

Gene regulatory network of the noncoding RNA *Qrr5* in the cytotoxicity of *Vibrio parahaemolyticus* during infection

Supplementary materials

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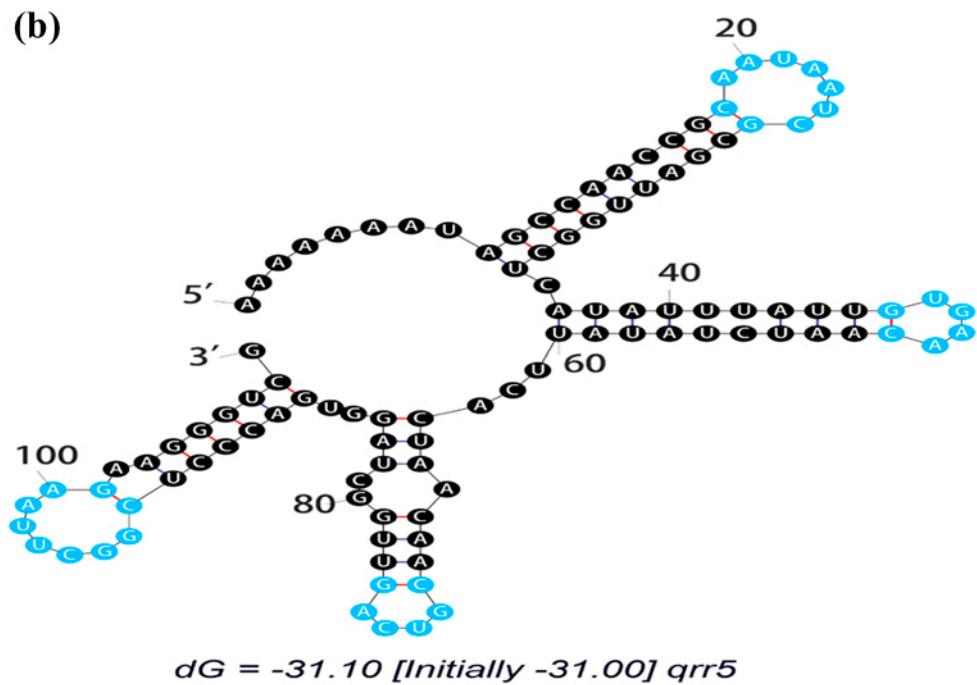
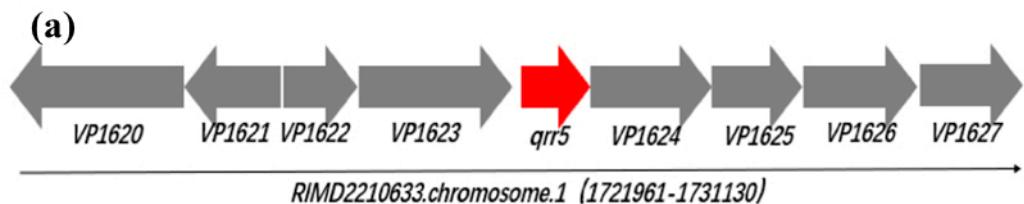


Figure S1. The genetic map and structure of sRNA *Qrr5* in *V. parahaemolyticus*.

(a) The position of *Qrr5* in the RIMD2210633 chromosome 1. (b) The secondary structure of *Qrr5* (109 bp) in *V. parahaemolyticus*.

Table S1. Oligonucleotide Primers

Primer	Sequence (5'—3')
For the construction of the	
<i>Qrr5</i> -deletion mutant	
<i>Qrr5</i> -U-F	CTTCTAGAGGTACCGCATGCGCTGCTAAAAA GAAG
<i>Qrr5</i> -U-R	ATGCATAATACTTCTGCCTGTCGTATATGA
<i>Qrr5</i> -D-F	TCATATACGACAGGCAGAAGTATTATGCAT
<i>Qrr5</i> -D-R	GTGGAATTCCCGGGAGAGCTGTGATCAGCT TCGCTAAGG
<i>Qrr5</i> -F100	AAGTGAAGCGCGCCGTACCAACCTCATTAC CGAT
<i>Qrr5</i> -R100	ATCGCGTTCTCTACCTGAC
<i>Qrr5</i> -pF	GCTCTAGAGTGAGGGAAGTGCAATTGC
<i>Qrr5</i> -pR	CCTCGAGCGACCCTTCTTAAGCCGAG
For quantitative PCR	
16s-F	AAGCGTGGGGAGCAAACAG
16-R	CGAAGGCACCAATCCATCTC
<i>qrr1</i> -F	CGACCCCTCGGGTCACCTAG
<i>qrr1</i> -R	AAAAAAAGAACCAATAGGCAGTCGG
<i>qrr2</i> -F	GACCCTTATTAAGCCGAGGG

<i>qrr2</i> -R	AGAAAAAAACGCCAATCACA
<i>qrr3</i> -F	TGACCCTTCTAACGCCGAG
<i>qrr3</i> -R	AAAAAAAAAGCCAACCACAAGG
<i>qrr4</i> -F	CGAGGGTCACCTAGCCA ACT
<i>qrr4</i> -R	AAATAGCCAACCGCAAAGAG
<i>qrr5</i> -F	AAAAAAAATAGCCAACCGCAA
<i>qrr5</i> -R	CGACCCTTCTTAAGCCG
VP1766-F	AAACAATGCGTGAGCCAGAC
VP1766-R	ACCAAACAGAACTCACCTCCAC
VPA1068-F	GAAAACACCCGCCAACATTAC
VPA1068-R	TCGCACTTCGGCATCATTG
VP2492-F	GAACAGCAAGAACATGACCAAAGC
VP2492-R	TGTACGTCGATAACGGAGAAGG
VP0485-F	GCAGTACGATGGCGAAAGC
VP0485-R	ACACCAAAGCGGGGACAA
VP2914-F	ATTTCACAAACCTTCCACATC
VP2914-R	GAGCAACAAGCTGGCTTCATC
VP0486-F	CAAGCGAACCTTGAACTTGAGA
VP0486-R	GCCCCGTAACAGTCGTTGTAATC
VPA0628-F	CGGTTGTGATTGCACTGGG
VPA0628-R	TCGCCTTTGGCAGATGAG
VP1665-F	TGTCGAGTGTGCGGAAGCG

VP1665-R

ATCAGTCGGCGAGCGTAT

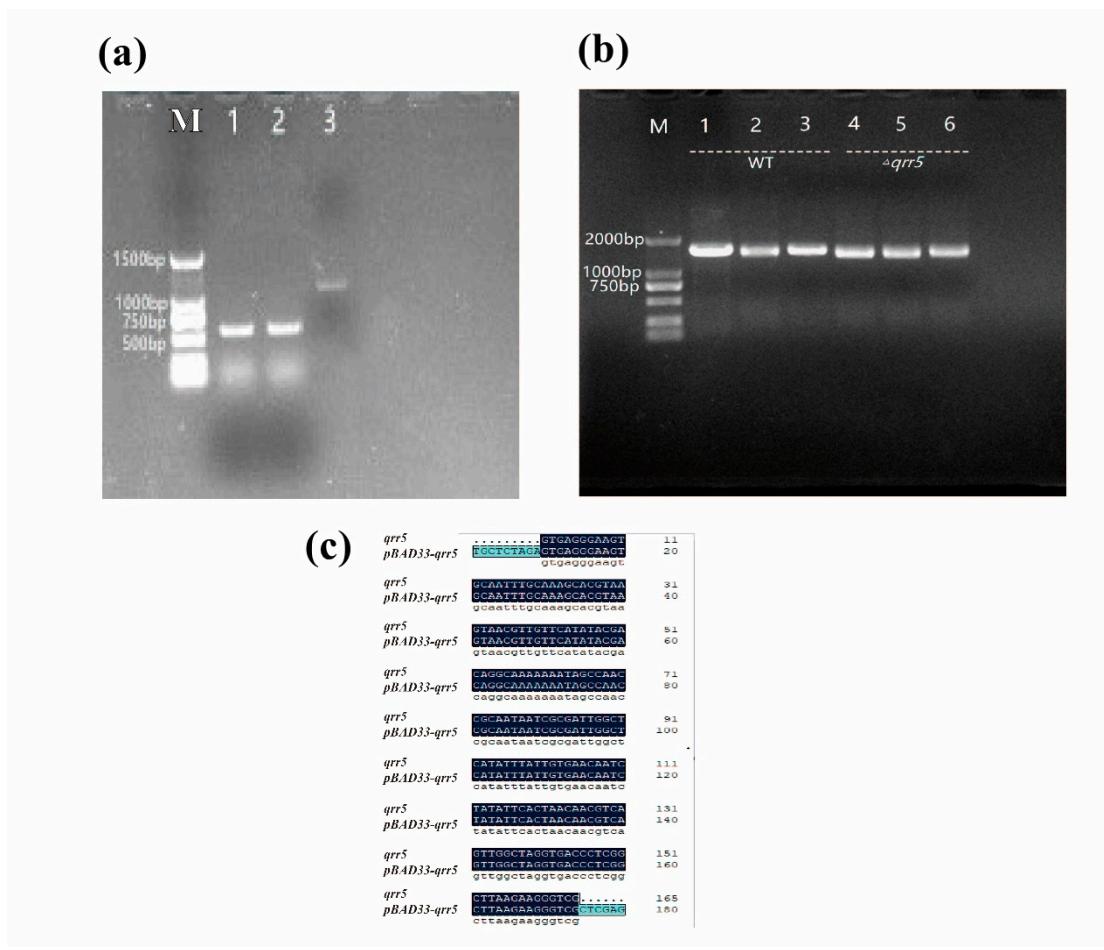


Figure S2. Construction and confirmation of $\Delta Qrr5$. (a) M: DNA marker; 1: The upstream DNA fragment (600 bp) of *Qrr5* was amplified by PCR with primers *Qrr5-U-F/Qrr5-U-R*; 2: The downstream DNA fragment (600 bp) of *Qrr5* was amplified by PCR with primers *Qrr5-D-F/Qrr5-D-R*; 3: The **confused** DNA fragment of upstream and downstream (1200 bp). (b) M: DNA marker; 1-3: the DNA fragments (1509 bp) of upstream-100bp to downstream +100 bp of *Qrr5* (109 bp) were amplified by PCR with primers *Qrr5-F100/Qrr5-R100* using the *V.parahaemolyticus* wild-type chromosomal DNA as templates; 4-6: The DNA fragments (1400 bp) of upstream -100 bp to downstream +100 bp of *Qrr5* (109 bp) was amplify by PCR with primers *Qrr5-F100/Qrr5-R100* using the *V.parahaemolyticus* $\Delta Qrr5$ chromosomal DNA as templates, respectively. (c) The sequence of DNA fragment (109 bp) of *Qrr5* was amplified by

PCR with primers *Qrr5-pF/Qrr5-pR* using the *V.parahaemolyticus*Δ*Qrr5::pBAD33-Qrr5* chromosomal DNA as templates compared to the sequence of *Qrr5*.

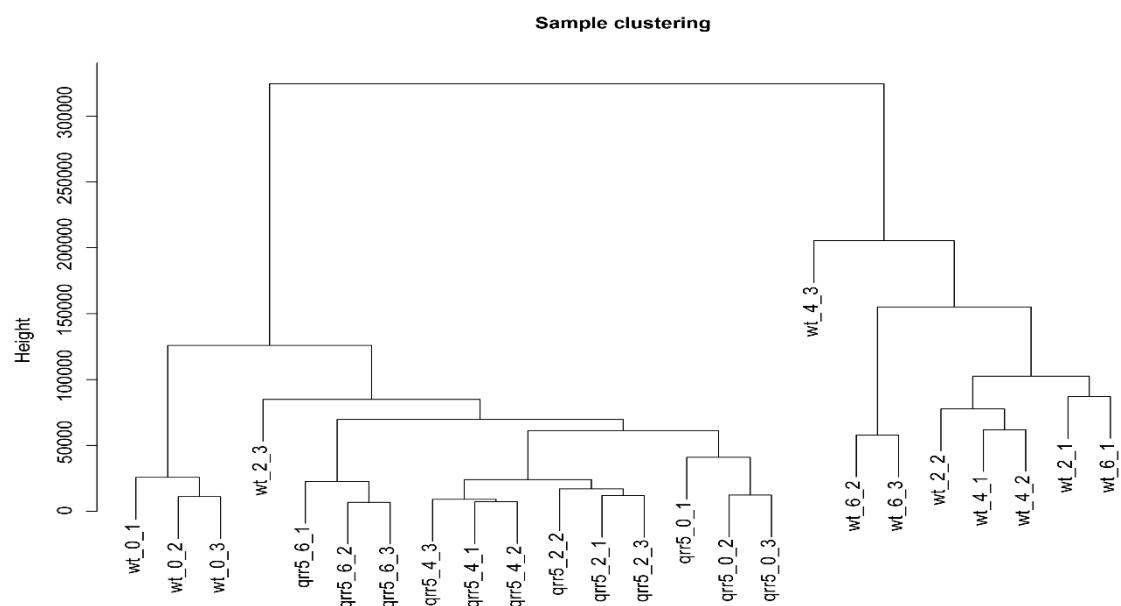


Figure S3. Each sample clustering of RNA-Seq data.

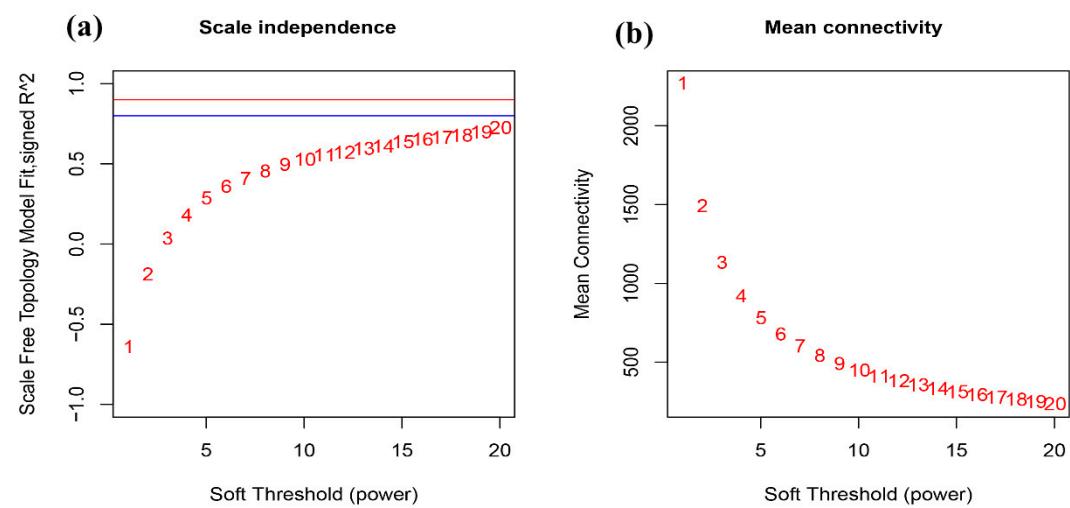


Figure S4. The scale independence (a) and mean connectivity (b) of RNA-Seq data.

Table S2. The function of genes in the WGCNA co-expression network

Name	Function
<i>VP1766</i>	Alanyl-tRNA editing protein
<i>VP1600</i>	Unknown
<i>VPA1068</i>	Hypothetical protein
<i>VP2914</i>	D-2-hydroxyacid dehydrogenase
<i>VP0027</i>	Hypothetical protein
<i>VP0807</i>	Unknown
<i>VP0486</i>	Diguanylate cyclase
<i>VPA1520</i>	Hypothetical protein
<i>VP2389</i>	Magnesium transporter
<i>VP2492</i>	Ammonium transporter
<i>VP2112</i>	Unknown
<i>VPA0631</i>	Heme synthase (cyoE)
<i>VPA1141</i>	Carboxymuconolactone decarboxylase
<i>VP0485</i>	TIGR01212 family radical SAM protein
<i>VPA0628</i>	Cytochrome ubiquinol oxidase subunit I (cyoB)
<i>VP1665</i>	Type III secretion I chaperone SycN
<i>VP2012</i>	Unknown

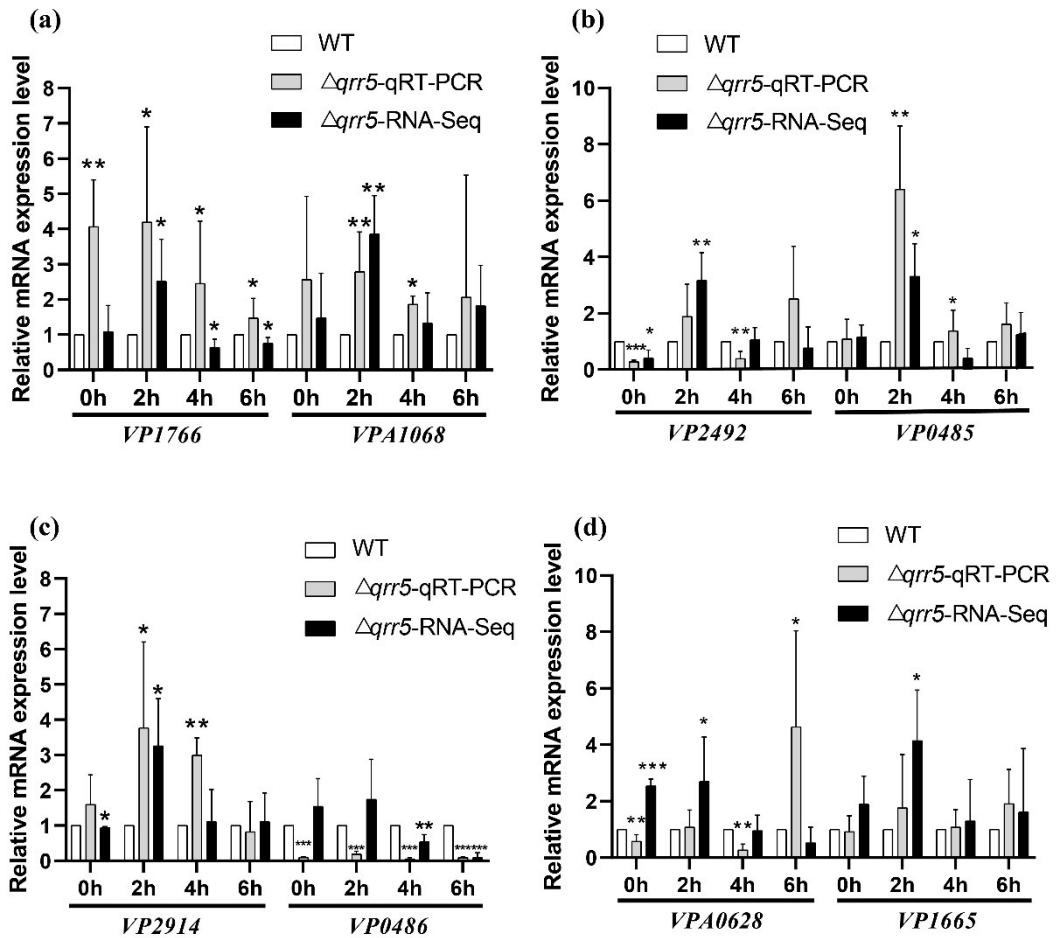


Figure S5. Relative expression of several differentially expressed genes ($\Delta Qrr5$ /WT) at the mRNA level determined by qPCR during various infection periods. (a) The expression of *VP1766* and *VPA1068* by qRT-PCR analysis. (b) The expression of *VP2492* and *VP0485* by qRT-PCR analysis. (c) The expression of *VP2914* and *VP0486* by qRT-PCR analysis. (d) The expression of *VPA0628* and *VP1665* by qRT-PCR analysis.