

Table S1. Plasmids used in this study

Plasmid name	Feature(s)	Reference or source
LacZ fusions		
pOG-R-125	The <i>icmR</i> regulatory region in pGS-lac-02	(6)
pOG-icmV-LR2	The <i>icmV</i> regulatory region in pGS-lac-02	This study
pOG-icmW-LR2	The <i>icmW</i> regulatory region in pGS-lac-02	This study
pEA-lpg0525-lacZ	The <i>lvgA</i> regulatory region in pGS-lac-02	This study
pGA-sidH-lacZ	The <i>sidH</i> regulatory region in pGS-lac-02	This study
pMR-sidM-lacZ	The <i>sidM</i> regulatory region in pGS-lac-02	This study
pGA-lepB-lacZ	The <i>lepB</i> regulatory region in pGS-lac-02	(5)
pEA-lpg0038-lacZ	The <i>legA10</i> regulatory region in pGS-lac-02	This study
pEA-lpg0436-lacZ	The <i>legA11</i> regulatory region pGS-lac-02	This study
pEA-lpg0227-lacZ	The <i>ceg7</i> regulatory region pGS-lac-02	This study
pEA-lpg0898-lacZ	The <i>ceg18</i> regulatory region pGS-lac-02	This study
pEA-lpg2591-lacZ	The <i>ceg33</i> regulatory region pGS-lac-02	This study
pEA-lpg0012-lacZ	The <i>cegC1</i> regulatory region pGS-lac-02	This study
pEA-lpg0126-lacZ	The <i>cegC2</i> regulatory region pGS-lac-02	This study
pEA-lpg1144-lacZ	The <i>cegC3</i> regulatory region pGS-lac-02	This study
pEA-lpg2200-lacZ	The <i>cegC4</i> regulatory region pGS-lac-02	This study
LacZ fusions with mutations		
pOG-V-cb-1	pOG-icmV-LR2 with a mutated CpxR binding site	This study
pOG-W-cb-1	pOG-icmW-LR2 with a mutated CpxR binding site	This study
pEA-lpg0525-mut-lacZ	pEA-lpg0525-lacZ with a mutated CpxR binding site	This study
pEA-sidH-mut-lacZ	pGA-sidH-lacZ with a mutated CpxR binding site	This study
pEA-sidM-mut-lacZ	pMR-sidM-lacZ with a mutated CpxR binding site	This study
pEA-lpg0898-mut-cpxR-lacZ	pEA-lpg0898-lacZ with a mutated CpxR binding site	This study
pEA-lpg0898-mut-pmrA-lacZ	pEA-lpg0898-lacZ with a mutated PmrA binding site	This study
pEA-lpg0012-mut-lacZ	pEA-lpg0012-lacZ with a mutated CpxR binding site	This study
pEA-lpg0126-mut-lacZ	pEA-lpg0126-lacZ with a mutated CpxR binding site	This study
pEA-lpg1144-mut-lacZ	pEA-lpg1144-lacZ with a mutated CpxR binding site	This study
pEA-lpg2200-mut-lacZ	pEA-lpg2200-lacZ with a mutated CpxR binding site	This study
Plasmid containing IPTG-inducible CpxR		
pEA-puc-CpxR	CpxR in pUC-18	This study
pEA-pTac-CpxR	CpxR under <i>Ptac</i> promoter in pMMB207	This study

pEA-pTac-CpxR-sidM-lacZ	<i>Ptac</i> -CpxR in pMR-sidM-lacZ	This study
pEA-pTac-CpxR-sidMmut-lacZ	<i>Ptac</i> -CpxR in pEA-sidM-mut-lacZ	This study
pEA-pTac-CpxR-lpg0525-lacZ	<i>Ptac</i> -CpxR in pEA-lpg0525-lacZ	This study
pEA-pTac-CpxR-lpg0525mut-lacZ	<i>Ptac</i> -CpxR in pEA-lpg0525-mut-lacZ	This study

CyaA fusions

pEA-lpg0038-cyaA	C-terminal fusion of <i>legA10</i> to CyaA	This study
pEA-lpg0436-cyaA	C-terminal fusion of <i>legA11</i> to CyaA	This study
pEA-lpg0227-cyaA	C-terminal fusion of <i>ceg7</i> to CyaA	This study
pEA-lpg0898-cyaA	C-terminal fusion of <i>ceg18</i> to CyaA	This study
pEA-lpg2591-cyaA	C-terminal fusion of <i>ceg33</i> to CyaA	This study
pEA-lpg0012-cyaA	C-terminal fusion of <i>cegC1</i> to CyaA	This study
pEA-lpg0126-cyaA	C-terminal fusion of <i>cegC2</i> to CyaA	This study
pEA-lpg1144-cyaA	C-terminal fusion of <i>cegC3</i> to CyaA	This study
pEA-lpg2200-cyaA	C-terminal fusion of <i>cegC4</i> to CyaA	This study
pEA-sidM-cyaA	C-terminal fusion of <i>sidM</i> to CyaA	This study
pEA-RR-lpg2200-cyaA	<i>cyaA-cegC4</i> fusion with the <i>cegC4</i> promoter	This study
pEA-RR-sidM-cyaA	<i>cyaA-sidM</i> fusion with the <i>sidM</i> promoter	This study

Additional plasmids

pOG-ECP-2	<i>L. pneumophila</i> CpxR in pET-15b	(1)
pMMB-cyaA-C	CyaA fusion vector	(5)
pRep4	<i>oriR</i> (p15A) <i>lacI^q</i> , Km ^r	QIAGEN
pGS-lac-02	<i>oriV</i> (RSF1010) with a promoterless <i>lacZ</i> gene Cm ^r	(2)
pMMB207	<i>oriV</i> (RSF1010) <i>IncQ lacI^q</i> Cm ^r <i>Ptac oriT</i> MCS	(3)
pUC-18	<i>oriR</i> (colEI) MCS Ap ^r	(4)

References for Table S1

1. Gal-Mor, O., and G. Segal. 2003. Identification of CpxR as a positive regulator of *icm* and *dot* virulence genes of *Legionella pneumophila*. J Bacteriol **185**:4908-4919.
2. Gal-Mor, O., T. Zusman, and G. Segal. 2002. Analysis of DNA regulatory elements required for expression of the *Legionella pneumophila icm* and *dot* virulence genes. J Bacteriol **184**:3823-3833.
3. Morales, V. M., A. Backman, and M. Bagdasarian. 1991. A series of wide-host-range low-copy-number vectors that allow direct screening for recombinants. Gene **97**:39-47.
4. Yanish-Perron, C., J. Viera, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103-119.

5. **Zusman, T., G. Aloni, E. Halperin, H. Kotzer, E. Degtyar, M. Feldman, and G. Segal.** 2007. The response regulator PmrA is a major regulator of the icm/dot type IV secretion system in *Legionella pneumophila* and *Coxiella burnetii*. *Mol Microbiol* **63**:1508-1523.
6. **Zusman, T., O. Gal-Mor, and G. Segal.** 2001. Characterization of a *Legionella pneumophila* *relA* insertion mutant and the role of RelA and RpoS in virulence gene expression. *J Bacteriol* **184**:67-75.

Table S2. Primers used in this study

Gene	Primer name	Sequence (5'-3')
Primers used for construction of LacZ translational fusions		
<i>icmV</i>	icmVW-F	GGGATCCCCTGATCCTGATTTCTTTTTCATATT
	icmW-R-long	CGGCGAATTCAGCAAACGTAAGCCTCTTCCTG
<i>icmW</i>	icmVW-R	GGGATCCCCTTCATGGCTTAAATCAGGCAT
	icmV-F-long	GCCGGAATTCGTATCATAAAAGCAGCGACAACC
<i>lvgA</i>	lpg0525-BamHI	GTGCGGATCCATTTTCGATATCGCCGTCTGCCAT
	lpg0525-EcoRI	GAGCGAATTCCTACACTCCGCTTTTGTGTTG
<i>sidH</i>	sidH-BamHI	GAGCGGATCCCCGGTTTCAATGGTTCTTTTCAT
	sidH-EcoRI	GACGGAATTCATAATTTTTACCGCAAACGC
<i>sidM</i>	sidM-BamHI	GACCGGATCCCCCTTGCTCTTCATTAACACTCATT
	sidM-EcoRI	GAGCGAATTCTGTACAAAGAGGCTGGATGC
<i>legA10</i>	lpg0038-BamHI	GTGCGGATCCCCATGGGGAATTGTTGCGGCCA
	lpg0038-EcoRI	GACGGAATTCAGCAAAGGACATACTATCGCC
<i>legA11</i>	lpg0436-BamHI	GACCGGATCCCCCTTTCATTTCACTTCTACCCATTT
	lpg0436-EcoRI	GCACGAATTCTCAGTCGTTTCCTTCGAAG
<i>ceg7</i>	lpg0227-BamHI	ACCGGATCCCCATTATCTATTGAATATGACATCTG
	lpg0227-EcoRI	GCAGGAATTCTTTTATGAAGGTGATAGCACG
<i>ceg18</i>	lpg0898-BamHI	GTGCGGATCCCCCGAAACAGCAGATAATATTGACAT
	lpg0898-EcoRI	GAGCGAATTCCTGCGCCTAGCGAAGAAC
<i>ceg33</i>	lpg2591-BamHI	GTGCGGATCCCCTGGATCTAATTGCGCGGCCAT
	lpg2591-EcoRI	GAGCGAATTCGCAGCAGGTTGCACACCATC
<i>cegC1</i>	lpg0012-BamHI	GTGCGGATCCCCTGTATGTTCCGTTGTGTTTCAT
	lpg0012-EcoRI	GAGCGAATTCGCGGAGATAATCCAATG
<i>cegC2</i>	lpg0126-BamHI	GTGCGGATCCCCAGTTGGGAACAAAGGTGTCAT
	lpg0126-EcoRI	GAGCGAATTCGTGAATTGGGCTTTGCACAG
<i>cegC3</i>	lpg1144-BamHI	GTGCGGATCCCCAGCGGGGGGGTTAAAGGCAT
	lpg1144-EcoRI	GAGCGAATTCGAATCGATGATCTGGAATCAGCC
<i>cegC4</i>	lpg2200-BamHI	GACCGGATCCCCCTCTTTTGTGTTTGCCTCATT

lpg2200-EcoRI GCACGAATTCGAAATGGAACGGGAAAGACA

Primers used for site-directed mutagenesis

<i>icmV</i>	VCB-1F	AAAAGATGAAATAATGCCTGATTTAAGCCATG
	VCB-1R	CAGGCATTATTTTCATCTTTTTTTACTGAGATACGTT
<i>icmW</i>	WCB-F	CATATTTTTTCATTTTTTCACAATTTTAAGTAATGG
	WCB-R	GTGAAAAATGAAAAAATATGAAAAAGAAATCAGGATCAAGG
<i>lvgA</i>	lvgA-mut-F	CGGCTTTTTTCACTTTTCTTAAGCTCTGTTTAATG
	lvgA-mut-R	AGAAAAGTGAAAAAAGCCGTAAAATCAATG
<i>sidH</i>	SidH-mut-F	TCAAAGTGAATGAAATTTTGAGAGAAAAAACCCT
	SidH-mut-R	CTCAAATTTTCATTCACCTTTGAATCAATATTGCGC
<i>sidM</i>	sidM-mut-F	GTGTAAATTTGAAATTATATGTAAAGACAATATCC
	sidM-mut-R	ACATATAATTTCAAATTTACACATCTAATACACAA
<i>ceg18</i>	lpg0898-CpxR-mut-F	TTTTAATTTCAATTTTTTTGTAAAGAAATATTTCA
	lpg0898-CpxR-mut-R	CAAAAAAATTGAAATTAAGTAATTGTTTCATTAT
	lpg0898-Pmra-mut-F	AACAATTCCTATATGTTCACTTAATGTTCTCTTCT
	lpg0898-Pmra-mut-R	AAGTGAACATATAGGAATTGTTAAGATTGAAATAT
<i>cegC1</i>	lpg0012-mut-F	TCTTAATTCACAATTAATTACTGTCTTATATTAAG
	lpg0012-mut-R	CAGTAATTAATTGTGAATTAAGATGAAAGCAATGG
<i>cegC2</i>	lpg0126-mut-F	CCGATTGATTCAAGACCATTTTTTTAATGATTATCC
	lpg0126-mut-R	AAAATGGTCTTGAATCAATCGAGAAACCAAACCC
<i>cegC3</i>	lpg1144-mut-F	CCAAGCTCTTGAAATTAATTGTAAACTTGACGC
	lpg1144-mut-R	AATTAATTTCAAGAGCTTGGTAGAGTAATTA
<i>cegC4</i>	lpg2200-mut-F	CTTATAATTTGAAGGAATTTGTAAAAAAATGAAC
	lpg2200-mut-R	ACAAATTCCTTCAAATTATAAGCTGCTCCTTTTAC

Primers used for RT PCR

<i>icmV</i>	icmV-RT-F	CCTCAGGAACCAACCCATATCGAATC
	icmV-RT-R	CAATGCGATCATAACCACAACCAAGC
<i>sidM</i>	SidM-RT-F	GCAGACCCAACCTTCGAAAATATGGATGC
	SidM-RT-R	GCCGTATAGCGGTGCTCTCTTC
<i>legA11</i>	lpg0436-RT-F	GGGTGGTTTGGAAAGCTGTTTCTTCTA
	lpg0436-RT-R	CAGTAGAGCCATGGATGGTTGGAGC
<i>ceg7</i>	lpg0227-RT-F	CAGCACACCCAGTAAGCGAGC
	lpg0227-RT-R	CCCGTTTGCCTTTACCAAAAACG
<i>ceg33</i>	Lpg2591-RT-F	TTGCAATCAGCTGCCCAAGGTTGC
	Lpg2591-RT-R	TTAACTCTTCAGCGAAGTCGGGAT
<i>cegC1</i>	Lpg0012-RT-F	CGAGTAACATCAGGAGCAGCCAG

	Lpg0012-RT-R	GCCTGAGCCAATGGAGCTGGAG
cegC2	lpg0126-RT-F	TCCGATAAGTTTTAGCAGGTGCCAA
	lpg0126-RT-R	GCAGCGATTTGGTCAGTAATACGC
sidF	sidF-RT-F	AGGTCTCGGGCTTGGGTTAC
	sidF-RT-R	CATACCCTCGCGAACAATGG
sdeB	sdeB-RT-F	CGCAATGAAAAAATTCGCATGC
	sdeB-RT-R	GAGGGTTCGGTAGATCCAGCA

Primers used for gel mobility shift assay

sidM	sidM-GS-F	GTGTAATAATCGAACGCATCG
	sidM-GS-R	CCTCGAGTACATGAGCATAATG
lvgA	lvgA-GS-F	GATTTGAGTACTTAAATTTGCCTC
	lpg0525-BamHI	GTGCGGATCCATTTCGATATCGCCGTCTGCCAT
cegC2	lpg0126-GS-F	TGTGCAAGCGTTTTCTTCTGTG
	lpg0126-BamHI	GTGCGGATCCCCAGTTGGGAACAAAGGTGTCAT

Primers used for construction of *Ptac-cpxR*

cpxR	CpxR-EcoRI	GAGCGAATTCATGAGCAGCTCTATTCTCATTATTG
	CpxR-pET-R	CCGGATCCTACAGACTACGCATTAAACATG

Primers used for construction of *CyaA* fusions

legA10	lpg0038-cyaA-F	GATATCTAGAAATGGCCGCAACAATTCCCCA
	lpg0038-cyaA-R	TCTCCTGCAGAAATTGCTAGTTGTAAGTGGG
legA11	lpg0436-cyaA-F	GAGAGAATTCGGTGATTAATAATGGGTAGAAGTG
	lpg0436-cyaA-R	GAGCGGATCCGGTGCTTCTTGACGACTTAA
ceg7	lpg0227-cyaA-F	GAGAGAATTCGATGTCATATTCAATAGATAATCCT
	lpg0227-cyaA-R	GAGCGGATCCCTGATGCATATTGAGTTCCC
ceg18	lpg0898-cyaA-F	GAGCGAATTCGATGTCAATATTATCTGCTGTTTCG
	lpg0898-cyaA-R	GACCGGATCCAAGGAGTTTATGAATGTGTT
ceg33	lpg2591-cyaA-F	GAGAGGATCCTATGGCCGCGCAATTAGATCC
	lpg2591-cyaA-R	GAGCCTGCAGTCTGACCACAAAGAAAAACC
cegC1	lpg0012-cyaA-F	GAGCGAATTCGATGAACACAACGGAACATAC
	lpg0012-cyaA-R	GACCGGATCCTTAAATAGTAACAATAGGCCAC
cegC2	lpg0126-cyaA-F	GAGCGGTACCCATGACACCTTTGTTCCCAAC
	lpg0126-cyaA-R	GACCGGATCCTGAAGCCTGACAACCAGTTC
cegC3	lpg1144-cyaA-F	GAGAGAATTCGATGCCTTTAACCCCCCCCCG
	lpg1144-cya-R	GAGCGGATCCCAGGTAAATACGGGGTGTCC
cegC4	lpg2200-cyaA-F	GAGAGAATTCGATGAGTGCAAAAACAAAAAGA

	lpg2200-cyaA-R	GAGCGGATCCTGATTATCGGCACTAAATCC
<i>sidM</i>	sidM-cyaA-EcoRI	GAGAGAATTCGATGAGTGTTAATGAAGAGCAATTT
	sidM-cyaA-BamHI	GACCGGATCCATAATTGAAATGCGCTGGT

Primers used for the construction of CyaA fusions under their natural promoter

<i>cegC4</i>	lpg2200-ApaI	GAGAGGGCCCGAAATGGAACGGGAAAGACA
	lpg2200-NdeI	GCACCATATGAGATATCTCCTCGCACTTGTTG
<i>sidM</i>	sidM-ApaI	GAGAGGGCCCTGTACAAAGAGGCTGGATGC
	sidM-NdeI	GCACCATATGAATTCTCCCCATTATGCTCAT
