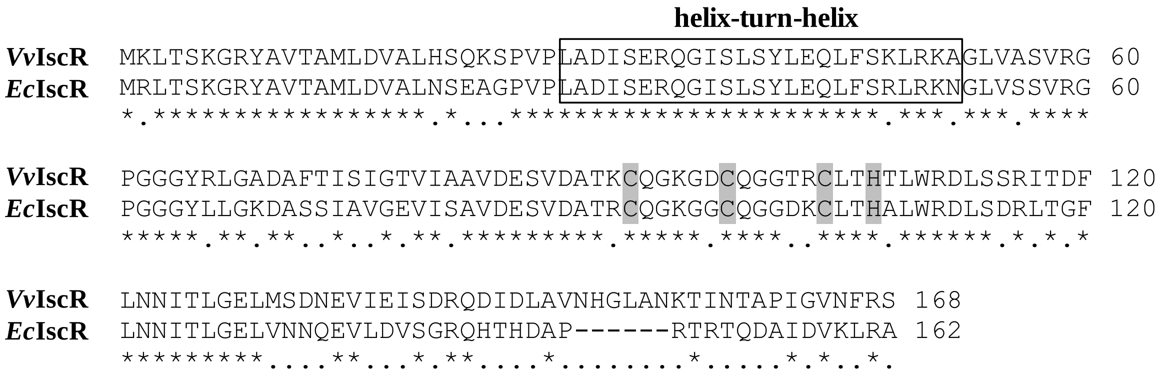
Supporting Information

Lim and Choi



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                                          helix-turn-helix
VvIscR  MKLTSKGRYAVTAMLDVALHSQKSPVPLADISERQGISLSYLEQLFSKLRKAGLVASVRG 60
EcIscR  MRLTSKGRYAVTAMLDVALNSEAGPVPLADISERQGISLSYLEQLFSRLRKNGLVSSVRG 60
        *.*****************.*...***********************.***.***.****

VvIscR  PGGGYRLGADAFTISIGTVIAAVDESVDATKCQGKGDCQGGTRCLTHTLWRDLSSRITDF 120
EcIscR  PGGGYLLGKDASSIAVGEVISAVDESVDATRCQGKGGCQGGDKCLTHALWRDLSDRLTGF 120
        *****.**.**..*..*.**.*********.*****.****..****.******.*.*.*

VvIscR  LNNITLGELMSDNEVIEISDRQDIDLAVNHGLANKTINTAPIGVNFRS 168
EcIscR  LNNITLGELVNNQEVLDVSGRQHTHDAP------RTRTQDAIDVKLRA 162
        *********....**...*.**....*........*.....*.*..*.
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Figure S1. Sequence analysis of IscR of *Vibrio vulnificus* and *Escherichia coli*. Identical sequences are indicated with asterisks (*), and dashed lines represent missing sequences. Conserved helix-turn-helix DNA binding motif (http://www.uniprot.org/uniprot/Q8DEY6) is indicated by open box. Three conserved cysteine residues (C92, C98, and C104) and one histidine residue (H107) (CCCH motif) which are essential for Fe-S cluster ligation are shaded in gray. Alignment was based on the amino acid sequences in the GenBank (NCBI) database and derived by the CLUSTALW alignment program (http://www.ch.embnet.org/software/ClustalW.html).

TABLE S1. Oligonucleotides used for deletion of *iscR* and amplification of *iscR* coding region and promoter regions of the IscR target genes

Oligonucleotide[a]	Sequence (5' → 3')[b]	Use
ISCR001F	TTGTGCGCTGGTTGTCGGTT	Deletion of *iscR*
ISCR001R	TAGGATCCCTCTAAGTAAGAGAGCGAAATC	
ISCR002F	AGGGATCCTATTGATCTTGCGGTAAAT	Deletion of *iscR*
ISCR002R	AGACTGCGCTGCATCGACGT	
ISCR003F	CATATGAAACTGACATCTAAAGGAAGATAT	Amplification of *iscR* ORF
ISCR003R	CTCGAGAGAGCGGAAATTTACAC	
ISCR004F	AAGCTTAGTGTGGATACGGTGTGATATG	Complementation of *iscR*
ISCR004R	TCTAGAGGCAGTTTCATTCTTCACTCC	
FLGE01F	TCTGGTTCACTTCCACGA	Amplification of the *flgE* promoter region
FLGE01R	TAACCTCTGCATTGCGTTCA	
GBPA01F	CGCCTCATCGTTATTCTCAT	Amplification of the *gbpA* promoter region
GBPA01R	GGCCAGTAAGGTTTTTTGCGGTTG	

VVHBA01F	GATAAAACAACAACGCCCACATTAATC	Amplification of the *vvhBA* promoter region
VVHBA01R	CTCGTAATGAGGAATCTATGCTTAAT	
PRX01F	CACCCAGTAAATATTGTTTGGC	Amplification of the *prx* promoter region
PRX01R	CTCAAGGCCAAACTTTACCTAA	
ISCR01F	GGTTGACGCAACTAAGTGTCAGG	Amplification of a part of the *iscR* coding region
ISCR01R	TTAAGAGCGGAAATTTACACCGAT	

[a] The oligonucleotides were designed using the genomic sequence of the *V. vulnificus* MO6-24/O (Park et al., 2011).

[b] Regions of oligonucleotides not complementary to corresponding genes are underlined.

TABLE S2. Oligonucleotides used for qRT-PCR

Locus tag[a]	**Oligonucleotide sequence (5' → 3')**[b]	
	Forward	**Reverse**
VVMO6_00199	ATGGAAGCGATTGAATACCTAA	GGAACTCTTGACCGACAG
VVMO6_00216	GAGCTTGTTTGGTGCAGGTG	ATGCCACATTGGTAAGGCCA
VVMO6_00218	GGTCAACGGAGTGCCAAAAC	ATCTTCTGGTTTGCGGTCGT
VVMO6_00219	CATCAAAGACAAAGGCAAGGTA	CGTAGAATCGCAGAGTGAAC
VVMO6_00314	AGTTGTGGCGGTGGATGGTG	GTGCCCCCTGGGGTTTCATA
VVMO6_00315	TTGTGCTGACTCGTGCCCCT	CATGCTGTCGTGGGCATTGA
VVMO6_00338	GGTCAGCCATCGGTCTTTCA	TTGTCCATCTCGTGAGCACC
VVMO6_00393	GCGCGTAATAACGTCGGCAA	CGGCCACGAGTTCTTCATCG
VVMO6_00423	GAAACGTGGTTTGCCATGCT	AAAGCAAAACAATCCGCCCC
VVMO6_00532	CTCGCCTAGATGGCGTTGAT	ATGGAACGGATACGACGCTC
VVMO6_00971	TGGTATCGCTGGTCTATCTAT	TCACGGATTGGTTTCACTTT
VVMO6_00997	GGGAATGAGAGCAACCAGCG	CCGACGTGGTTTTGGCTGTT
VVMO6_01063	CCGATTACCCAGCCAATGGT	GCTTTTTGAACGCCATCGGT
VVMO6_01074	GGCAACAAAGAGTATGAGTTTC	GCACAGACCACATCACAA
VVMO6_01129	ACCAAGCGCACAATGCCAGT	CGGCTTCAATGGCAGCATTC
VVMO6_01149	CGCCATTGGGGTGGTCAGTA	GTCTTGCCGTGCTTGTTGGC
VVMO6_01150	AGCTTTTGCGAACGCTGCAC	AGCGCACAATACCAGCGGAA
VVMO6_01249	CTTTGAAACGGGCATCGCAG	AAACTCGGCGTAACGCGACA
VVMO6_01954	TGAAAGAGCAAGTCGACGCT	TTTGCCTTTTCCATTGCCCG
VVMO6_02043	CGAGCACCCAGAAACTCACT	TCGTCAGTAACAACCGCGAA
VVMO6_02138	CAAGCCTTTAGCGATGAAA	CAGCAATAATCCTCTTCCAT
VVMO6_02203	GCAACAGAAGATGAGCATATC	GTCGCCAGAGGAACATAA
VVMO6_02205	GTGGTTTACACCTCAGGCCA	ACCACTGGCTTCCAATACCG
VVMO6_02243	CTGCTGACGAAGATGGAA	AATCACGAGATACGGAACC
VVMO6_02433	TGGATCCAAAAACGGTGCGT	TCCCATTCATCCAACCAGCAA
VVMO6_02434	AGCATGGGGGCTAGAGCCTG	TCCGATGCGTGGTTTAGCGT
VVMO6_02435	CATTCCTCGCAATACCACCATTCC	ACGACAGTCATCCACCATCTCAC
VVMO6_02436	ATCAAACGCAGCCAAAGCACTC	GAAATCCGAGGTGAGCAGCAAAC
VVMO6_02437	TTAACAGAAACCGCAGCAAGTCG	TCACACCTAAACGCAAACCAACG
VVMO6_02438	GCAAATCAAAGTAACGCCAGAAGG	GCAATCGCACTACCACAACCG
VVMO6_02439	TTGTTGAAGGTGAGTCGTTACTGATG	CGAGAGCACGCAATACATAAGAAGG
VVMO6_02440	GATATGCGGTAACGGCAATGCT	TAAGAGAGCGAAATCCCCTGACG

VVMO6_02482	ACAGATGAAGTTCGAGGCCGTG	GCTCGACGTGCATCAGGCTT
VVMO6_02521	ATCGGTTCCTTTGAAGCGCC	GGCTCGAGCGACCGTTTCTT
VVMO6_02539	AAGTGGCTATCGAAGCAGCA	TCACGCTGTTGTTCTGGTGT
VVMO6_03020	AAGCGTGGATGGTGAGTA	GGAAATGAAGAAAGAGAGAAAGC
VVMO6_03043	GGCTCGTTTACGGTTTGGGG	TGGCAGATGGTGTGGGTTCC
VVMO6_03179	GGCGACAGCATCAAGATCGG	GGTGCTTCGCGCACCTCTAA
VVMO6_03472	TAAAGACGCAGCAGACAGCA	AAACCTGCATTGACGTTGCC
VVMO6_03494	TAGACAAAGACGGCACGCTGCA	CCCGCCGTGTACGAGCTCAA
VVMO6_03502	GGCAGGCCAAAACGAACAAT	CCGCGACGTCTCTTAAGTCT
VVMO6_03758	TGTTGGCGATGCCTTTGCTT	GAGCGATGCTCCCGTCACTG
VVMO6_03816	GTTTTGCCATCGCACGAACT	TCGATGCCCAACTTGTACCC
VVMO6_03848	CAACAATGCCACGAAACGCA	CCATGGCATCCACCACTCGT
VVMO6_03878	TCGACCGCTTGCATGTGATG	CTGCGTTGCCTGCAATTTCC
VVMO6_03880	CACGCTGTTTAACGGCCAGC	ATCACCACGGTGCTACCGGA
VVMO6_03881	CGCCAAGAGCTTGGGTGCTA	AGACACGGGCGCTTCGTACA
VVMO6_04018	TTTAAGGCGCGTGCAGAGTA	GTCTTGGCTCTACTGCCGTT
VVMO6_04141	TGAAAGCCTGGGGTGAAGCA	ATCGCGTAGCGTTGAGAGCG
VVMO6_04152	CGTTTGATCTCGCCGGTTTG	GCAGCCATCTCGCCTTTTGA
VVMO6_04166	GCCTTTTCGCCAATTGTGGT	CCACAACACGGAGACGGTAA
VVMO6_04170	CTGCAAAATCGGCGGCATAG	GCGAAGCGTGGCTTTGCTTA
VVMO6_04184	CGTCGCTAAAGCAGAGGCGA	AAAGCGGCGGTAGGCTCATC
VVMO6_04185	TGACGCAGCAATCTCAGCGA	GCAAATGACACGAAAGCCGC
VVMO6_04186	GGCCAACTACTGCGCTGGTG	CGCGAATGGTCTGAGAAGCG
VVMO6_04222	ATCACCCGCGAAAGCTACAA	TTTGAAGCAAAGCAGACCGC
VVMO6_04284	CTACACGCTAGAAGGCATC	CGAATTTCAAGACCCACAC
VVMO6_04286	GCGGTACTCTTCACCCTCAC	AGACGAACATCACGGCAACT
VVMO6_04436	CGTGAAATCGGAACTTGGCG	CGAGTGTTGGCAGTTTGGTG
VVMO6_04467	CCAAACATGGCGCTCGCTAC	GTTAAACCGGGGCTGATGGC
VVMO6_04468	CCCATTTTGTGCTCGCGTTC	GAGGGCTTGGCCGTCATTTT
VVMO6_04469	CCCGGCGTTTATACCGATGA	TGGAACACTGGGTGCGATAC
VVMO6_04473	GACGCGATCAAGATTGGCAC	AGCTCTTCAACGCTGCTGAT
VVMO6_04475	GCAACGGCACTTATGTCTA	GAGTAGGTCATCGCATCG
VVMO6_04488	TGTCAGTCGGTATGGTCA	GGGTATCCTGCGTGATTC
VVMO6_04555	CCGTGGTGGTGGTGCTCAAT	TGGCGGTTTGATTTCCCCTT

[a] Locus tags are based on the database for the *V. vulnificus* MO6-24/O genome, which was retrieved from GenBank (accession number CP002469 and CP002470).

[b] The oligonucleotides were designed using the genomic sequence of the *V. vulnificus* MO6-24/O (Park et al., 2011).

TABLE S3. Genes whose expression is up-regulated by IscR[a]

Locus tag	**Product name**	**Microarray**		**qRT-PCR**	
		Mean Log$_2$ ratio[b]	***P*-value**	**Mean Log$_2$ ratio[b]**	***P*-value**

Transport and metabolism					
VVMO6_00199	Aspartate ammonia-lyase	-1.047	7.51E-03	-1.310	2.60E-05
VVMO6_00314	Argininosuccinate synthase	-2.515	8.63E-03	-3.520	1.01E-04
VVMO6_00315	Argininosuccinate lyase	-1.622	2.64E-03	-2.373	1.68E-05
VVMO6_00338	Glucose-6-phosphate isomerase	-1.221	1.81E-03	-1.697	1.69E-04
VVMO6_00393	Ornithine carbamoyltransferase	-1.732	9.09E-03	-1.552	4.37E-05
VVMO6_01954	Cob(I)alamin adenosyltransferase	-1.184	4.83E-06	-1.007	2.96E-04
VVMO6_03020	Na+/H+ antiporter NhaD type	-1.214	5.06E-05	-1.040	2.33E-05
VVMO6_03043	Putrescine/proton symporter, Putrescine/ornithine antiporter PotE	-1.548	1.28E-02	-1.717	1.40E-04
VVMO6_03179	Argininosuccinate synthase	-1.304	5.93E-03	-2.177	1.45E-03
VVMO6_03816	Ribonucleotide reductase of class III (anaerobic) large subunit	-1.561	1.23E-02	-4.070	2.84E-06
VVMO6_04184	Aarginine ABC transporterATP-binding protein ArtP	-1.162	2.60E-02	-1.437	7.28E-05
VVMO6_04185	Arginine ABC transporter periplasmic arginine-binding protein ArtI	-3.511	4.15E-03	-3.520	1.44E-05
VVMO6_04186	Arginine ABC transporter permease ArtQ	-2.482	8.15E-03	-3.393	3.01E-05
VVMO6_04222	Tryptophanase	-1.152	2.75E-02	-2.483	1.99E-05
VVMO6_04284	Acetylornithinedeacetylase	-1.498	1.26E-02	-7.083	7.79E-07
VVMO6_04467	Dehydrogenases with different specificities (short-chain alcohol dehydrogenases)	-1.727	1.04E-07	-2.213	1.07E-04
VVMO6_04473	Ascorbate-specific PTS system, EIIA component	-1.548	1.63E-03	-3.480	8.15E-04
VVMO6_04488	Sulfate permease	-1.427	3.51E-05	-1.307	4.12E-03
Energy production and conversion					
VVMO6_00216	Fumarate reductase subunit D	-1.224	4.17E-02	-4.873	1.48E-07
VVMO6_00218	Succinate dehydrogenase flavoprotein subunit	-1.231	7.04E-03	-5.377	5.25E-06
VVMO6_00219	Succinate dehydrogenase flavoprotein subunit	-1.130	1.38E-02	-5.583	1.85E-07
VVMO6_00971	Pyruvate formate-lyase	-1.392	2.18E-03	-2.690	1.25E-04
VVMO6_01074	D-Lactate dehydrogenase	-2.245	4.53E-06	-2.380	1.54E-05
VVMO6_02043	Alcohol dehydrogenase; Acetaldehyde dehydrogenase	-1.114	1.80E-03	-3.347	3.78E-03
VVMO6_02203	Pyridine nucleotide-disulfide oxidoreductase; NADH dehydrogenase	-1.152	3.38E-02	-1.600	1.81E-04
VVMO6_03472	Alcohol dehydrogenase	-1.704	7.85E-03	-9.143	3.03E-05
VVMO6_03502	Cytochrome c553	-1.149	8.31E-05	-1.967	2.10E-05
VVMO6_04166	L-Lactate permease	-1.014	4.28E-05	-1.973	1.24E-03
VVMO6_04170	D-Lactate dehydrogenase, Fe-S protein, FAD/FMN-containing	-1.099	1.10E-03	-2.143	3.40E-05
VVMO6_04469	2,4-Dienoyl-CoA reductase	-2.133	1.75E-06	-1.758	1.82E-04
VVMO6_04475	Hydrolase	-1.548	2.21E-04	-2.810	9.34E-05
Virulence					
VVMO6_03494	*N*-acetylglucosamine-binding protein GbpA	-1.690	1.76E-05	-1.300	7.84E-04
VVMO6_03880	Cytolysin secretion protein VvhB	-2.133	3.30E-04	-2.367	5.42E-06
VVMO6_03881	Cytolysin / Hemolysin VvhA	-1.966	1.27E-03	-2.467	2.39E-06
Oxidative stress					
VVMO6_04141	Peroxiredoxin	-2.344	1.51E-08	-3.157	8.40E-05
VVMO6_04468	Glutaredoxin 2	-2.506	2.01E-07	-1.738	6.73E-05
Cell motility and chemotaxis					
VVMO6_02263	Flagellar hook protein FlgE	-1.003	2.12E-04	-1.277	5.24E-03
VVMO6_01129	Methyl-accepting chemotaxis protein II	-1.015	3.04E-05	-1.117	5.39E-04

VVMO6_01149	Signal transduction histidine kinase	-1.685	8.56E-03	-4.653	3.63E-05
VVMO6_01150	Signal transduction protein CheY	-2.184	4.58E-03	-4.697	9.51E-06
VVMO6_03848	Methyl-accepting chemotaxis protein I	-1.139	1.44E-03	-1.383	1.28E-04
VVMO6_03878	Methyl-accepting chemotaxis protein HylB	-1.377	3.63E-03	-2.440	3.41E-04
VVMO6_04555	Signal transduction protein CheW	-1.635	1.99E-04	-1.403	2.38E-03
Translation					
VVMO6_02205	Endoribonuclease L-PSP	-1.092	1.05E-03	-1.583	4.29E-05
VVMO6_02482	Ribosome hibernation protein YfiA	-1.123	3.23E-05	-5.473	3.77E-06
Replication, recombination and repair					
VVMO6_00423	A/G-specific adenine glycosylase	-1.544	1.50E-04	-1.247	6.24E-04
Function unknown					
VVMO6_00997	Hypothetical protein	-1.366	1.42E-05	-1.040	9.19E-05
VVMO6_01063	Hypothetical protein	-1.003	8.20E-04	-2.277	1.19E-05
VVMO6_02138	Hypothetical protein	-2.737	7.64E-06	-4.887	1.47E-03
VVMO6_02243	*N*-acetylglucosamine-regulated outer membrane porin	-1.308	3.70E-05	-1.340	2.19E-02
VVMO6_03758	Hypothetical protein	-1.411	8.68E-05	-4.663	6.32E-04
VVMO6_04286	Hypothetical protein	-2.184	1.67E-03	-7.127	2.88E-04

[a] Locus tag numbers, functional categories, and annotation of gene products are based on the database of the *V. vulnificus* MO6-24/O genome (Park et al., 2011; GenBank accession CP002469 and CP002470). Functional categories in boldface are shown above the first gene in each category.

[b] The M value represents the log2 ratio of mRNA expression of each gene in the *iscR* mutant versus the parental wild type. The values shown are the mean from three independent experiments. The genes with M ≥ 1.0 or M ≤ -1.0 (expression ratios of ≥ 2.0, *P* ≤ 0.05) were considered as the IscR regulon. Negative numbers show up-regulation by IscR.

TABLE S4. Genes whose expression is down-regulated by IscR[a]

		Microarray		**qRT-PCR**	
	Product name	**Mean Log_2 ratio**[b]	***P*-value**	**Mean Log_2 ratio**[b]	***P*-value**
Fe-S cluster biogenesis					
VVMO6_02433	Hypothetical protein	2.821	1.48E-07	3.300	1.86E-05
VVMO6_02434	Ferredoxin (Fdx)	3.537	4.17E-07	3.313	3.60E-05
VVMO6_02435	Chaperone protein HscA	3.163	2.27E-07	3.037	1.81E-06
VVMO6_02436	Chaperone protein HscB	3.690	3.74E-07	3.047	3.54E-05
VVMO6_02437	Iron binding protein IscA for iron-sulfur cluster assembly	3.032	6.47E-07	2.963	1.02E-04
VVMO6_02438	Iron-sulfur cluster assembly scaffold protein IscU	3.637	8.81E-09	2.813	6.28E-04
VVMO6_02439	Cysteine desulfurase IscS subfamily	3.413	8.01E-08	3.710	1.08E-06
VVMO6_02440	Iron-sulfur cluster regulator IscR[c]	6.043	2.65E-07	4.310	1.77E-06
Energy production and conversion					

VVMO6_00532	Pyruvate dehydrogenase E1 component	1.209	3.71E-02	1.910	1.29E-03
VVMO6_02539	Pyruvate formate-lyase	1.140	1.05E-05	1.630	3.56E-06
Signal transduction mechanisms					
VVMO6_01249	Diguanylate cyclase	1.249	1.44E-04	1.087	1.49E-05
VVMO6_02521	VpsR family transcriptional regulator	1.441	3.76E-03	1.413	6.29E-05
Membrane protein					
VVMO6_04018	Outer membrane protein A	1.938	7.03E-04	3.683	3.62E-05
Function unknown					
VVMO6_04152	Hypothetical protein	2.846	3.52E-07	3.380	2.23E-05
VVMO6_04436	Hypothetical protein	1.156	5.33E-03	2.710	3.71E-03

[a] Locus tag numbers, functional categories, and annotation of gene products are based on the database of the *V. vulnificus* MO6-24/O genome (Park et al., 2011; GenBank accession CP002469 and CP002470). Functional categories in boldface are shown above the first gene in each category.

[b] The M value represents the log2 ratio of mRNA expression of each gene in the *iscR* mutant versus the parental wild type. The values shown are the mean from three independent experiments. The genes with $M \geq 1.0$ or $M \leq -1.0$ (expression ratios of ≥ 2.0, $P \leq 0.05$) were considered as the IscR regulon. Positive numbers show down-regulation by IscR.

[c] The probe (in microarray) and a set of primers (in qRT-PCR) used for the *iscR* gene (VVMO6_02440) expression analysis were designed to hybridize to the *iscR* coding region upstream from the deletion site of the *iscR* mutant.

References

1. **Park JH, Cho YJ, Chun J, Seok YJ, Lee JK, Kim KS, Lee KH, Park SJ, Choi SH.** 2011. Complete genome sequence of *Vibrio vulnificus* MO6-24/O. J. Bacteriol. **193:**2062-2063.