SUPPLEMENTAL MATERIAL

Bacillus subtilis Fur is a transcriptional activator for the PerR-repressed *pfeT* gene encoding an iron efflux pump

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Table S1)

STRAIN	GENOTYPE	REFERENCE
B. subtilis		
CU1065	W168 *attSP*β *trpC2*	Laboratory stock
ZB307A	W168 SPβ *c2Δ2*::Tn*917*::pSK10Δ6	Laboratory stock
HB18022	CU1065 SPβ *c2Δ2*::Tn*917*::ϕ(*pfeT-cat-lacZ*)	This work
HB2118	CU1065 *perR*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(*pfeT-cat-lacZ*)	(1)
HB2111	CU1065 *fur*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(*pfeT-cat-lacZ*)	(1)
HB2139	CU1065 *perR*::*spc fur*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(*pfeT-cat-lacZ*)	(2)
HB8116	CU1065 *pfeT*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(*pfeT-cat-lacZ*)	Laboratory stock
HB18068	CU1065 SPβ *c2Δ2*::Tn*917*::ϕ(PI**pfeT-cat-lacZ*)	This work
HB18075	CU1065 SPβ *c2Δ2*::Tn*917*::ϕ(FII**pfeT-cat-lacZ*)	This work
HB18076	CU1065 SPβ *c2Δ2*::Tn*917*::ϕ(FIII**pfeT-cat-lacZ*)	This work
HB18074	CU1065 SPβ *c2Δ2*::Tn*917*::ϕ(FF***pfeT-cat-lacZ*)	This work
HB18127	CU1065 SPβ *c2Δ2*::Tn*917*::ϕ(FII*PI**pfeT-cat-lacZ*)	This work
HB18125	CU1065 SPβ *c2Δ2*::Tn*917*::ϕ(FIII*PI**pfeT-cat-lacZ*)	This work
HB18126	CU1065 SPβ *c2Δ2*::Tn*917*::ϕ(3**pfeT-cat-lacZ*)	This work
HB18228	CU1065 Δ*katA*, *ahpCF*::*kan*	This work
HB18220	CU1065 Δ*katA*, *ahpCF*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(*pfeT-cat-lacZ*)	This work
HB18221	CU1065 Δ*katA*, *ahpCF*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(PI**pfeT-cat-lacZ*)	This work
HB18222	CU1065 Δ*katA*, *ahpCF*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(FII**pfeT-cat-lacZ*)	This work
HB18223	CU1065 Δ*katA*, *ahpCF*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(FIII**pfeT-cat-lacZ*)	This work
HB18224	CU1065 Δ*katA*, *ahpCF*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(FF***pfeT-cat-lacZ*)	This work
HB18225	CU1065 Δ*katA*, *ahpCF*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(FII*PI**pfeT-cat-lacZ*)	This work
HB18226	CU1065 Δ*katA*, *ahpCF*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(FIII*PI**pfeT-cat-lacZ*)	This work
HB18227	CU1065 Δ*katA*, *ahpCF*::*kan* SPβ *c2Δ2*::Tn*917*::ϕ(3**pfeT-cat-lacZ*)	This work
E. coli		
DH5α	ϕ80*lacZ*ΔM15 *recA1 endA1 gyrA96 thi-1 hsdR17*(rK- mK+)*supE44 relA1 deoR* Δ(*lacZYA-argF*)U169	Laboratory stock
PLASMID	**DESCRIPTION**	**REFERENCE**
pJPM122	*cat-lacZ* operon fusion vector for SPβ.	(3)
pDR244	For removal of the erythromycin resistance cassette to generate in-frame deletions.	(4)
pET17b	Used as template for the IVT ladder.	Laboratory stock

Table S2)

NUMBER	NAME	SEQUENCE
6927	IVT pfeT-F	GCAAACAAGCTGACGTTTCCGATTGTCCTT
6928	IVT pfeT-R	TTTCCCTCTTGTCTGTTTTCAATGGCTCATG
7115	IVT frvA-F	CAGTGGTTGGTACAATGAATCCAGGAAGAA
7116	IVT frvA-R	CAACATCACTACCGACCAAACAGCCAAT
9052	IVT ladder F1	GCCGCAGTGTTATCACTCATGGT
9053	IVT ladder R1	TGGTTGAGTACTCACCAGTCACAGAA
9054	IVT ladder R2	AGCACTTTTAAAGTTCTGCTATGTGGCG
9055	IVT ladder R3	TGCTGAAGATCAGTTGGGTGCAC
7277	bgal-pfeT-F	GCCAAGCTTCCCAACATCATTTTTGCTGAAT
7278	bgal-pfeT-R	GCGGATCCGGGTCGCGTTGAACGATAA
9002	EMSA pfeT-F	GCTGAGAGCATAGACTCTCAGCTTT
7032	pointPI-F	TGATAATTATTATCAAAAAGAAATTAATTAATAATTAATTGAAATTCTCTTCGT
7174	pointPI-R	TTAATTATTAATTAATTTCTTTTTGATAATAATTATCATTAATGTTTATTCACTTC
7352	pointFIII-F	GTTTTTATAAGAGAAACTTAGTAGTAATAAGTTCTCAATTAGAGA
7353	pointFIII-R	CTTATTACTACTAAGTTTCTCTTATAAAAAC
7440	pointFII-F	GAAGAAGTGAATAAACATTAACTTTATATTACAAAAAAAAGAAA
7351	pointFII-R	TTTTGTAATATAAAGTTAATGTTTATTCACTTCTTC
9094	katA check-F	CACTTACTCTGCTTGTTTCGCAA
9093	katA check-R	GAACACCGAAGGCTCTTATCGTT
6326	BKE MLS check R	TTTTCTCGTTCATAGTAGTTCCTCC
535a	pJPM122 check F	GTACATATTGTCGTTAGAACGCGGC
366	pJPM122 check R	ACTCTCCGTCGCTATTGTAACCAG

Table S3)

Fur regulated genes	OPERATOR SEQUENCE
dhbA	T**GA**TAATCATTA**TC**A
ykuN1	T**GA**AAATCATTA**TC**A
ykuN2	T**GA**AAATCATTA**TC**A
yuiI (*besA*)	T**GA**AAATCATTA**TC**A
feuABCybbA	T**GA**TAATAGTTA**TC**A
yxeB	T**GA**TAATGATAA**TC**A
ydbN	T**GA**TTATCAATA**TC**G
yfiY	T**GA**TAATGAATT**TC**A
ybbB (*btr*)	T**GA**AAATGATTA**TC**A
fhuB/D	A**GA**GAATCATTA**TC**A
ywjA	T**GA**GAAATATTA**TC**A
yhfQ	T**GA**TAATGATTC**TC**A
yoaJ	T**GA**TAATGATTC**TC**A
ywbL (*efeU*)	T**GA**TAATCATTT**TC**A
yfmC (*fecC*)	T**GA**TAATGATTC**TC**A
yfkM	TT**A**GGCTAAGTA**TC**A
yclN (*fpbN*)	T**GA**TAATGATAA**TC**A
yfiZ	T**GA**GAATAATCC**TC**A
yusV1	T**GA**AAATGATTT**TC**A
yusV2	TCGGAATCATTTG**C**A
yfhC	T**GA**TAATCATTT**TC**A
pfeT1	T**GA**TAATTATTA**TC**A
pfeT2	T**GA**AGATGATTTA**C**G

Table S3) List of Fur operator sites used to generate logo sequences. 23 Fur operator sites from Fur-regulated genes (numbers indicate the presence of multiple sites in a single regulatory region) were used to generate the sequence logo. The letters in bold represent the conserved signature bases that distinguish Fur operator sites from those regulated by PerR and Zur (2).

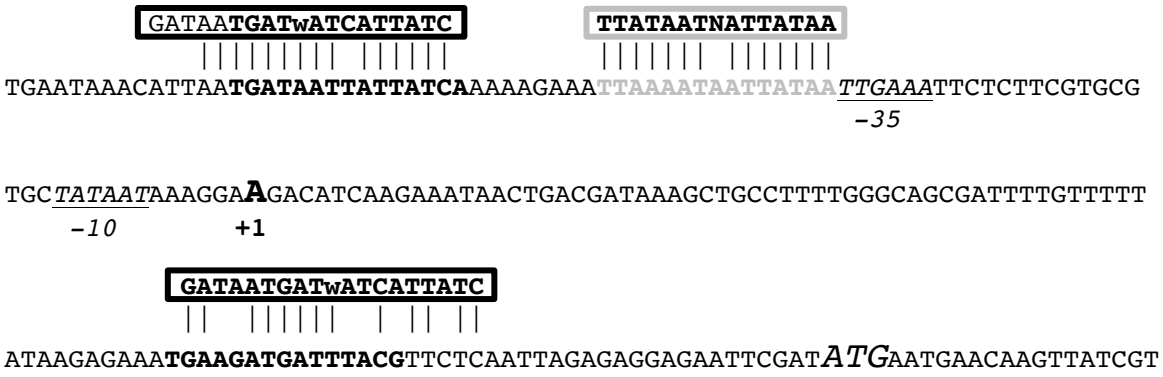
Table S4)

PerR regulated genes	OPERATOR SEQUENCE
mrgA	T**TA**TAATTATTA**TA**A
ahpC1	T**TA**GAATTATTA**T**TG
ahpC2	TA**A**TAATTCATA**TA**T
ahpC3	A**TA**TATTAATTAATA
katA	T**TA**TAATAATTA**TA**A
fur	T**TA**TAATAATTA**TA**G
perR1	T**TA**CACTAATTA**TA**A
perR2	T**TA**TAAACATTAC**A**A
hemAXCDBL1	T**TA**TAATTATTA**TA**A
hemAXCDBL2	T**TA**GAATGATTA**TA**A
pfeT	T**TA**AAATAATTA**TA**A

Table S4) List of PerR operator sites used to generate logo sequences. 11 PerR operator sites from PerR-regulated genes (numbers indicate the presence of multiple sites in a single regulatory region) were used to generate the sequence logo. The letters in bold represent the conserved signature bases that distinguish PerR operator sites from those regulated by Fur and Zur (2).

Figure S1)

A) ***pfeT* (*Bacillus subtilis*):**



B) ***frvA* (*Listeria monocytogenes*):**

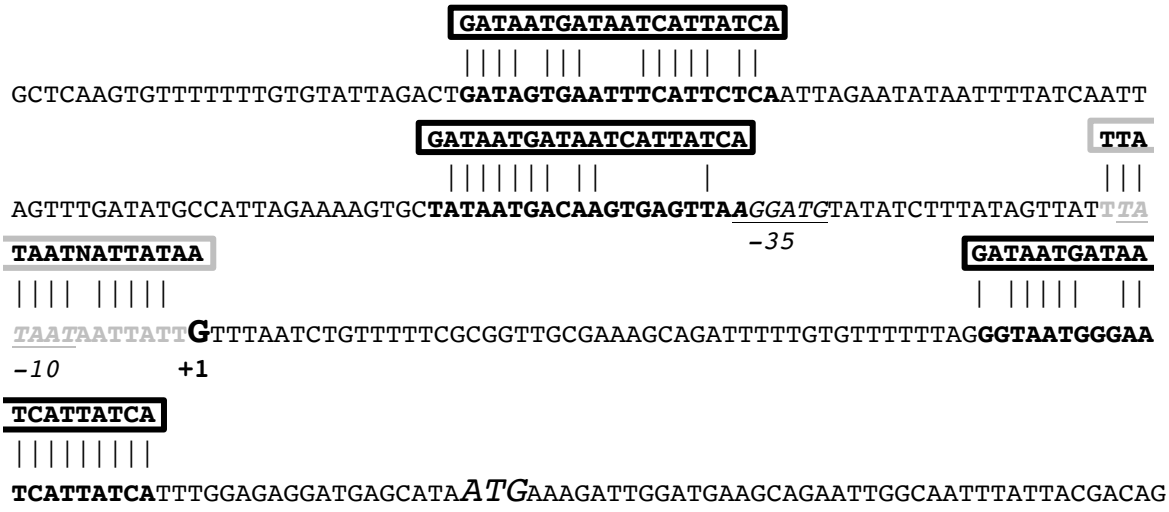


Figure S1) Full promoter sequences of *pfeT* and *frvA*.

A) Sequence of the *B. subtilis pfeT* promoter showing the designated Fur and PerR boxes in bold and grey, respectively. Operator sites are aligned with the conserved 7-1-7 operator sites for these regulators. Fur boxes show the full, classical 19 bp sequence both in the sequence and for the conserved alignment sequence. The -35 and -10 regions are in italics and underlined. The transcriptional start site is in bold black.

B) Complete sequence of the *L. monocytogenes frvA* promoter showing the designated Fur and PerR boxes boxes in bold and grey, respectively. Fur boxes show the full, classical 19 bp sequence both in the sequence and for the consensus. The -35 and -10 regions are in italics and underlined. The transcriptional start site is in bold.

Figure S2)

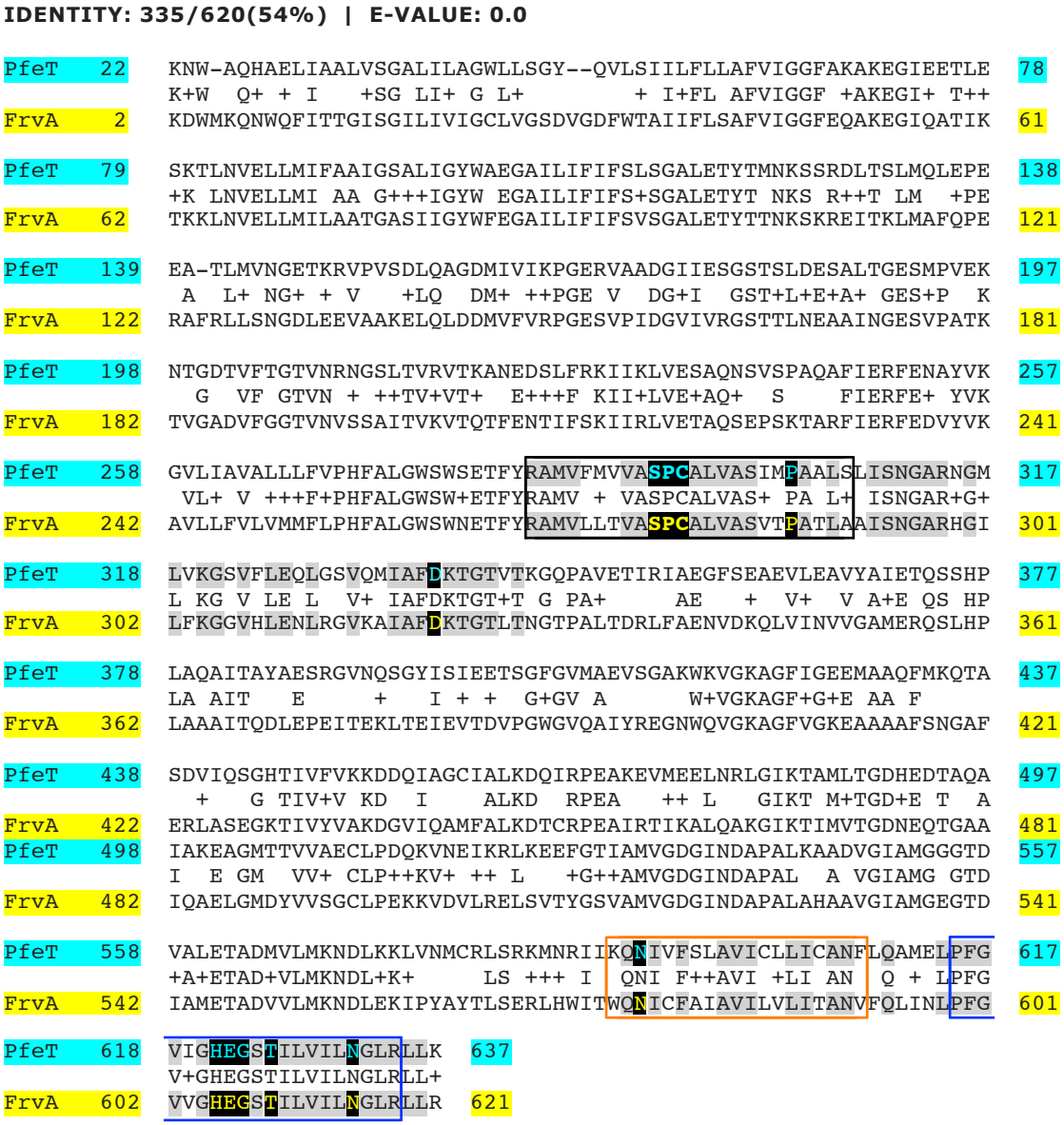


Figure S2) Protein sequence identity between PfeT and FrvA. BLAST alignment was used to illustrate protein sequence identity between *B. subtilis* PfeT and *L. monocytogenes* FrvA. H6, H7 and H8 conserved transmembrane segments characteristic of P_{1B4}-ATPases (5) are highlighted by black, orange and blue boxes, respectively.

References:

1. Fuangthong M, Herbig AF, Bsat N, Helmann JD. 2002. Regulation of the *Bacillus subtilis fur* and *perR* genes by PerR: not all members of the PerR regulon are peroxide inducible. J Bacteriol 184:3276-86.

2. Fuangthong M, Helmann JD. 2003. Recognition of DNA by three ferric uptake regulator (Fur) homologs in *Bacillus subtilis*. J Bacteriol 185:6348-57.

3. Slack FJ, Mueller JP, Sonenshein AL. 1993. Mutations that relieve nutritional repression of the *Bacillus subtilis* dipeptide permease operon. J Bacteriol 175:4605-14.

4. Koo BM, Kritikos G, Farelli JD, Todor H, Tong K, Kimsey H, Wapinski I, Galardini M, Cabal A, Peters JM, Hachmann AB, Rudner DZ, Allen KN, Typas A, Gross CA. 2017. Construction and Analysis of Two Genome-Scale Deletion Libraries for *Bacillus subtilis*. Cell Syst 4:291-305 e7.

5. Argüello JM. 2003. Identification of ion-selectivity determinants in heavy-metal transport P_{1B}-type ATPases. J Membr Biol 195:93-108.