

**SUPPLEMENTARY TABLE 1** – Primers used in this study

Primer Name	Sequence (5' → 3')	Description
<b>Methyl HTM-seq primers</b>		
ABD013	CCCTACACGACGCTCTTCCGATCTACGTATCACG	Top strand of tIL1 adaptor
ABD013D	gatcCGTGATACGTAGATCG	Bottom strand of tIL1 adaptor for Dam Methyl HTM-seq (lower case nucleotides indicate the 5' overhang generated when annealed to ABD013)
ABD013B	ccggCGTGATACGTAGATCG	Bottom strand of tIL1 adaptor for VchM Methyl HTM-seq (lower case nucleotides indicate the 5' overhang generated when annealed to ABD013)
OLJ 131	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	Forward primer for amplification of Methyl HTM-seq samples
OLJ 573	CAAGCAGAAGACGGCATAACGAGATcacacaGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGGGGGGGGGGGGG	Reverse primer for amplification of Dam Methyl-HTM seq samples (unique barcode sequence is shown in lower case)
BC33G	CAAGCAGAAGACGGCATAACGAGATcgtgatGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGGGGGGGGGGGGG	Reverse primer for amplification of VchM Methyl-HTM seq samples (unique barcode sequence is shown in lower case)
<b>MeSR digestion and qPCR primers</b>		
ABD061	TCGCGATTATCCTGATGCTGGTC	Forward primer for <i>E. coli</i> Dam positive control
ABD062	GCTGGCGGGAATGCTGGC	Reverse primer for <i>E. coli</i> Dam positive control
ABD104	GCGTCACCCTTCAAATAGTC	Forward primer for K139 Ig-I
ABD105	ATTGACAACGTAAATGCCAAACAAC	Reverse primer for K139 Ig-I
ABD106	TGTTTGGCATTACAGTTGTCAATTG	Forward primer for K139 Ig-II
ABD107	CCTTAAACGTTGTTAAATCATCGGTTC	Reverse primer for K139 Ig-II
ABD049	GTGATTCCCTTTTCATTGCATGGAATCC	Forward primer for P <sub>VC1231</sub>
ABD050	CGTCACATCTCATACACTTGCAATCG	Reverse primer for P <sub>VC1231</sub>
ABD009	TATATGCCTTTAGGCATTAAGTACTTCCGTC	Forward primer for P <sub>VC1784</sub>
ABD010	TGAAGTCATCTTGATTGACAAGTCTCCATCGAATG	Reverse primer for P <sub>VC1784</sub>
ABD047	TGGGGTTTCGTGGTGTATATCAAG	Forward primer for P <sub>VCA0063</sub>
ABD048	GATGTCGTTAACAGCAATACATTGAGC	Reverse primer for P <sub>VCA0063</sub>
ABD172	GGTCCATGAGTTCACCGTCCAG	Forward primer for P <sub>VC0286/7</sub>
ABD173	CCAAACTTGC GCGTGATCGC	Reverse primer for P <sub>VC0286/7</sub>

ABD180	GCGCTAACTATCCGAAATGGGATTTTG	Forward primer for P <sub>VC1280/1</sub>
ABD181	CATACTGGTAGACATAACCAGCACTACAGC	Reverse primer for P <sub>VC1280/1</sub>
ABD188	GGCAATTCATTTTTAGCCAGTTCAAACG	Forward primer for P <sub>VC1558/9</sub>
ABD189	GAGTCTAGACACGGATCCCCAC	Reverse primer for P <sub>VC1558/9</sub>
<b>SOE PCR primers for generation of mutant strains (overlap for Kan cassette in R1 and F2 primers are shown in lower case)</b>		
ABD146	TTCGGCGGCTGCTAGAGC	F1 primer for $\Delta$ <i>lrp</i>
ABD147	gtcgacggatccccggaatCATTTTTTATTCACCTTATTACTTCCTTGC	R1 primer for $\Delta$ <i>lrp</i>
ABD148	gaagcagctccagcctacaTAACTTAAAGCGAATGTTGGAGATCTTTC	F2 primer for $\Delta$ <i>lrp</i>
ABD149	CCGAAGGCGTTTCTTCAAGCTC	R2 primer for $\Delta$ <i>lrp</i>
ABD138	ACCACAGCCGTGGTTGCTC	F1 primer for $\Delta$ <i>rpiR</i>
ABD139	gtcgacggatccccggaatCACTTGGAGTCCTTAATATTCTTTTGACTAC CATAAAAG	R1 primer for $\Delta$ <i>rpiR</i>
ABD140	gaagcagctccagcctacaTAAGCAGTATTTAACAAGTTTGAAACCCATC AGG	F2 primer for $\Delta$ <i>rpiR</i>
ABD141	TTGTTACCACAGACTGTATAGGCAGC	R2 primer for $\Delta$ <i>rpiR</i>
ABD117	CAAAGCCTCTGAGCGCCTGTTTGTG	F1 primer for $\Delta$ <i>fur</i>
ABD118	gtcgacggatccccggaatCATATACTTTCCTGTTGATGTTCTGCAG	R1 primer for $\Delta$ <i>fur</i>
ABD119	gaagcagctccagcctacaTAACCATAGGCTTTACGCTCTGTAAACC	F2 primer for $\Delta$ <i>fur</i>
ABD120	CTTAAACAAGCGGATGTGATCACTCTG	R2 primer for $\Delta$ <i>fur</i>
ABD175	CTGATCGTCGCCCAATGG	F1 primer for $\Delta$ <i>Vc0289</i>
ABD176	gtcgacggatccccggaatCATAGGTTATTGAGTCTTTTATAAAATGTTA TGAG	R1 primer for $\Delta$ <i>Vc0289</i>
ABD177	gaagcagctccagcctacaTAACACCAGCACTCCACAAAGGATAAAGC	F2 primer for $\Delta$ <i>Vc0289</i>
ABD178	GCCGAGTACGGCCATAACCG	R2 primer for $\Delta$ <i>Vc0289</i>
ABD183	TGGGTGATGGCGCCAATTC	F1 primer for $\Delta$ <i>Vc1286</i>
ABD184	gtcgacggatccccggaatCATATTAGAGCCTATAGGAAGTCAGAAACTT C	R1 primer for $\Delta$ <i>Vc1286</i>
ABD185	gaagcagctccagcctacaTAAACGCCCTTAGTGATTTGAAGTTTACAG	F2 primer for $\Delta$ <i>Vc1286</i>
ABD186	GCCGCTCGCATCTATGGTCC	R2 primer for $\Delta$ <i>Vc1286</i>
ABD191	GAGATGCAAGCTGGTGATGAGC	F1 primer for $\Delta$ <i>Vc1557</i>
ABD192	gtcgacggatccccggaatCAAAAATTAAGCGGCGACCCAAAG	R1 primer for $\Delta$ <i>Vc1557</i>
ABD193	gaagcagctccagcctacaTGAGTAAACAGCAACGTGTCAGTAA	F2 primer for $\Delta$ <i>Vc1557</i>
ABD194	GTGGCCAGTGTTGTACAACAGTTG	R2 primer for $\Delta$ <i>Vc1557</i>
FRT-Kan-F	ATTCCGGGGATCCGTCGAC	Forward primer for Kan cassette
FRT-Kan-R	TGTAGGCTGGAGCTGCTTC	Reverse primer for Kan cassette

Primers for Fur expression construct		
ABD084	TATAcatatgTCAGACAATAACCAAGCGC	Forward primer to amplify <i>fur</i> gene containing an engineered NdeI site (lower case)
ABD085	ATAggatccTTATTTCTTCGGCTTGTGAGCG	Reverse primer to amplify <i>fur</i> gene containing an engineered BamHI site (lower case)
Primers for EMSA probes		
ABD116	ATAACACAAATGATAATTGATCTTATTTAGATTTG	Forward oligonucleotide for P <sub>VCA0063</sub> (Cy5 end labeled)
ABD115	CAAATCTAAATAAGATCAATTATCATTGTGTTAT	Reverse oligonucleotide for P <sub>VCA0063</sub>
ABD113	TTTACTTTACCTCTGGCGAGCG	Forward primer for P <sub>SodA</sub>
ABD114	CTTGGCTATCAGCAAGGCGG	Reverse primer for P <sub>SodA</sub>
ABD111	GAATGCGCAATACTGGTTAAC	Forward primer for P <sub>AphA</sub>
ABD112	CTGATTAGTCGAAAACTGACATTATTC	Reverse primer for P <sub>AphA</sub>
qRT-PCR primers		
ABD033	GCTCAATGTATTGCTGTTAACGACATC	Forward primer for <i>VCA0063</i>
ABD034	ATCGGTTTTTCGATTATGCTCAGCC	Reverse primer for <i>VCA0063</i>
ABD031	CGTCCATTGTAGCAAGTAGCGTAA	Forward primer for <i>VC1784</i>
ABD032	TCGGTATCCCAAGTTATACCGCC	Reverse primer for <i>VC1784</i>
RPB2FD	CTGTCTCAAGCCGTTACAA	Forward primer for <i>rpoB</i>
RPB2RV	TTTCTACCAGTGCAGAGATGC	Reverse primer for <i>rpoB</i>

**SUPPLEMENTARY TABLE 2** – Undermethylated sites in *V. cholerae* identified by Methyl HTM-seq

Chromosome	Position <sup>a</sup>	Locus <sup>a</sup>	Frequency <sup>b</sup>	Fold Overrepresented <sup>c</sup>
<b>Dam Data</b>				
chrI	54096	VCr001	1.58E-04	5.9
chrI	54120	VCr001	2.32E-04	8.8
chrI	90598	VC0092	1.49E-04	5.6
chrI	90603	VC0092	1.33E-04	5.0
chrI	151332	VCr004	1.51E-04	5.7
chrI	151356	VCr004	2.36E-04	8.9
chrI	205531	VC0199	1.59E-04	6.0
chrI	205534	VC0199	1.47E-04	5.6
chrI	324420	VCr007	1.48E-04	5.6
chrI	324444	VCr007	2.45E-04	9.2
chrI	391875	VC0376	1.42E-04	5.4
chrI	391878	VC0376	1.26E-04	4.7
chrI	402024	VCr010	1.31E-04	4.9
chrI	402048	VCr010	2.36E-04	8.9
chrI	763048	VCr013	1.69E-04	6.4
chrI	763072	VCr013	2.25E-04	8.5
chrI	1306007	ig-VC1231-VC1232	6.96E-04	26.2
chrI	1306010	ig-VC1231-VC1232	5.74E-04	21.6
chrI	1458917	VC1369	1.60E-04	6.0
chrI	1458920	VC1369	1.34E-04	5.1
chrI	1886030	VC1751	1.36E-04	5.1
chrI	1886069	VC1751	1.83E-04	6.9
chrI	1933145	ig-VC1783-VC1784	3.28E-04	12.4
chrI	1933148	ig-VC1783-VC1784	4.60E-04	17.3
chrI	2364224	ig-K139p05-K139p04	2.02E-04	7.6
chrI	2364255	ig-K139p05-K139p04	1.22E-04	4.6
chrI	2416094	VC2229	1.25E-04	4.7
chrI	2416112	VC2229	1.69E-04	6.4
chrI	2918860	VC2712	1.79E-04	6.7
chrI	2918875	VC2712	1.62E-04	6.1
chrI	2993372	VC2775	1.16E-04	4.4
chrI	2993388	VC2775	1.54E-04	5.8
chrI	2993756	VC2775	1.16E-04	4.4
chrI	2993766	VC2775	2.17E-04	8.2

chrII	68810	ig-VCA0062-VCA0063	3.11E-04	11.7
chrII	68813	ig-VCA0062-VCA0063	1.07E-03	40.3
chrII	1027744	VCA1074	1.84E-04	6.9
chrII	1027772	VCA1074	1.57E-04	5.9
chrII	1051025	VCA1095	1.23E-04	4.6
chrII	1051058	VCA1095	1.29E-04	4.9
<b>VchM Data</b>				<b>5.9</b>
chrI	296268	ig-VC0286-VC0287	1.10E-02	8.8
chrI	296271	ig-VC0286-VC0287	9.66E-03	5.6
chrI	1356851	ig-VC1280-VC1281	5.55E-03	5.0
chrI	1356854	ig-VC1280-VC1281	2.72E-03	5.7
chrI	1670727	ig-VC1558-VC1559	5.27E-03	8.9
chrI	1670730	ig-VC1558-VC1559	3.80E-03	6.0

Grayed lines indicate undermethylated intergenic sites

<sup>a</sup> The Position and Locus is based on the annotated N16961 genome containing the K139 prophage genome in chromosome I. The unmethylated site is located in the intergenic region between the loci indicated.

<sup>b</sup> Indicates the frequency of reads mapping to the indicated site relative to the total number of reads mapped

<sup>c</sup> Indicates how overrepresented the reads are at the indicated site relative to the mean number of reads obtained for all sites