

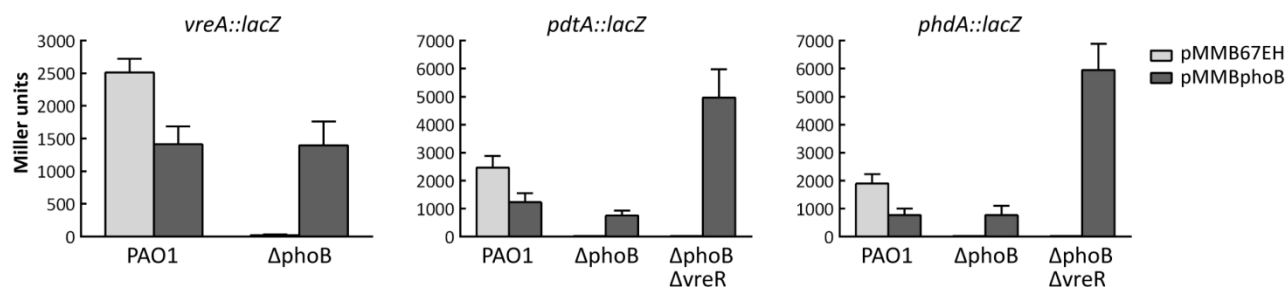
## Supplementary Material

# The activity of the *Pseudomonas aeruginosa* virulence regulator $\sigma^{\text{VreI}}$ is modulated by the anti- $\sigma$ factor VreR and the transcription factor PhoB

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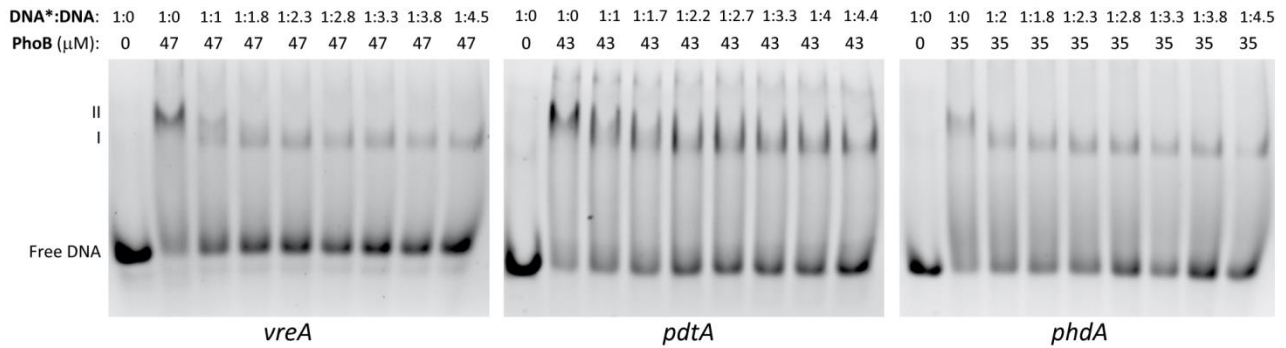
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### Supplementary Figure 1



**Figure S1. Complementation of the *P. aeruginosa* *phoB* mutants.**  $\beta$ -galactosidase activity of the indicated *P. aeruginosa* strains bearing the *vreA*, *pdtA* or *phdA lacZ* transcriptional fusion and the pMMB67EH (empty) or pMMBphoB (containing the *phoB* gene) plasmids upon growth in Pi starvation conditions.

## Supplementary Figure 2



**Figure S2. Competition EMSA reactions with unlabelled probes.** EMSA gels using fluorescein-labelled dsDNA probes and increasing amounts of an unlabelled competitor (Table S2) containing the indicated *P. aeruginosa* promoter. Upper numbers indicate the ratio of labelled-DNA (\*) and unlabelled-DNA, and the concentration of phosphorylated PhoB protein used in the assay (in μM). The position of the free DNA and of the PhoB-DNA complexes (I and II) are indicated.

**Table S1. Sequence of the primers used in this study**

Amplified (or deleted) gene and promoter region	Plasmid/assay	Name	Sequence (5' → 3') <sup>a</sup>
<i>vreA</i> (PA0674)	pKΔ <i>vreA</i>	PA0673F-X	CATTCTAGACAACCTGCTGACCAACGAG
		ΔPUMA3R-E	CCAGAATTCATTCCAGTCCGAACTACCC
		<i>vreIF</i> -E	GATGAATTCTGACGGAGGGAGTGGGAGGG
		C- <i>VreRR</i> -B	AATGGATCCTCAGCCGAGCAGCACCACC
<i>vreI</i> (PA0675)	pET- <i>vreI</i>	<i>VreIF</i> -Nd	ACACATATGAGCGATTGCGGGCAAAGC
		PA0675R-B	TATGGATCCCCTGCTTATGCTTATGACGG
<i>vreR</i> (PA0676)	pKΔ <i>vreR</i>	pUCMA3F-X	TCATCTAGAGTCCACTCGTCCG
		Δ <i>vreRR</i> -E	ACTGAATTCGACTGTGCTGTACGGACAC
		Δ <i>vreRF</i> -E	AAAGAATTCGGGGTGGTGTCTGCTCGGC
		PA0678R-B	TTCGGATCCATCTTCGTCGGGCACTCTG
	pMMB- <i>VreR</i> and pBBR <i>vreR</i>	PA0676F_4-Kp	GAATGGTACCATGACAGCCTCAGACTCCGC
		PA0676R_960-H	ACACAAGCTTTCAGCCGAGCAGCACCACC
	pMMB- <i>VreR</i> 43	PA0676F_4-Kp	GAATGGTACCATGACAGCCTCAGACTCCGC
		PA0676R_43-H	ACAAAGCTTACGCACACCACTGGCGGAAGG
	pMMB- <i>VreR</i> 86	PA0676F_4-Kp	GAATGGTACCATGACAGCCTCAGACTCCGC
		PA0676R_85-H	TTAAAGCTTAGCGACGACCGAAACGCCTC
	pMMB- <i>VreR</i> 110	PA0676F_4-Kp	GAATGGTACCATGACAGCCTCAGACTCCGC
		PA0676R_110-H	TTAAAGCTTAGCCATGGTCGAGCAGCGACG
	pMMB/ <i>VreR</i> -HA	PA0676Fa-E	AAAGAATTCATGACAGCCTCAGACTCCGCCGCC
		CHA-PA0676-X	TTTTCTAGATTAGCACGCGTAGTCCGGCACGTC GTACGGGTAGCCGAGCAGCACCACCCCGCCCGG
<i>pdta</i> (PA0690)	pMP0690	PR0690F-E	TTAGAATTCTCATGAGCGCCTTCATCACTGGTA
		PR0690R-X	TAATCTAGAAACGCTGCAACTGCTGGTTGA
	pTOPO-Pr0690	PR0690 F-E	TTAGAATTCTCATGAGCGCCTTCATCACTGGTA
		MCS220R	ATCAACGGTGGTATATCC
	5' RACE	GSP1B-0690	GAACATCCTGCGGGAGATTC

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		GSP2-0690	TGCTGGTTGACCCGCTGCTGCT
	Primer extension	PA0690R	GACGGCGAACGGGACGGCA
<i>phdA</i> (PA0691)	pTOPO-Pr0691b	PR0691F2Bg	<b>TAAAGATCT</b> GATCGCCGCGCTGTTCCCCGAG
		MCS220R	ATCAACGGTGGTATATCC
	5' RACE	GSP1-0691	CCGACGAACCAGCGATACAG
		GSP2-0691	CGCCCCCTGCCAATCTTCCTG
	Primer extension	PA0691R	CGTATTCACCGGAGTGACCA
<i>phoB</i> (PA5360)	pET-phoB	PhoBF-Nd	<b>TAGCATATG</b> GTTGGCAAGACAATCCTC
		PhoBR-B	AAC <b>GGATC</b> CCTCAGCTCTTGGTGGAGAAACG
	pMMB-phoB	1147F-E	AGAG <b>GAA</b> TTCAACCTGTTGAGCATAGCTC
		1148R-H	AGAG <b>AAGCTT</b> TTCAGCTCTTGGTGGAGAAAC

<sup>a</sup> The sequences of the restriction sites are indicated in bold

**Table S2. Oligonucleotides used in the EMSA reactions**

Name	Sequence (5' → 3') <sup>a</sup>
Flu-PvreAF	[Flc]-ACCGCAGGT <u>ACCGTCACACCACAGTCACACAGT</u> GCCATCAGGATGTCCCTCCTGGGTGATGGCCATCTGG
PvreAF	ACCGCAGGT <u>ACCGTCACACCACAGTCACACAGT</u> GCCATCAGGATGTCCCTCCTGGGTGATGGCCATCTGG
PvreAR	CCAGATGGCCATCACCCAGGAGGGACATCCTGATGGCACTGTGTGACTGTGGTGTGACGGTACCTGCGGT
Flu-PpdtAF	[Flc]-CGCTCCGCT <u>CATGAAC</u> TTTCCATGACAAGTCTTCGGCCACCCTCCGCCAGGCCGTCTACCAGTAA
PpdtAF	CGCTCCGCT <u>CATGAAC</u> TTTCCATGACAAGTCTTCGGCCACCCTCCGCCAGGCCGTCTACCAGTAA
PpdtAR	TTACTGGTAGACGGCCTGGCGGAGGGTGGGCCGAAGACTTGTTCATGGAAAGTTCATGGAGCGGAGCG
Flu-PpdtAmutF1	[Flc]-CGCTCCGCTC <u>ACCACCA</u> TTTCCATGACAAGTCTTCGGCCACCCTCCGCCAGGCCGTCTACCAGTAA
PpdtAmutR1	TTACTGGTAGACGGCCTGGCGGAGGGTGGGCCGAAGACTTGTTCATGGAAATGGTGGTGGAGCGGAGCG
Flu-PpdtAmutF2	[Flc]-CGCTCCGCTC <u>ACCACCA</u> TTTCTGGTGTAAAGTCTTCGGCCACCCTCCGCCAGGCCGTCTACCAGTAA
PpdtAmutR2	TTACTGGTAGACGGCCTGGCGGAGGGTGGGCCGAAGACTTACACCAGAAAATGGTGGTGGAGCGGAGCG
Flu-PphdAF	[Flc]-CGGCCGGTCGCATGAAGTTTTTCATGACAAAAGTTCGGTGGTGC GGCGGGGTGCGCGTCAAACAGGTGT
PphdAF	CGGCCGGTCGCATGAAGTTTTTCATGACAAAAGTTCGGTGGTGC GGCGGGGTGCGCGTCAAACAGGTGT
PphdAR	ACACCTGTTTGACGGCAACCCCGCCGACCACCGAACTTTTGTTCATGAAAAC TTCATGCGACCGGCCG

<sup>a</sup>Flc indicates fluorescein. The *pho box* is underlined and nucleotide substitutions relative to the wild-type sequence are in bold.