

## SUPPLEMENTAL TABLES

### **Two nucleotide second messengers regulate the production of the *Vibrio cholerae* colonization factor GbpA**

*Running title: c-di-GMP and cAMP regulation of GbpA*

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**Table S1. Strains used in this study.**

Strain	Strain Description	Reference
<i>Escherichia coli</i>		
DH5α	F <sup>-</sup> φ80 <i>lacZ</i> ΔM15 Δ( <i>lacZYA-argF</i> )U169 <i>recA1 endA1 hsdR17</i> (rK <sup>-</sup> , mK <sup>+</sup> ) <i>phoA supE44 thi-1 gvrA96 relA1 λ- tonA</i>	Invitrogen; [1]
DH5αλpir	F <sup>-</sup> Δ( <i>lacZYA-argF</i> )U169 <i>recA1 endA1 hsdR17 supE44 thi-1 gvrA96 relA1 λ.pir</i>	[1]
SM10λpir	<i>thi thr leu tonA lacY supE recA::RPA-2-Te::Mu λpirR6K</i> , Km <sup>r</sup>	[2]
<i>Vibrio cholerae</i>		
C6706	Wild type, O1 El Tor	[3]
C6706 Δ <i>gbpA</i>	C6706 with in-frame deletion in <i>gbpA</i> (VCA0811)	This study
C6706 Δ <i>lacZ</i>	C6706 with in-frame deletion in <i>lacZ</i>	[4]
C6706 Δ <i>lacZ</i> pP <sub><i>gbpA</i></sub> <sup>-</sup> Vc1- <i>lacZ</i>	C6706 Δ <i>lacZ</i> bearing translational reporter of the <i>gbpA</i> promoter and 5' untranslated region (UTR) to <i>E. coli lacZ</i>	This study
C6706 Δ <i>lacZ</i> pP <sub><i>gbpA</i></sub> <sup>-</sup> ΔVc1- <i>lacZ</i>	C6706 Δ <i>lacZ</i> bearing translational reporter of the <i>gbpA</i> promoter and 5' UTR, with Vc1 deleted, to <i>E. coli lacZ</i>	This study
C6706 Δ <i>lacZ</i> pP <sub><i>gbpA</i></sub> <sup>-</sup> Vc1- <i>lacZ</i> pBAD33	C6706 Δ <i>lacZ</i> bearing translational reporter of the <i>gbpA</i> promoter and 5' untranslated region (UTR) to <i>E. coli lacZ</i> , with pBAD33	This study
C6706 Δ <i>lacZ</i> pP <sub><i>gbpA</i></sub> <sup>-</sup> Vc1- <i>lacZ</i> pPDE	C6706 Δ <i>lacZ</i> bearing translational reporter of the <i>gbpA</i> promoter and 5' untranslated region (UTR) to <i>E. coli lacZ</i> , with pPDE	This study
C6706 pBAD33	C6706 bearing vector pBAD33	This study
C6706 pPDE	C6706 pBAD33:: <i>vieA</i> ("pPDE") (encodes EAL domain PDE)	This study
C6706 pPDE <sup>mut</sup>	C6706 pBAD33:: <i>vieAE170A</i> ("pPDE <sup>mut</sup> ") (encodes enzymatically inactive PDE)	This study
C6706 pVC1592	C6706 pBAD33::VC1592 (encodes EAL domain PDE)	This study
C6706 ΔVc1	C6706 with in-frame deletion of Vc1 element upstream of <i>gbpA</i> open reading frame	This study
C6706 ΔVc1 pBAD33	C6706 ΔVc1 bearing vector pBAD33	This study
C6706 ΔVc1 pPDE	C6706 ΔVc1 with pBAD33:: <i>vieA</i> (pPDE)	This study
C6706 Δ <i>nagC</i>	C6706 with in-frame deletion of <i>nagC</i>	This study
C6706 Δ <i>nagC</i> pBAD33	C6706 Δ <i>nagC</i> with vector pBAD33	This study
C6706 Δ <i>nagC</i> pPDE	C6706 Δ <i>nagC</i> with pBAD33:: <i>vieA</i> (pPDE)	This study
C6706 Δ <i>crp</i>	C6706 with in-frame deletion of <i>crp</i>	This study
C6706 Δ <i>crp</i> pBAD33	C6706 Δ <i>crp</i> with vector pBAD33	This study
C6706 Δ <i>crp</i> pPDE	C6706 Δ <i>crp</i> with pBAD33:: <i>vieA</i> (pPDE)	This study
C6706 Δ <i>cpdA</i>	C6706 with in-frame deletion of <i>cpdA</i>	This study
C6706 Δ <i>cpdA</i> pBAD33	C6706 Δ <i>cpdA</i> with vector pBAD33	This study
C6706 Δ <i>cpdA</i> pPDE	C6706 Δ <i>cpdA</i> with pBAD33:: <i>vieA</i> (pPDE)	This study
C6706 Δ <i>cya</i>	C6706 with in-frame deletion of <i>cya</i>	This study
C6706 Δ <i>cya</i> pBAD33	C6706 Δ <i>cya</i> with vector pBAD33	This study
C6706 Δ <i>cya</i> pPDE	C6706 Δ <i>cya</i> with pBAD33:: <i>vieA</i> (pPDE)	This study

C6706 $\Delta vpsT$	C6706 with in-frame deletion of <i>vpsT</i>	This study
C6706 $\Delta vpsT$ pBAD33	C6706 $\Delta vpsT$ with vector pBAD33	This study
C6706 $\Delta vpsT$ pPDE	C6706 $\Delta vpsT$ with pBAD33:: <i>vieA</i> (pPDE)	This study
C6706 $\Delta vpsR$	C6706 with in-frame deletion of $\Delta vpsR$	[5]
C6706 $\Delta vpsR$ pBAD33	C6706 $\Delta vpsR$ with vector pBAD33	This study
C6706 $\Delta vpsR$ pPDE	C6706 $\Delta vpsR$ with pBAD33:: <i>vieA</i> (pPDE)	This study
C6706 $\Delta flrA$	C6706 with in-frame deletion of $\Delta flrA$	This study
C6706 $\Delta flrA$ pBAD33	C6706 $\Delta flrA$ with vector pBAD33	This study
C6706 $\Delta flrA$ pPDE	C6706 $\Delta flrA$ with pBAD33:: <i>vieA</i> (pPDE)	This study

**Table S2. Plasmids used in this study.**

Plasmid Name	Plasmid Description	Reference
pP <i>lacthiM#2-lacZ</i>	The TPP riboswitch encoded in the 5' UTR of <i>E. coli thiM</i> was mutated to function as an ON switch and cloned into pP <i>lac-lacZ</i> . Origin of replication <i>colEI</i> . Amp <sup>R</sup>	[6]
pP <sub><i>gbpA</i></sub> -Vc1- <i>lacZ</i>	<i>gbpA</i> promoter and 5'UTR cloned into plasmid pP <i>lacthiM#2-lacZ</i> ; translational fusion	This study
pP <sub><i>gbpA</i></sub> -ΔVc1- <i>lacZ</i>	<i>gbpA</i> promoter and 5'UTR, minus the Vc1 sequence (Δ 17-202 bp) cloned into plasmid pP <i>lacthiM#2-lacZ</i> ; translational fusion	This study
pBAD33	Expression vector, P <sub><i>ara</i></sub> promoter, Cm <sup>R</sup>	[7]
pPDE	pBAD33:: <i>vieA-His6</i>	[8]
pPDE <sup>mut</sup>	pBAD33:: <i>vieA-E170A</i> , allele encodes inactive PDE	[8]
pVC1592	pBAD33::VC1592 (EAL domain PDE gene)	This study
pBAD33:: <i>crp-his6</i>	Expression vector for purification of CRP	This study
pCVD442	<i>oriR6K</i> plasmid with a polylinker, <i>mobRP4</i> , <i>bla</i> , and <i>sacB</i>	[9]
pCVD442::Δ <i>gbpA</i>	Allelic exchange vector for in-frame deletion of <i>gbpA</i>	This study
pCVD442::ΔVc1	Allelic exchange vector for in-frame deletion of Vc1	This study
pCVD442::Δ <i>nagC</i>	Allelic exchange vector for in-frame deletion of <i>nagC</i>	This study
pCVD442::Δ <i>crp</i>	Allelic exchange vector for in-frame deletion of <i>crp</i> (VC2614)	This study
pCVD442::Δ <i>cya</i>	Allelic exchange vector for in-frame deletion of <i>cya</i> (VC0122)	This study
pCVD442::Δ <i>cpdA</i>	Allelic exchange vector for in-frame deletion of <i>cpdA</i>	This study
pCVD442::Δ <i>vpsT</i>	Allelic exchange vector for in-frame deletion of <i>vpsT</i>	This study
pCVD442::Δ <i>vpsR</i>	Allelic exchange vector for in-frame deletion of <i>vpsR</i>	[5]
pCVD442::Δ <i>fliA</i>	Allelic exchange vector for in-frame deletion of <i>fliA</i>	This study

**Table S3. Primers used in this study.**

<b>Primer Name</b>	<b>Oligonucleotide sequence (5' to 3') *</b>	<b>Reference</b>
pCVDseqF	CTGTTGCATGGGCATAAAGTTGCC	This study
pCVDseqR	ACACAGGAACACTTAACGGCTGAC	This study
Vc1F1	CCTCTAGATACTCGTCAGGTCTTTGG	This study
Vc1R1	TTGCATGCTTGCCAAAGTGTGACCGAGAG	This study
Vc1F2	TTGCATGCCAGCTAACAAAGAAGAGTCTGTG	This study
Vc1R2	AAGAGCTCCGAGTGTACACGGTATCG	This study
gbpAF1	TTGCATGCTACTCGTCAGGTCTTTGG	This study
gbpAR1	TTGGTACCCATCACAGACTCTTCTTTG	This study
gbpAF2	TTGGTACCTAAGTTATCCTCCCTCTTAC	This study
gbpAR2	TTGAGCTCTTTCTCTGGATGGGAGTC	This study
gbpAF0	GCAAACGGTAGCAAGAAG	This study
nagCF1	GAGCATGCCTGACGATACGATGATTGATAC	This study
nagCR1	GACCATGGCATTATCAATTCTGCTCGTATTGTC	This study
nagCF2	GACCATGGGAAGACTGAGCTTTTTAGTTAAGC	This study
nagCR2	GAGAGCTCGAGCATCATCCCTAAGATCAG	This study
nagCF0	CGATACCATGCACAAAGC	This study
crpF1	CCTCTAGATTTATGAAGGCTTACACGGC	This study
crpR1	CCGCATGCCATAATAATCTCACTTCCTCTGCAG	This study
crpF2	CCGCATGCTAAGTGCCCCGATAACCC	This study
crpR2	CCGAGCTCTTGAACATCCCGATCCTTTG	This study
crpF0	CCCTACTTACTGGCGATGAT	This study
cyaF1	CCGAGCTCCATTGACGGCGTAAACTG	This study
cyaR1	CCGAATTCCAAGTTTGCTTCCCTGATATG	This study
cyaF2	CCGAATTCTAACTCGTTGACGTCTCAG	This study
cyaR2	CCTCTAGACATAAAGCGGTGTACCGTAC	This study
cyaF0	CAAGCTTACTTAGGCGAGTC	This study
cpdAF1	CCTCTAGAGCTGACTGTAGGCAGAATTG	This study
cpdAR1	GGGCATGCCAAAATCGGTAAACCTAACTCTG	This study
cpdAF2	GGGCATGCTGATATGACTGCTCGTCTG	This study
cpdAR2	AAGAGCTCGTCTAAGGGCAACATAGTCTG	This study
cpdAF0	GTCGAGCGGGAAATGTTTG	This study
vpsTF1	CCGCATGCGCGAAGTTTCACGTA CTG	This study
vpsTR1	CCGGTACCCATTTACCCCTCCTAACAC	This study
vpsTF2	CCGGTACCTAATTCGTTGTGTAATGTCTCTTCG	This study
vpsTR2	CCGAGCTCCATCCCACACATACCAACC	This study
vpsTF0	CGATGATATCTTGGCTCAACTC	This study
vpsTF1	TGCATGCCTACAACCCAAATCACGC	This study
vpsTR1	AATCAGCAAACTTACATGAACCTATATTCCTT	This study
vpsTF2	GAATATAGGTTTCATGTAAGTTTTGCTGATTTAC	This study
vpsTR2	TTTCTAGAGGTAACTCAAGCCGATT	This study
vpsTF0	CTCTGTGGCGTTAGAAG	This study
FLRAF1	CCGCATGCCAACTTGTGGAACAGATGCAG	This study

FLRAR1	<u>CCGGTACCCTGCATAGGTGAGATTATTTGCC</u>	This study
FLRAF2	<u>CCGGTACCAACATGCAACGCTAGGG</u>	This study
FLRAR2	<u>CCGAGCTCTCGACCATAGGCATAAATTCG</u>	This study
flrAF0	GTTAGGCTATTTGGCCGAG	This study
RPB2F	CTGTCTCAAGCCGTTACAA	[10]
RPB2R	TTTCTACCAGTGCAGAGATGC	[10]
gyrAqF	AATGTGCTGGGCAACGACTG	This study
gyrAqR	GAGCCAAAGTTACCTTGGCC	This study
gbpAqF	CAGTGGATTAGCGTATGGACAC	This study
gbpAqR	GTATTGAATCGCGCCACAGT	This study
crpQF	GGTGAGAAAGCGGAAACGCTGTACTA	This study
crpQR	CTTCAAACAAGCCAAGCTCACCGA	This study
VC1046qF	GCCTTGATCGAGCGGATTAT	This study
VC1046qR	TCACAGGCGATGATGTTGAG	This study
VC2013qF	CTATCGTCGGTTACGGCATTAT	This study
VC2013qR	ACCCAGATATTCAGGCAGTTG	This study
VC2544qF	TTCGCTTGGCACCTTCTATC	This study
VC2544qR	GTCCCTCTTCTGGCACATTT	This study
CRPeF	<u>CCGAATTCGCAGAGGAAGTGAGATTATTATGG</u>	This study
CRPeH6R	<u>CCGTGCACTTAATGGTGATGGTGATGGTGCGAGTGCCGTAA</u> ACCA	This study
VC1592eF	<u>CCGAGCTCTTTAGGATACATTTTTATGAATCACATCCACCCC</u>	This study
VC1592eH6R	<u>AACTGCAGCTAATGATGATGATGATGATGTAACGCAGACATAC</u> GTGG	This study

\* The underlined sequences represent restriction enzyme recognition sites. Italicized sequences encode a 6-histidine tag.

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