.36
VSsrna156	1.08	0.71	0.83	13.00	11.99	10.00	14.02	17.67	20.38
VSsrna159	0.00	0.00	1.04	0.00	0.00	21.22	16.47	17.00	16.30
VSsrna16	1.25	1.14	1.25	51.00	45.38	52.67	48.85	139.67	112.25
VSsrna161	1.24	1.16	1.18	14.11	12.96	27.64	23.50	10.00	8.48
VSsrna165	0.81	0.85	0.87	8898.67	10887.98	14485.67	16622.71	17270.67	19567.63
VSsrna166	0.88	1.03	0.90	11.43	12.98	14.00	15.10	16.00	17.78
VSsrna167	1.90	1.48	2.52	28.33	15.02	37.67	24.88	120.00	58.19
VSsrna169	1.19	1.27	1.47	100.33	86.13	125.67	96.56	179.00	128.16
VSsrna17	1.02	0.90	1.01	24.00	23.37	25.00	27.69	65.00	66.35
VSsrna170	0.84	0.83	0.90	12.19	12.28	12.00	14.43	15.33	17.67
VSsrna172	0.99	0.78	0.90	273.67	278.74	317.00	411.85	771.33	878.24
VSsrna173	0.93	1.03	0.81	14.14	17.51	0.00	0.00	11.00	13.44
VSsrna174	1.35	1.40	1.82	12.97	12.93	21.38	20.90	18.00	10.20
VSsrna177	1.22	1.17	1.19	12.99	12.88	13.42	12.54	19.00	15.96

VSsrna18	1.19	1.03	1.12	12.05	21.12	12.02	12.31	17.00	15.22
VSsrna180	0.86	0.78	0.97	13.20	15.72	13.00	16.61	27.67	28.26
VSsrna182	1.18	0.92	1.12	28.00	25.22	31.00	33.75	57.00	53.52
VSsrna183	0.82	0.87	0.82	35.00	41.87	32.00	37.26	81.67	96.04
VSsrna184	1.30	1.23	1.21	17.00	13.03	14.00	11.39	29.33	24.19
VSsrna185	1.83	1.36	2.09	801.67	443.50	767.00	606.36	3936.00	2392.86
VSsrna187	1.24	0.91	0.94	385.33	320.99	624.33	720.26	3052.00	3196.71
VSsrna19	0.00	0.63	1.24	13.61	15.01	12.00	18.98	31.50	30.07
VSsrna190	1.56	1.57	1.63	128.00	85.93	118.33	81.32	185.00	119.75
VSsrna191	1.13	0.96	0.78	13.00	11.47	14.00	14.48	16.33	20.44
VSsrna192	1.03	1.04	1.16	0.00	0.00	0.00	0.00	12.50	10.79
VSsrna193 CsrB3	1.38	1.18	2.03	19259.00	14016.52	19588.00	17267.58	29911.00	14824.78
VSsrna194 CsrB2	1.83	1.21	1.24	6176.67	3428.99	9837.67	8145.92	20130.33	16370.39
VSsrna195	1.16	0.90	1.08	40.33	34.72	34.67	39.25	95.00	90.91
VSsrna196	1.04	0.90	1.14	88.67	83.84	84.33	95.10	135.33	120.52
VSsrna20	1.22	0.80	0.58	16.00	14.66	15.00	19.03	45.33	80.27
VSsrna21	0.74	0.49	0.26	66.94	75.51	53.75	48.75	18.50	71.53
VSsrna22	0.91	1.16	0.97	16.00	17.57	14.00	12.12	14.00	14.39
VSsrna23 Spot42	2.28	1.69	1.10	192.00	91.45	94.00	54.67	17.00	15.77
VSsrna24	2.84	1.42	1.78	126.67	44.00	380.33	319.76	4346.33	2485.47
VSsrna25	1.02	0.94	1.03	16.38	18.32	21.00	22.30	23.00	21.89
VSsrna28	1.00	1.19	1.00	33.67	34.73	31.33	26.16	64.00	62.41
VSsrna3	1.15	1.03	1.23	14.00	12.00	14.45	13.56	27.00	25.03
VSsrna30	1.14	0.97	1.62	12.00	10.51	11.00	11.33	32.33	21.14
VSsrna31	1.18	0.90	1.44	150.67	132.76	111.00	129.40	461.33	309.10
VSsrna32	1.47	1.77	1.31	14.00	9.55	18.00	10.08	40.33	32.39
VSsrna33	0.77	0.74	1.01	92.33	119.82	109.00	145.51	240.67	247.43
VSsrna35	1.22	0.70	0.92	11.00	9.02	12.55	13.48	20.67	22.05
VSsrna37	0.97	1.09	1.07	12.00	14.55	12.01	11.38	23.00	21.53
VSsrna41	0.99	0.96	1.02	15.70	18.71	14.56	15.20	22.00	21.52
VSsrna42	1.88	1.32	1.07	1678.67	968.57	2063.33	1593.50	2904.00	2755.01
VSsrna43	1.34	1.15	1.15	1512.00	1152.64	1342.67	1225.70	1950.67	1698.16
VSsrna44	1.89	1.58	1.08	76.33	47.13	73.00	51.54	150.67	135.65
VSsrna46	0.00	1.08	1.12	0.00	0.00	0.00	0.00	16.00	14.31
VSsrna47	1.10	1.17	1.12	15.29	14.98	18.10	17.27	20.50	18.16
VSsrna48	0.81	0.78	0.69	0.00	0.00	0.00	0.00	10.00	14.41
VSsrna49	1.05	0.89	1.53	192.67	180.52	256.67	292.12	497.33	426.26
VSsrna52	2.19	1.85	2.14	378.33	186.42	581.67	314.11	3535.00	1912.21
VSsrna53	1.70	1.15	1.62	41.00	24.51	45.33	40.50	191.67	119.71
VSsrna55 CrsB1	2.21	1.34	1.21	5772.00	2630.69	11503.33	8524.71	19599.00	16250.42
VSsrna56	1.20	1.03	1.12	12.00	9.99	9.00	8.72	24.00	22.06
VSsrna57	1.56	1.41	1.35	13.34	14.08	0.00	0.00	16.50	12.24
VSsrna59	1.06	1.06	0.92	12.57	14.62	14.46	15.39	14.00	15.29

VSsrna60	1.23	0.93	0.68	36.33	29.80	33.00	34.74	69.67	100.28
VSsrna61	1.27	1.14	1.29	14.11	11.35	13.00	11.42	30.00	23.25
VSsrna62	1.04	0.90	1.09	18.00	17.40	16.67	19.03	30.33	28.90
VSsrna63	1.10	1.11	1.01	16.23	15.51	34.56	28.02	11.00	10.86
VSsrna64	1.04	0.93	0.97	0.00	0.00	0.00	0.00	13.00	13.59
VSsrna65	1.06	1.45	1.46	0.00	0.00	78.27	50.73	22.00	15.09
VSsrna66	0.00	0.00	0.91	13.99	15.67	16.16	15.58	17.00	18.62
VSsrna67	1.77	1.49	1.26	718.67	403.05	502.33	342.70	535.67	418.31
VSsrna68	0.00	0.00	1.75	0.00	0.00	0.00	0.00	16.00	9.12
VSsrna7	1.96	1.32	2.44	34.00	18.95	31.67	24.88	214.67	102.95
VSsrna70	1.36	1.05	0.00	14.00	10.33	11.00	10.44	0.00	0.00
VSsrna71	1.20	1.15	1.18	11.39	12.91	11.00	9.57	18.00	15.63
VSsrna72	0.97	0.84	1.20	18.00	18.53	19.67	23.55	43.33	35.99
VSsrna75	1.77	1.02	1.58	137.33	79.77	137.00	139.01	331.67	209.33
VSsrna76	1.55	1.42	1.24	13.50	9.07	12.50	8.81	15.50	12.71
VSsrna77	1.49	1.57	1.12	20.67	13.65	27.00	20.08	62.67	55.63
VSsrna78	1.61	1.53	1.72	74.33	47.42	60.33	39.63	111.00	66.71
VSsrna81	1.30	1.14	1.40	17.00	13.03	13.00	11.42	25.00	17.92
VSsrna83	1.40	1.49	1.25	168.67	119.44	227.33	163.00	401.67	310.39
VSsrna85	0.93	1.02	1.16	10.00	10.71	18.00	17.61	20.50	17.61
VSsrna87	1.15	1.08	1.08	0.00	0.00	0.00	0.00	13.50	13.03
VSsrna88	1.19	1.21	1.32	14.94	13.44	28.86	23.22	12.00	9.08
VSsrna89	0.87	0.78	0.99	38.67	46.34	38.33	49.83	133.00	134.72
VSsrna9	0.85	0.70	0.77	28.00	33.38	39.67	57.97	181.67	227.70
VSsrna93	1.87	1.90	1.47	69.00	38.44	133.33	67.76	123.33	88.21
VSsrna95	1.17	0.90	1.10	24.81	18.68	13.21	12.06	18.50	16.48
VSsrna97	1.35	0.97	1.16	90.67	67.84	104.33	145.64	199.00	173.42
VSsrna99	0.76	0.85	1.01	11.00	14.53	20.00	23.59	99.67	107.77

Supplement file 2 (Table S2) - Statistics

	# genes with intensity in any of three cond on microarray	# genes with intensity >50 (WT - any of three conditions)	# genes with intensity >75 (WT - any of three conditions)	# number genes with intensity >100 (WT - any of three conditions)	# genes differentially expressed (> 2 fold) (any cond)	# genes differentially expressed (> 1.5 fold) (any cond)
DP	134	85	68	52	7	22
HP	142	91	62	50	4	26
LitR	152	72	60	48	15	50